PTPIP51 in adipose tissue

INAUGURAL-DISSERTATION
(Cumulative Thesis)
in partial fulfillment of the requirements
for the Doctoral degree
of the Faculty of Medicine
of the Justus-Liebig-University Giessen

by
Manuel Anton Bobrich
of Bamberg

Giessen 2012

From the Institute of Anatomy and Cell Biology
Managing Director: Prof. Dr. med. W. Kummer
Faculty of Medicine, Justus-Liebig-University Giessen

1. Reviewer: Frau Prof. Dr. Wimmer

2. Reviewer: Prof. Dr. Bellusci

Day of doctoral defense: 18.10.2012

To my father, who will always be with me;

To my mother, who will always take care of me;

To my uncle, who will always be there for me;

To my siblings, who will always be part of me;

To my niece, who will always be sunshine for me;

To Janika, who will always mean everything to me.

“I declare that I have completed this dissertation single-handedly without the unauthorized help of second party and only with the assistance acknowledged therein. I have appropriately acknowledged and referenced all text passages that are derived literally from or based on the content of published or unpublished work of others, and all information that relates to verbal communications. I have abided by the principles of good scientific conduct laid down in the character of the Justus-Liebig-University of Giessen in carrying out the investigations described in the dissertation.“

Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten oder nicht veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.

Manuel Anton Bobrich

This dissertation is submitted as a cumulative thesis according to the doctoral degree regulations of the Faculty of Medicine of the Justus-Liebig-University of Giessen. The thesis includes an interconnection of three original papers.
A general survey and summary of the state of the art of PTPIP51 implications in adipose tissue will be given here as the introduction.

Table of contents

I.	**List of papers submitted for thesis**	**1**
II.	**Introduction**	**2**
1.	**Introduction**	**2**
1.1.	Scientific background of PTPIP51	2
1.2.	PTPIP51 in different cellular processes	3
1.3.	PTPIP51 in cancer	5
2.	**PTPIP51 – Gene, mRNA and protein**	**5**
2.1.	PTPIP51 gene	5
2.2.	PTPIP51 mRNA	6
2.3.	PTPIP51 protein	7
3.	**Interaction partners of PTPIP51**	**8**
3.1.	Interaction with phosphatases	9
3.2.	Interaction with 14-3-3β	10
3.3.	Interaction with kinases	10
4.	**Adipose tissue**	**11**
4.1.	Energy storage	11
4.2.	Obesity	13
4.3.	Adipocyte metabolism	13
4.4.	Two important pathways in adipocytes	14
5.	**Experimental setup to determine the role of PTPIP51 in adipose tissue**	**15**
6.	**Role of PTPIP51 in adipose tissue**	**17**

7.	Prospect	19
8.	References	20
III.	Summary	29
IV.	Zusammenfassung	30
V.	List of publications	31
VI.	Acknowledgements	33
VII.	Curriculum vitae	34
VIII.	Annexes (Original papers)	36

I. List of papers submitted for thesis

Original papers

(1) Bobrich M, Brobeil A, Mooren FC, Krüger K, Steger K, Tag C, Wimmer M (2011). PTPIP51 interaction with PTP1B and 14-3-3β in adipose tissue of insulin-resistant mice. Int J Obes (Lond) 35(11): 1385-1394.

(2) Brobeil A, Bobrich M, Wimmer M (2011a). Protein tyrosine phosphatase interacting protein 51--a jack-of-all-trades protein. Cell Tissue Res 344(2): 189-205.

(3) Bobrich M, Schwabe SA, Brobeil A, Viard M, Kamm M, Mooren FC, Krüger K, Tag C, Wimmer M (2012). PTPIP51 – a new interaction partner of the insulin receptor and PKA in adipose tissue. Metabolism, under review

II. INTRODUCTION

1. Introduction

1.1 Scientific background of PTPIP51

Protein tyrosine phosphatase interacting protein 51 (PTPIP51) was discovered about ten years ago. Using the yeast two hybrid screening method, it was initially identified as an interaction partner of T-cell protein tyrosine phosphatase (TcPTP) and later, to a much higher extent, Protein tyrosine phosphatase 1B (PTP1B) (Stenzinger et al., 2009). To reveal the function of PTPIP51 several studies dealt with its expression pattern and its interaction partners in different tissues and cell culture systems.

PTPIP51 protein is expressed in epithelial cells of various organs. Highest evidence of PTPIP51 in epithelia can be found in skin where the expression of PTPIP51 is most likely associated with differentiation processes. The protein was also described in ciliated cells of the trachea, the fallopian tube and ependyma of the brain (Stenzinger et al., 2009). Recently, PTPIP51 was detected in non-keratinized epithelia of the mouth. Furthermore it is expressed in neurons in the brain and several brain tumours. In brain tissue, the expression is restricted to distinct functional nuclei, for example nucleus subthalamicus and nucleus paraventricularis. Additionally, PTPIP51 could be found in Purkinje cells of the cerebellum (Koch et al., 2009b).

Stenzinger et al. (2005) described a fast-fibre-type specific expression pattern of PTPIP51 in skeletal muscle, here being significantly associated to fast-contracting type-2a-fibres (Stenzinger et al., 2005).

PTPIP51 is also expressed in male reproductive tissue, where it can be detected throughout the complete differentiation process, from the primary spermatocytes up to the spermatid stage (Stenzinger et al., 2009). In placenta, PTPIP51 is expressed in syncytiotrophoblast and cytotrophoblast layer from the first, second, and third trimesters (Stenzinger et al., 2009). Stenzinger et al. (2007) investigated the expression of PTPIP51 in liver tissue. The tissue featured PTPIP51 protein in non-parenchymal cells, namely Kupffer cells, stellate cells and natural killer cells. Despite that specific association with non-parenchymal cells, a subset of hepatocyte nuclei

and cholangiocytes of the bile tract also expressed PTPIP51 (Stenzinger et al., 2007).

PTPIP51 also was seen in all investigated primary and secondary cell lines. Different studies revealed that PTPIP51 is expressed in a multitude of cell lines, such as glucagon-producing cells (INR1G9), human keratinocytes (HaCat), human placental cells (BeWo), acetylcholine-producing cells (NS20Y), Jurkat T-lymphoma cells (Stenzinger et al. 2005) and human skeletal muscle cells (Barop et al. 2009) as well as in a mouse fibroblast cell line (3T3) (Fig. 1) and a leukemia cell line (HL60) (Brobeil, personal communication).

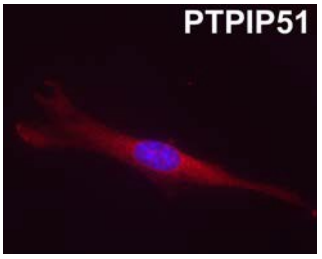


Fig. 1: PTPIP51 in proliferating 3T3 mouse fibroblast

These observations gave basis for further functional studies.

1.2 PTPIP51 in different cellular processes

First studies indicated an involvement of PTPIP51 in differentiation processes, as the protein was solely expressed in suprabasal cells of the epidermis (Koch et al., 2008). Further experimental exploration was conducted by investigating human keratinocyte cell culture (HaCat cells). Several experiments revealed that PTPIP51 expression can be upregulated by stimulation with Epithelial Growth Factor (EGF) or $1,25OH_2D_3$, two factors stimulating differentiation and inhibiting proliferation (Hashimoto, 2000; Stenzinger et al., 2009). Recently, a phosphorylation dependent activation of the MAPK pathway was proven by Brobeil et al. (2012). The MAPK pathway is important for proliferation and differentiation of cells. Activation of the MAPK pathway can be induced by several receptors like epithelial growth factor receptor (EGFR) (Petri et al., 2011) or downstream by the receptor kinases. One possibility of such a downstream activation is the activation of raf-1. 14-3-3β is able to interact with raf-1 and thus activate the MAPK pathway. PTPIP51 seems to play an important role in this interaction (Brobeil et al., 2012).

Barop et al. (2009) investigated the expression of PTPIP51 during muscle cell differentiation. Proliferating myoblasts displayed low expression levels of both mRNA and protein. Induction of differentiation to myotubes led to a sharp increase in PTPIP51 expression. In differentiated multinuclear myotubes PTPIP51 expression was highest. Re-induction of proliferation again decreased the level of PTPIP51

protein in the myotubes, so a differentiation dependent expression profile seems likely.
Further experiments by Brobeil et al. (2010) described PTPIP51 expression in differentiated cells of the immune system. Bone marrow specimen, umbilical cord blood and peripheral venous blood were all positively tested for PTPIP51 expressing cells. Especially neutrophil granulocytes showed high PTPIP51 expression levels in all investigated samples. In human bone marrow specimen PTPIP51 could also be traced in myeloid precursor cells of neutrophils (Brobeil et al., 2010).

Based on the observation of a nucleic localization and an increased expression in several types of cancer, experiments were conducted to investigate whether or not PTPIP51 is also involved in proliferation processes. PTPIP51 exhibits partial sequence homology with the microtubule-associated protein regulator of microtubule dynamics 1 (RMD-1). Therefore, PTPIP51 has been internalized in a family of proteins which are named regulator of microtubule dynamics (RMD), according to their assumed function. PTPIP51 is also called regulator of microtubule dynamics 3 (RMD-3) (Oishi et al., 2007; Brobeil et al., 2011a).
In vivo experiments associated PTPIP51 with the cell cycle. Important regulators of mitotic processes, CGI-99 and Nuf-2, were found to interact with PTPIP51 through a two-hybrid screen that used PTPIP51 as bait (Stenzinger et al., 2009). The interaction with CGI-99 hints towards an involvement of PTPIP51 in the regulation of polymerization of microtubules, whereas the interaction with Nuf-2 indicates a modulating function on the spindle apparatus (Brobeil et al., 2012). Therefore, PTPIP51 seems to be an important protein in proliferating cells, which is also shown by its expression in tumour tissue.

Additionally, PTPIP51 seems to be involved in apoptosis. Overexpression of PTPIP51 in HEK293T and HeLa cells led to the induction of apoptosis (Lv et al., 2006). Transfection of these cells with PTPIP51 led to a significant increase in the active caspase 3 and its substrate poly ADP ribose polymerase (Lv et al., 2006), two indicators of apoptosis. These findings were accompanied by a reduction of the mitochondrial membrane potential and cytochrome c release.

1.3 PTPIP51 in cancer

Studies on different kinds of cancer revealed an association of PTPIP51 with proliferating cells.

In glioblastoma cells, PTPIP51 expression was significantly upregulated compared to healthy glial cells. The expression of PTPIP51 was linked to 14-3-3β expression and to the tumour grade (Petri et al., 2011).

In prostate cancer, PTPIP51 mRNA levels were slightly increased compared to benign prostate hyperplasia (Koch et al., 2009a). Furthermore, the gene promoter region of prostate cancer was unmethylated. In benign prostate hyperplasia, 70% of CpG-rich islands were methylated.

Malignant melanoma cells displayed specific expression patterns of PTPIP51. The protein was also detected in peritumoural tissue. Koch et al. (2008) assumed that PTPIP51 might play a role in the development of keratinocyte tumours.

Current data concerning PTPIP51 in cancer reveals an increased expression in several leukemia cells and in squamous cell carcinoma (SCC) of the oropharynx (Brobeil et al., 2010; Planz, personal communication)

These observations of several different functions result in the question how this protein is capable of influencing oppositely directed signal pathways.

2. PTPIP51 - Gene, mRNA and protein

2.1 PTPIP51 gene

The gene of PTPIP51 is located on chromosome 15 (15q15.1, position 41,028,086-41,047,458), encompasses 13 exons and has a length of 19,373 base pairs (bp). Up to now, information about the gene and its regulation are rare.

Three mechanisms for the regulation of PTPIP51 gene transcription have been described so far.

Koch et al. (2009a) investigated the methylation status of the gene promoters and disclosed methylation dependence in PTPIP51 gene expression. In comparison to benign prostate hyperplasia, the PTPIP51 gene is hypomethylated in prostate cancer, whereas PTPIP51 protein expression is upregulated. Methylation of CpG-rich

islands associated with gene promoters leads to gene silencing through inhibition of binding sites for relevant transcription factors. Demethylation leads to the opposite effect: Cells display increased transcription and translation of demethylated genes (Ellis et al., 2009). In benign prostate hyperplasia, eighteen investigated CpG-rich regions are hypermethylated compared to their methylation status in prostate cancer (Koch et al., 2009a).

Another regulatory mechanism for increasing PTPIP51 expression is the regulation by the cAMP responsive element binding protein (CREB). A CREB binding site on the PTPIP51 gene has been identified by recent database research (Brobeil et al., 2011a). Several factors that are influenced by PTPIP51 seem to increase its expression by interaction with CREB, namely PKA and p90 ribosomal S6 kinase (p90RSK), leading to the regulation of the MAPK pathway (Zhang et al., 2005; Shaywitz and Greenberg, 1999; McCubrey et al., 2000).

Roger et al. (2007) identified the ciliary neurotrophic factor (CNTF) to inhibit PTPIP51 expression during retinal differentiation. CNTF is a neuroprotective factor (De Almeida et al., 2001) that possibly protects the differentiating retina cells from the pro-apoptotic effects of PTPIP51 (Brobeil et al., 2011a)

2.2. PTPIP51 mRNA

PTPIP51 mRNA consists of 2251 basepairs (bp), distributed over 13 exons. Exon 1 forms the untranslated 5' region and is a noncoding exon. Protein synthesis ends at a triplet on exon 13 at position 1596. This position holds a stop-codon related sequence (UAA) (Brobeil et al., 2011a). Initiation of translation starts at particular sequences, namely Kozak-sequences (GCCRCCaugG, whereas R stands for purine bases) (Kozak et al., 2005). AUG triplets mostly start Open Reading Frames (ORFs). Five ORFs have been identified so far. One of them is located upstream of the coding exons. This upORF is possibly able to regulate translation of PTPIP51, as observed in several other proteins (Kozak et al., 2005). As PTPIP51 holds different functions, an expression of different isoforms of the protein is assumed. Two different reasons for isoforms could be important for PTPIP51: leaky scanning and alternative splicing. Leaky scanning results in N-truncated isoforms (Kochetov 2008). Alternative splicing is a second possibility to explain isoforms. It is estimated that about 95-100% of pre-mRNAs from multi-exon-genes are alternatively spliced (Nilsen and Graveley 2010).

2.3 PTPIP51 protein

The full length form of human PTPIP51 consists of 470 amino acids with a molecular weight of 52,118 kDa. Calculations revealed several potential isoforms with different molecular weights (45 kDa, 38 kDa and 30 kDa). They display partially different expression patterns than the full length form. These isoforms are possibly generated through alternative splicing or leaky scanning, two mechanisms accounting for variations in protein length and weight.

The full length protein possesses several domains that are important for its functions but lack in some of its isoforms (Fig. 2).

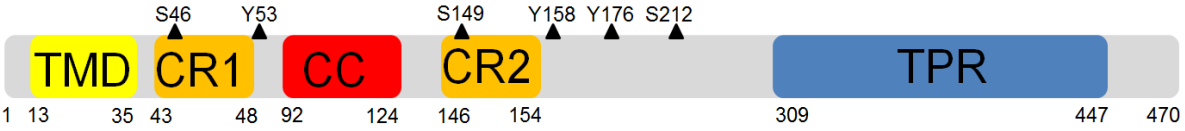


Fig. 2: Domains and phosphorylation sites of PTPIP51
S: Serine; Y: Tyrosine
TMD: transmembrane domain; CR1 and CR2: Conserved region 1 and 2; CC: Coiled-coil domain; TPR: Tetratricopeptide repeat domain
Numbers: amino acids

The N-terminal transmembrane domain is required for its association to mitochondria. The association of PTPIP51 to mitochondria seems to induce apoptosis as seen in HEK293T cells and HeLa cells (Lv et al. 2006).

Conserved region 1 (CR1) and 2 (CR2) are two crucial domains of the protein. CR1, spanning amino acid (aa) 43 to aa 48 and CR2, spanning aa 146 to aa 154, are binding sites for 14-3-3β. Yu et al. (2008) indicated that, after deletion of one of the conserved regions, the other one alone is able to bind 14-3-3β and activate the MAPK pathway. These findings indicate that there might be more functions for these regions than only the MAPK pathway activation.

PTPIP51 also possesses a coiled-coil domain, spanning aa 92 to aa 124. Such a coiled-coil domain indicates an involvement of the protein in vesicle trafficking in general and in hormone release in particular, as seen in hypothalamic neurons (Gillingham and Munro, 2003; Fotheringham et al. 1991; Koch et al. 2009b). Proteins

with coiled-coil domains are also important during mitosis. Nuf-2, for example, is a protein with a coiled-coil domain (Brobeil et al., 2011a). The interaction of Nuf-2 and PTPIP51 seems to be important during mitosis (Brobeil et al., 2012).

A tetratricopeptide repeat (TPR) like domain at aa303 to aa447 also seems to be associated with the cell cycle (Sikorski et al. 1990). Proteins containing several TPR-like domains seem to be especially involved in mitosis (Goebl and Yanagida, 1991; Oishi et al., 2007).

An important mechanism to regulate protein action in cells is phosphorylation and dephosphorylation. As PTPIP51 seems to be involved in a multitude of signal pathways, its activity needs to be controlled tightly. Therefore, several serine and tyrosine phosphorylation sites can be found in the protein. Tyrosines are at positions 53, 158, 176 und 300, whereas serines are at positions 44, 46, 50, 212, 225 (Brobeil et al., 2011a).

3. Interaction partners of PTPIP51

PTPIP51 is a multifunctional protein which can accomplish different functions by participating in several signalling cascades. Up to now, several interaction partners of PTPIP51 have been identified, either hypothetically or practically (Fig. 3).

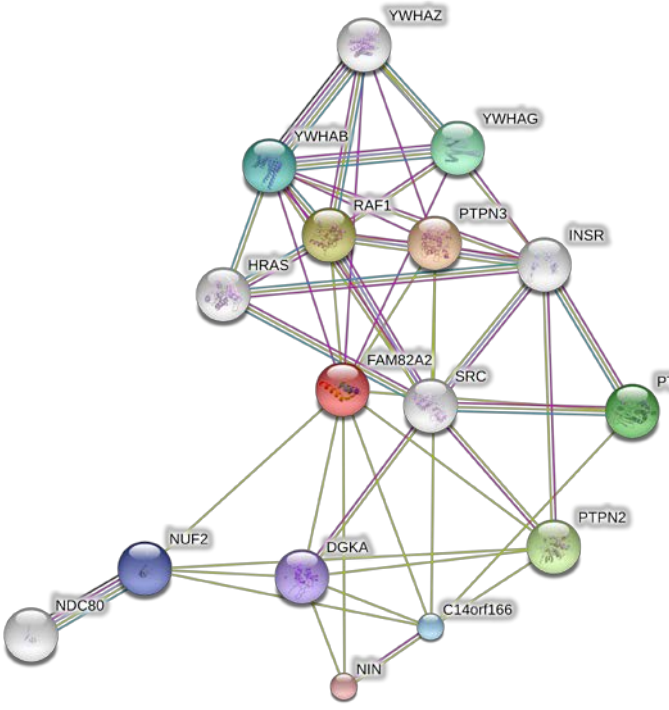


Fig. 3: Interaction partners of PTPIP51, as determined by string software:
FAM82A2: PTPIP51; C14orf166: UPF0568 protein C14orf166; DGKA: Diacylglycerolkinase α; NIN: Ninein; NUF: Nuf2; PTPN1-3: Protein Tyrosine Phosphatase non-receptor type 1-3; RAF1: v-raf-1 murine leukemia viral oncogene homolog 1; YWHAB: tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, β polypeptide; YWHAG: tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide;. Many these predicted interaction partners have already been proofed to interact with PTPIP51 in vivo.Calculated with string software, version 9.0

3.1 Interaction with phosphatases

TcPTP was the first interaction partner of PTPIP51 to be identified (Stenzinger et al., 2009). TcPTP is involved in apoptotic signal pathways, especially by increasing the expression of p53, a strong pro-apoptotic factor (Gupta et al., 2002; Radha et al., 1999). The interaction between TcPTP and PTPIP51 has not been investigated further yet.

PTP1B is a protein tyrosine phosphatase that dephosphorylates several important proteins, thus influencing intracellular signalling pathways. It is upregulated in different tumours, so it seems to be involved in tumourigenic processes (Wang et al., 2011; Lessard et al., 2012). Overexpression of PTP1B leads to hypophosphorylation of several receptor tyrosine kinases, thus decreasing the activity of the associated signal pathways (Stenzinger et al., 2009).

We could also find an association between PTPIP51 and PTP1B in adipose tissue which could be related to the insulin sensitivity of the investigated animals. PTP1B is already known to interact with the insulin receptor. Knockout of PTP1B in mice prevented them from adiposity, even after high fat diet (Klamann et al., 2000). This effect seems to take place mainly due to knockout of PTP1B in liver and skeletal muscle. PTP1B in adipose tissue seems to play a minor role in this action. Recent research in this area revealed that PTP1B has an influence on lipogenesis in adipose tissue (Owen et al., 2012).

Our experiments concerning the role of PTP1B interaction with PTPIP51 revealed that it is an essential factor in the interaction of PTPIP51 with 14-3-3β. Those experiments showed that PTP1B mediated dephosphorylation on tyrosine 53 or tyrosine 158 lead to an interaction of PTPIP51 with 14-3-3β and raf-1, whereas phosphorylation of these two sites resulted in dissociation of the complex (Brobeil et al., 2012).

Therefore, PTP1B seems to be one of the most important regulators of PTPIP51 function. Its impact on the action of PTPIP51 in adipose tissue has been investigated recently (Bobrich et al., 2011). The interaction of both proteins seems to be correlated to the status of insulin sensitivity, but the exact impact of this interaction is still not well known.

3.2 Interaction with 14-3-3β

14-3-3β is a crucial protein for MAPK pathway activation. Activation of this pathway leads to several changes in the cells, for example an increase in proliferation and differentiation (Yu et al., 2008). Thus, an increase in 14-3-3β can be found in different tumours, for example hepatocellular carcinoma or glioblastoma multiforme (Liu et al., 2012; Petri et al., 2011). In glioblastoma multiforme, 14-3-3β expression correlates to the grade of malignancy (Petri et al., 2011). Recent investigation concerning the relation between PTPIP51 and 14-3-3β in glioblastoma multiforme revealed an association of both, hence PTPIP51 expression seems also to correlate with the grade of malignancy. Controversially, the MAPK pathway seems to be involved in both lipogenesis and lipolysis in adipocytes (Gehart et al., 2010). An association between PTPIP51 and 14-3-3β was found in several tissues and cell culture systems. In adipose tissue, the interaction between both depends on the grade of insulin sensitivity. Several studies revealed that the interaction of PTPIP51 and 14-3-3β influences the MAPK pathway (Yu et al., 2008; Stenzinger et al., 2009; Bobrich et al., 2009; Brobeil et al., 2012). Interaction leads to an activation of raf-1. This activation stimulates the MAPK pathway, which is an important regulator of proliferation and differentiation (Brobeil et al., 2012).

3.3 Interaction with kinases

C-src is a kinase that plays an important role in mitosis (Brobeil et al., 2012). It is overexpressed in several tumours and in healthy tissue. Recently, c-src was found to influence the MAPK pathway through phosphorylation of tyrosine residue 176 in PTPIP51. Thus raf-1 is activated to stimulate the MAPK pathway (Brobeil et al., 2012)

Protein kinase A (PKA) fulfils a multitude of functions. It regulates calcium signalling (Vang et al., 2001) as well as the MAPK pathway (Wu et al., 1993). It also plays a role in apoptosis (Lizcano et al., 2000). In adipocytes, PKA mediates the β-adrenergic signal of catecholamines which leads to lipolysis (Collins et al., 2004). Its interaction with PTPIP51 seems to downregulate the inhibitory action of the IR on adenylylcyclase and to increase the lipolytic activity of the MAPK pathway (Bobrich et al., 2012).

Several receptor tyrosine kinases have been found to interact with PTPIP51. The epithelial growth factor receptor (EGFR) promotes proliferation. It is important in malignant transformation of glioblastoma multiforme and HNSCC (head and neck squamous cell carcinoma) of the oropharynx. In glioblastoma multiforme, a correlation between PTPIP51 and EGFR could be found (Petri et al., 2011). In HNSCC, a colocalization between PTPIP51 and the membranous EGF receptor seems likely (Planz, personal communication). The Insulin receptor (IR) activates several pathways following activation by insulin. Its functions in different organs such as liver or skeletal muscle aim to decrease blood glucose levels. Thus, it induces the translocation of specific glucose transporters to the plasma membrane. In adipose tissue it also increases lipogenesis and suppresses lipolysis (Bobrich et al., 2012). These effects can, at least partially, be mediated through PTPIP51 by direct phosphorylation. The tyrosine 176 phosphorylation of PTPIP51 leads to decreased interaction with 14-3-3β. Without being bound to PTPIP51, 14-3-3β does not stimulate the MAPK signal pathway and is able to inhibit PKA through interaction with phosphodiesterase 3B (PDE-3B) (Onuma et al., 2002; Bobrich et al., 2012).

The MAPK pathway is an important pathway in adipose tissue where it can either promote lipolysis or lipogenesis (Gehart et al., 2010). 14-3-3β is an interesting protein in adipocytes as it is able to interact with phosphodiesterase 3B (PDE-3B), thus decreasing the intracellular cAMP levels (Onuma et al., 2002). This leads to decreased lipolysis. As the interaction partners of PTPIP51 are important in adipocytes, we investigated the expression and interaction profile of PTPIP51 in adipose tissue in relation to the metabolism of the investigated individuals. The main focus of these investigations lies on the balance between lipolysis and lipogenesis, as imbalance between both can cause obesity.

4. Adipose tissue

4.1 Energy storage

Excessive energy in the body is stored in the form of fat, consisting of esters of glycerin and fatty acids. Fatty acids are absorbed in the intestine and are further

processed in the liver. They are converted to triglycerides and transported through the hepatocyte cell membrane to the blood as very low density lipoproteins (VLDL). VLDLs are transported via the blood stream to the adipose tissue, especially to the visceral adipose tissue. Several enzymes convert triglycerides into free fatty acids which can get incorporated into fat molecules and stored as fat droplets (Dallinga-Thie et al., 2010).

Adipocytes are highly specialized cells that represent the most important energy storage of the body. In case of energy demand fatty acids are transferred to the liver where they are converted into energy in form of ATP. To ensure a constant high energy level in the body, adipose tissue needs to be a dynamic storage which easily and quickly can take up and release fatty acids. These functions need to be controlled tightly, e.g. by hormones. Several hormones, such as insulin, epinephrine or glucagon influence adipocytes by binding to specialized receptors on the cellular surface.

Insulin, a multifunctional hormone, especially influences muscle, liver and adipose tissue where it induces several intracellular changes. One important function of insulin is its ability to increase glucose uptake by the translocation of the glucose transporter 4 (GLUT-4) to the cell membrane (Choi et al., 2010). In adipocytes, insulin acts lipogenic by tyrosine phosphorylation of several proteins, especially insulin receptor substrate 1 (IRS-1) and antilipolytic by the inhibition of the lipolytic cAMP-activated kinase A (PKA) (Gual et al., 2005; Choi et al., 2010). PKA inactivation is mainly conducted by phosphodiesterase 3B (PDE-3B), a protein that is activated via Akt signalling (Ahmad et al., 2007; Kitamura et al., 1999). PDE-3B is not only activated via Akt but also through 14-3-3β (Onuma et al., 2007). The 14-3-3β protein (Onuma et al., 2002) interacts with the MAPK pathway not only in adipose tissue but also in several other tissues. As PTPIP51 interacts with 14-3-3β in adipose tissue, we assume that it might play a role in the metabolism of adipocytes.

The opponents of insulin, epinephrine and glucagon, bind to different receptors, namely β-adrenergic receptors resp. glucagon receptors. Both act lipolytic through the activation of adenylylcyclase, thus increasing intracellular cAMP levels and PKA activity (Duncan et al., 2007).

Lipolysis and lipogenesis can only be regulated by interplay of both regulation mechanisms. Thus, the lipolytic and lipogenic pathways are connected via several proteins which can act as switchers between both pathways. Protein Tyrosine

Phosphatase Interacting Protein 51 (PTPIP51) is involved in a broad panel of regulations. It could possibly play a role in the modulation of both pathways.

4.2 Obesity

The number of obese people in developed countries steadily increases. Obesity is defined through a body-mass-index (BMI) of above 30 kg/m^2. Metabolic changes in the obese individual abet several diseases such as diabetes type II, atherosclerosis or impaired wound healing (Sibal et al., 2011; Tirosh et al., 2011; Xing et al., 2011). Recent findings point out that adipocytes play an important role in energy balance, although adipocyte differentiation does not seem to cause obesity itself (Rosen et al. 2006). However, adipocytes seem to play an important role in the development of the metabolic syndrome (Barth, 2011). Adipose tissue does not only possess the ability to store fatty acids, it is also an important regulatory and hormone producing organ (Wozniak et al., 2008). Adipose tissue produces several cytokines like adiponectin and leptin. Both are mediators that influence the energy state of individuals (Li, 2011; Ahima and Osei 2008; Hotta et al., 2008; Rosen and Spiegelman 2006).

Adipokines, produced by adipocytes, are suspected to play an outstanding role in the development of the metabolic syndrome, especially when the balance of adipokines is disturbed (Tersigni et al., 2011). The adipokine production is influenced by the metabolism of the adipocyte and by the activation levels of several signalling pathways, such as the PKA signalling pathway (Than et al., 2011). Insulin and leptin seem to exhibit overlapping actions through activation of different pathways in the body (Scherer et al., 2011).

4.3 Adipocyte metabolism

Fatty acids are stored as triacylglycerids (TAG). There are two ways to produce TAG in adipocytes. One way is de novo lipogenesis. In human adipocytes, de novo lipogenesis seems to play a minor role in TAG genesis, as it has been found in several investigations (Sjostrom 1973; Lafontan, 2008; Roberts et al., 2009). Recent findings by Collins et al. (2009) revealed that de novo lipogenesis might play a more important role in the membrane lipid generation.

The more frequented way in the generation of TAGs is its formation from fatty acids that accumulate within the bloodstream as chylomicrons or very low density

lipoproteins (VLDLs). The lipolysis of these particles takes place in the lumen of the blood vessels (Goldberg, 1996). The lipoprotein lipase (LPL) is secreted by adipocytes and released into the luminal space where it is bound to glycosaminoglycans on endothelial cells. The hydrolysis of both lipoproteins results in non-esterified fatty acids (NEFA) which can be absorbed by adipocytes.

FATP1 is one of the membrane-bound transporters for fatty acids. It does not play an important role in the basal fatty acid uptake, but it is important in the insulin stimulated fatty acid uptake (Lobo et al., 2006). Its translocation is stimulated by insulin, whereas its expression is increased by PPARγ and decreased by insulin (Lobo et al., 2006). These in vivo findings have recently been corroborated by in vitro experiments (Lenz et al., 2011).

Besides FATP1, another fatty acid transporter protein namely FATP4 has been detected in adipocytes. The role of FATP4 has not been fully determined yet. Its knockout does not influence the fatty acid mobilization in adipocytes, but lipolysis seems to be downregulated (Lenz et al., 2011).

4.4 Two important pathways in adipocytes

Important regulatory hormones in adipose tissue metabolism are on the one hand insulin and on the other hand epinephrine and glucagon. They fulfil opposing functions. Insulin increases glucose uptake and promotes lipogenesis, whereas epinephrine and glucagon induce lipolysis.

Insulin acts by activation of the insulin receptor. Extracellular binding of insulin leads to autophosphorylation of the two intracellular β-subunits, thus activating these subunits. The insulin receptor itself activates several molecules by tyrosine phosphorylation in favour of the following signalling cascades. One of them is the insulin receptor substrate 1 (IRS-1), a protein which induces most of the insulin actions in adipocytes. For example, IRS-1 is able to induce trafficking of intracellular glucose transporters to the plasma membrane (Peres et al., 2009). These GLUT-4 transporters increase glucose uptake into the cell. The insulin receptor also plays a role in the MAP kinase pathway, the Cbl-associated protein (CAP)/Cbl pathway, PI3K/Akt pathway or PTEN (Siddle, 2011). To sum up, the insulin receptor controls the metabolism in adipocytes in many different ways.

Adrenaline, as an antagonist of insulin, augments blood glucose levels and lipolysis. Its function in adipose tissue is mediated by two different receptors, α- and β-adrenergic receptors.

Adrenaline is able to either provoke lipolysis, mediated by β2-adrenergic receptors or, to a minor percentage, to initiate antilipolytic pathways by the activation of α2-adrenergic receptors. B2-adrenergic receptors induce lipolysis through activation of adenylyl-cyclase. Activated adenylyl-cyclase transforms ATP into cyclic AMP (cAMP) which in turn activates the cAMP dependent protein kinase A (PKA) (Mantovani et al., 2009; Lafontan, 2008). Protein kinase A thereupon initiates lipolysis (Mantovani et al., 2009). A2-adrenergic activation leads to opposite effects by inhibition of adenylyl-cyclase and PKA. (Lafontan, 2008).

PKA activity is the first step of the following downstream pathway. Thus, PKA is a constitutive kinase for the regulation of adipocyte energy status (Omar et al., 2009).

PKA activity is inhibited by the activated insulin receptor (Aitken, 2006). Amongst other effects insulin signalling results in the activation of phosphodiesterase 3B, which in turn hydrolyses cAMP, thus reducing the lipolytic activity of cAMP activated PKA (Lafontan, 2008).

PTPIP51 is involved in several signalling cascades. It interacts with 14-3-3β, influences the MAPK pathway and is controlled by receptor kinases such as the insulin receptor. All those reasons make PTPIP51 a possibly interesting target in diabetes and obesity, as the named proteins play important roles in the metabolism of adipocytes. Therefore, we investigated PTPIP51 in adipose tissue.

5. Experimental setup to determine the role of PTPIP51 in adipose tissue

To enclose the role of PTPIP51 especially in the metabolism of adipocytes we created mouse models with different states of insulin sensitivity. To accomplish these models, several groups of 10 mice each were distributed to different types of feeding and physical activity. The control group was fed a standard diet. Another group was fed a standard diet and trained on a running wheel at 80% of individual oxygen consumption to obtain a significant endurance training effect (Fig. 4). The third group was fed a high fat diet, and the fourth group was fed a high fat diet and submitted to the same training protocol as the second group.

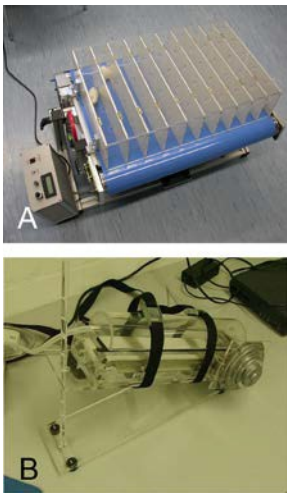


Fig.4: Experimental setup
A: Running wheel
B: Spirometry to determine the maximum oxygen consumption.

After a defined time interval, a glucose tolerance test was performed. The test revealed that the high fat diet mice had high blood glucose levels, even 2 hours after application of glucose. Training in these mice lowered the values. Normal fed mice displayed normal blood glucose levels, independent of the training state. After the glucose tolerance tests, the mice were killed and the visceral adipose tissue was investigated. PTPIP51, PTP1B, 14-3-3β, PKA and the IR in adipocytes were identified by immunofluorescence. To confirm possible interactions, a Duolink proximity ligation assay was performed. To review the principle of the Duolink proximity ligation assay, please refer to figure 5. PCR experiments detected PTPIP51 mRNA in adipose tissue of all investigated groups and could link its expression to the expression of PTP1B and 14-3-3β.

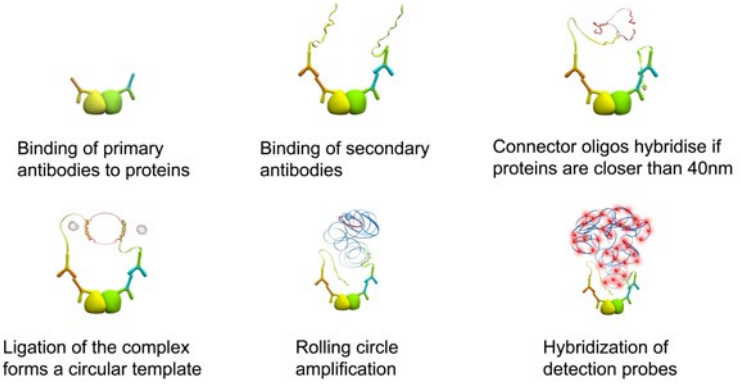


Fig. 5: Principle of the Duolink proximity ligation assay

The hybridization of the detection probes leads to a point-shaped signal, which can be quantified in special computer programmes. The amount of dots per cm^3 tissue can be compared to other probes of the same tissue.

Our experiments revealed that PTPIP51 interacts with PTP1B, 14-3-3β, PKA and the insulin receptor (IR), in correlation with the state of insulin sensitivity.

A partial colocalization in the immunofluorescent labelled probes could be detected for PTPIP51 with all investigated proteins. Interaction profiles differed widely. Animals fed a high fat diet displayed reduced interaction of PTPIP51 with PTP1B and 14-3-3β, whereas interaction with the IR was significantly increased. Submitting high fat diet animals to endurance training led to a slight increase in the interaction with PTP1B and the IR, a strong increase in 14-3-3β interaction and no significant difference in PKA interaction. Endurance training in normal fed animals led to a

further decrease of interaction with the IR compared to all other groups. PKA interaction showed a very strong decrease.

6. Role of PTPIP51 in adipose tissue

Basing on our results, we assume that PTPIP51 acts as a switcher between lipolytic and antilipolytic pathways in adipocytes.

The insulin receptor interacts with PTPIP51, probably phosphorylating PTPIP51 at Tyrosine 176. This phosphorylation site is known to influence the interaction profile of PTPIP51, especially with 14-3-3β. Phosphorylation at Tyrosine 176 decreased the interaction of PTPIP51 with 14-3-3β in HaCaT cells (Brobeil et al., 2012). In consequence the activation of the MAPK pathway was reduced in those cells. This mechanism is probably transferable to the regulation of the MAPK pathway in adipocytes. Phosphorylation of PTPIP51 at Tyrosine 176 could lead to a decrease in the interaction between PTPIP51, 14-3-3β and raf-1 thus reducing the MAPK pathway activity. Instead, 14-3-3β could interact with other proteins, most likely phosphodiesterase 3B (PDE-3B). Stimulated PDE-3B is able to influence PKA. The interaction of 14-3-3β with PDE-3B is known to decrease the activity of PKA, thus inhibiting lipolysis.

As has been known for long, PTP1B plays a crucial role in the development of diabetes and obesity. PTP1B knockout mice are unable to develop adiposity, not even when submitted to high fat diet (Ali et al., 2009). So far, PTP1B in adipocytes seems to play a minor role in the development of adiposity. Previous knockout experiments identified especially liver PTP1B and muscle PTP1B to be important. Although the definite role of adipocyte PTP1B still remains unclear, it seems to dephosphorylate proteins in the insulin signalling pathway (Venable et al., 2000). Recent investigations concerning the role of PTP1B in adipocytes state that adipose tissue specific deletion of PTP1B increases lipogenesis (Owen et al., 2012).

PKA interacts with PTPIP51 by phosphorylating it at Ser46. This phosphorylation site is crucial for binding of PTPIP51 to 14-3-3β and its interaction with raf-1. These interactions result in activation of the MAPK pathway (Bobrich et al., 2012). The interaction pattern of PKA and PTPIP51 leads to the assumption that it increases the lipolytic action of the MAPK pathway in adipocytes by phosphorylation of PTPIP51.

Our experiments showed that, in states of physical training, interaction between PKA and PTPIP51 is reduced. This seems controversial, especially if PTPIP51 is a mediator of the lipolytic action of PKA. But there is another, very potent interaction partner of PKA. The hormone sensitive lipase (HSL) is a strong activator of lipolysis (Campbell et al., 2009; Collins et al., 2004). A switch of PKA from PTPIP51 to HSL to force lipolysis seems most likely. We hypothesize that PTPIP51 acts as a switcher between the lipolytic action of PKA and the antilipolytic action of the IR (Fig. 6).

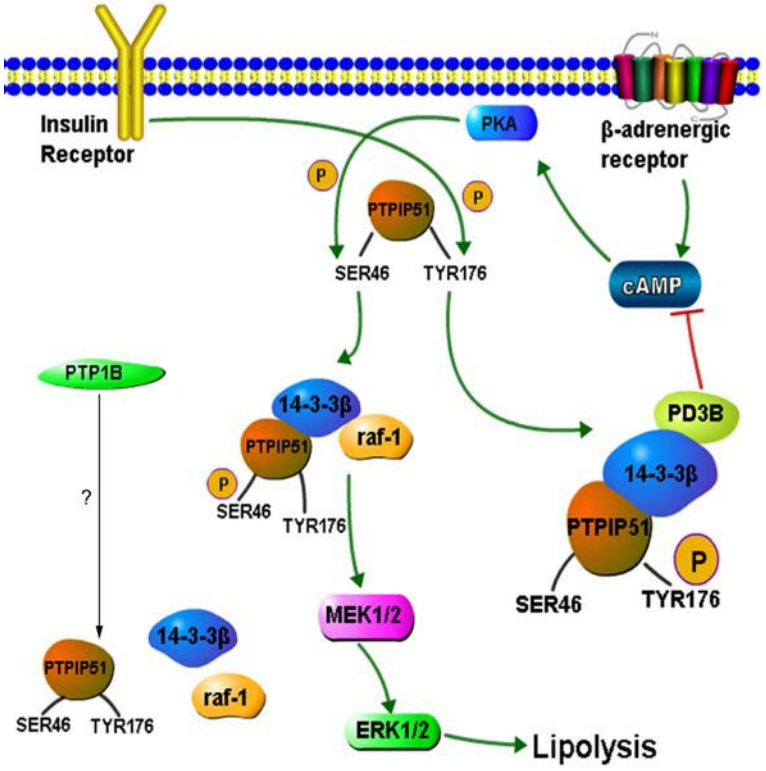


Fig. 6: Possible function of PTPIP51 in adipose tissue
The insulin receptor phosphorylates PTPIP51 at tyrosine 176. This leads to interaction of the PTPIP51-14-3-3β-complex with PDE3B, thus leading to a decrease in the PKA-activating cAMP-levels. β-adrenergic receptor activation in contrast leads to an increase in cAMP-levels and PKA activation. PKA phosphorylates PTPIP51 at serine 46, thus activating the lipolytic MAPK pathway through activation of raf-1. The impact of PTP1B dephosphorylation of PTPIP51 in adipose tissue is not yet clear.

7. Prospect

The impact of PTPIP51 on adipose tissue metabolism is still not fully understood. Our investigations linked PTPIP51 to insulin and PKA signalling in adipocytes. Yet, there are numerous other pathways, so the definite role and importance of PTPIP51 in adipocytes is not completely understood. Functional assays in PTPIP51 knockout models could enclose the role of the protein in adipocyte metabolism. Furthermore, PTPIP51 expression in other insulin dependent organs like liver, pancreas or skeletal muscle will enrol more details of PTPIP51 effects on fatty acid metabolism. Thus, further investigations are needed to identify the role of PTPIP51 in the development of metabolic syndrome and diabetes.

8. References

Ahmad F, Lindh R, Tang Y, Weston M, Degerman E, Manganiello VC (2007). Insulin-induced formation of macromolecular complexes involved in activation of cyclic nucleotide phosphodiesterase 3B (PDE3B) and its interaction with PKB. Biochem J 404(2): 257-268.

Ahima RS, Osei SY (2008). Adipokines in obesity. Front Horm Res 36: 182-197.

Aitken A (2006). 14-3-3 proteins: a historic overview. Semin Cancer Biol 16(3): 162-172.

Ali MI, Ketsawatsomkron P, Belin de Chantemele EJ, Mintz JD, Muta K, Salet C et al. (2009). Deletion of protein tyrosine phosphatase 1b improves peripheral insulin resistance and vascular function in obese, leptin-resistant mice via reduced oxidant tone. Circ Res 105: 1013–1022.

Barop J, Sauer H, Steger K, Wimmer M (2009). Differentiation-dependent PTPIP51 expression in human skeletal muscle cell culture. J Histochem Cytochem 57(5): 425-435.

Barth RJ (2011). Insulin resistance, obesity and the metabolic syndrome. S D Med Spec No: 22-7.

Bobrich M, Brobeil A, Mooren FC, Krüger K, Steger K, Tag C, Wimmer M et al. (2011). PTPIP51 interaction with PTP1B and 14-3-3β in adipose tissue of insulin-resistant mice. Int J Obes (Lond) 35(11): 1385-1394.

Bobrich M, Schwabe SA, Brobeil A, Viard M, Kamm M, Mooren FC et al. (2012). PTPIP51 – a new interaction partner of the insulin receptor and PKA in adipose tissue. Metabolism, submitted manuscript

Brobeil A, Graf M, Oeschger S, Steger K, Wimmer M (2010). PTPIP51-a myeloid lineage specific protein interacts with PTP1B in neutrophil granulocytes. Blood Cells Mol Dis 45(2): 159-168.

Brobeil A, Bobrich M, Wimmer M (2011a). Protein tyrosine phosphatase interacting protein 51--a jack-of-all-trades protein. Cell Tissue Res 344(2): 189-205.

Brobeil A, Bobrich M, Graf M, Kruchten A, Blau W, Rummel M, Oeschger S, Steger K, Wimmer M (2011b). PTPIP51 is phosphorylated by Lyn and c-Src kinases lacking dephosphorylation by PTP1B in acute myeloid leukemia. Leuk Res 35(10):1367-1375.

Brobeil A, Bobrich M, Tag C, Wimmer M (2012). PTPIP51 in Protein Interactions: Regulation and In Situ Interacting Partners. Cell Biochem Biophys; Epub ahead of print.

Campbell JE, Fediuc S, Hawke TJ, Riddell MC (2009). Endurance exercise training increases adipose tissue glucocorticoid exposure: adaptations that facilitate lipolysis. Metabolism 58(5): 651-660.

Choi SM, Tucker DF, Gross DN, Easton RM, DiPilato LM, Dean AS, Monks BR, Birnbaum MJ (2010). Insulin regulates adipocyte lipolysis via an Akt-independent signalling pathway. Mol Cell Biol 30(21): 5009-5020.

Collins S, Cao W, Robidoux J (2004). Learning new tricks from old dogs: β-adrenergic Receptors teach new lessons on firing up adipose tissue metabolism. Mol Endocrinol 18(9): 2123-2131.

Collins JM, Neville MJ, Hoppa MB, Frayn KN (2010). De novo lipogenesis and stearoyl-CoA desaturase are coordinately regulated in the human adipocyte and protect against palmitate-induced cell injury. J Biol Chem 285(9): 6044-6052.

Dallinga-Thie GM, Franssen R, Mooij HL, Visser ME, Hassing HC, Peelman F, Kastelein JJ, Péterfy M, Nieuwdorp M (2010). The metabolism of triglyceride-rich

lipoproteins revisited: new players, new insight. Atherosclerosis 211(1): 1-8.

De Almeida LP, Zala D, Aebischer P, Déglon N (2001). Neuroprotective effect of a CNTF-expressing lentiviral vector in the quinolinic acid rat model of Huntington's disease. Neurobiol Dis 8: 433–446

Duncan RE, Ahmadian M, Jaworski K, Sarkadi-Nagy E, Sul HS (2007). Regulation of lipolysis in adipocytes. Annu Rev Nutr 27: 79-101.

Ellis L, Atadja PW, Johnstone RW (2009). Epigenetics in cancer: targeting chromatin modifications. Mol Cancer Ther 8: 1409–1420

Fotheringham AP, Davidson YS, Davies I, Morris JA (1991). Ageassociated changes in neuroaxonal transport in the hypothalamoneurohypophysial system of the mouse. Mech Ageing Dev 60: 113–121

Gehart H, Kumpf S, Ittner A, Ricci R (2010). MAPK signalling in cellular metabolism: stress or wellness? EMBO Rep 11(11): 834-840.

Gillingham AK, Munro S (2003). Long coiled-coil proteins and membrane traffic. Biochim Biophys Acta 1641: 71–85

Goebl M, Yanagida M (1991). The TPR snap helix: a novel protein repeat motif from mitosis to transcription. Trends Biochem Sci 16: 173–177

Goldberg IJ (1996). Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. J Lipid Res 37(4): 693-707.

Gual P, Le Marchand-Brustel Y, Tanti JF (2005). Positive and negative regulation of insulin signalling through IRS-1 phosphorylation. Biochimie 87(1): 99-109.

Gupta S, Radha V, Sudhakar CH, Swarup G (2002). A nuclear protein tyrosine phosphatase activates p53 and induces caspase-1-dependent apoptosis. FEBS Lett 532, 61–66.

Hashimoto K (2000). Regulation of keratinocyte function by growth factors. J Dermatol Sci 24 Suppl 1: S46-50.

Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y et al. (2000). Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 20: 1595–1599.

Kabir M, Stefanovski D, Hsu IR, Iyer M, Woolcott OO, Zheng D, Catalano et al. (2011). Size Cells in the Visceral Adipose Depot Predict Insulin Resistance in the Canine Model. Obesity (Silver Spring); Epub ahead of print

Kitamura, T, Kitamura Y, Kuroda S, Hino Y, Ando M, Kotani K et al. (1999). Insulin-induced phosphorylation and activation of cyclic nucleotide phosphodiesterase 3B by the serine-threonine kinase. Akt Mol Cell Biol 19: 6286–6296.

Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM et al. (2000) Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol Cell Biol 20: 5479–5489.

Koch P, Stenzinger A, Viard M, Märker D, Mayser P, Nilles M et al. (2008). The novel protein PTPIP51 is expressed in human keratinocyte carcinomas and their surrounding stroma. J Cell Mol Med 12(5B): 2083-2095.

Koch P, Petri M, Paradowska A, Stenzinger A, Sturm K, Steger K, Wimmer M (2009a). PTPIP51 mRNA and protein expression in tissue microarrays and promoter methylation of benign prostate hyperplasia and prostate carcinoma. Prostate 69(16): 1751-1762.

Koch P, Viard M, Stenzinger A, Brobeil A, Tag C, Steger K, Wimmer M (2009b). Expression profile of PTPIP51 in mouse brain. J Comp Neurol 517: 892–905

Kochetov AV (2008). Alternative translation start sites and hidden coding potential of eukaryotic mRNAs. Bioessays 30: 683–691.

Kozak M (2005). Regulation of translation via mRNA structure in prokaryotes and eukaryotes. Gene 361: 13–37

Lafontan M (2008). Advances in adipose tissue metabolism. Int J Obes (Lond) 32 Suppl 7: S39-51.

Lenz LS, Marx J, Chamulitrat W, Kaiser I, Gröne HJ, Liebisch G et al. (2011). Adipocyte-specific inactivation of the acyl-CoA-synthetase fatty acid transport protein 4 (Fatp4) in mice causes adipose hypertrophy and alterations in metabolism of complex lipids under high-fat diet. J Biol Chem; Epub ahead of print

Lessard L, Labbé DP, Deblois G, Bégin LR, Hardy S, Mes-Masson AM et al. (2012). PTP1B is an androgen receptor-regulated phosphatase that promotes the progression of prostate cancer. Cancer Res72(6): 1529-1537.

Li MD (2011). Leptin and beyond: an odyssey to the central control of body weight. Yale J Biol Med 84(1): 1-7.

Liu TA, Jan YJ, Ko BS, Chen SC, Liang SM, Hung YL et al. (2012). Increased expression of 14-3-3β promotes tumor progression and predicts extrahepatic metastasis and worse survival in hepatocellular carcinoma. Am J Pathol 179(6): 2698-2708.

Lizcano JM, Morrice N, Cohen P (2000). Regulation of BAD by cAMP-dependent protein kinase is mediated via phosphorylation of a novel site, Ser155. Biochem J 349, 547–557.

Lobo S, Wiczer BM, Smith AJ, Hall AM, Bernlohr DA (2007). Fatty acid metabolism in adipocytes: functional analysis of fatty acid transport proteins 1 and 4. J Lipid Res 48(3): 609-620.

Lv BF, Yu CF, Chen YY, Lu Y, Guo JH, Song QS et al. (2006). Protein tyrosine phosphatase interacting protein 51 (PTPIP51) is a novel mitochondria protein with an

N-terminal mitochondrial targeting sequence and induces apoptosis. Apoptosis 11(9): 1489-1501.

Mantovani G, Bondioni S, Alberti L, Gilardini L, Invitti C, Corbetta S et al. (2009). Protein kinase A regulatory subunits in human adipose tissue: decreased R2B expression and activity in adipocytes from obese subjects. Diabetes 58(3): 620-626.

McCubrey JA, May WS, Duronio V, Mufson A (2000). Serine/threonine phosphorylation in cytokine signal transduction. Leukemia 14: 9–21

Nilsen TW, Graveley BR (2010). Expansion of the eukaryotic proteome by alternative splicing. Nature 463: 457–463

Oishi K, Okano H, Sawa H (2007). RMD-1, a novel microtubule-associated protein, functions in chromosome segregation in Caenorhabditis elegans. J Cell Biol 179: 1149-1162.

Omar B, Zmuda-Trzebiatowska E, Manganiello V, Göransson O, Degerman E (2009). Regulation of AMP-activated protein kinase by cAMP in adipocytes: roles for phosphodiesterases, protein kinase B, protein kinase A, Epac and lipolysis. Cell Signal 21(5): 760-766.

Onuma H, Osawa H, Yamada K, Ogura T, Tanabe F, Granner DK, Makino H (2002). Identification of the insulin-regulated interaction of phosphodiesterase 3B with 14-3-3 β protein. Diabetes 51(12): 3362-3367.

Owen C, Czopek A, Agouni A, Grant L, Judson R, Lees EK et al. (2012). Adipocyte-specific protein tyrosine phosphatase 1B deletion increases lipogenesis, adipocyte cell size and is a minor regulator of glucose homeostasis. PLoS One 7(2): e32700.

Peres SB, de Moraes SM, Costa CE, Brito LC, Takada J, Andreotti S et al. (2005). Endurance exercise training increases Insulin responsiveness in isolated adipocytes through IRS/PI3-kinase/Akt pathway. J Appl Physiol 98(3): 1037-1043.

Petri MK, Koch P, Stenzinger A, Kuchelmeister K, Nestler U, Paradowska A, et al. (2011). PTPIP51, a positive modulator of the MAPK/Erk pathway, is upregulated in glioblastoma and interacts with 14-3-3β and PTP1B in situ. Histol Histopathol 26(12): 1531-1543.

Radha V, Sudhakar C, Swarup G (1999). Induction of p53 dependent apoptosis upon overexpression of a nuclear protein tyrosine phosphatase. FEBS Lett 453, 308–312.

Roberts R, Hodson L, Dennis AL, Neville MJ, Humphreys SM, Harnden KE et al. (2009). Markers of de novo lipogenesis in adipose tissue: associations with small adipocytes and insulin sensitivity in humans. Diabetologia 52(5): 882-890.

Roger J, Goureau O, Sahel JA, Guillonneau X (2007) Use of suppression subtractive hybridization to identify genes regulated by ciliary neurotrophic factor in postnatal retinal explants. Mol Vis 13: 206–219

Rosen ED, Spiegelman BM (2006). Adipocytes as regulators of energy balance and glucose homeostasis. Nature 14;444(7121): 847-853.

Scherer T, Buettner C (2011). Yin and Yang of hypothalamic insulin and leptin signalling in regulating white adipose tissue metabolism. Rev Endocr Metab Disord 12(3): 235-243.

Shaywitz AJ, Greenberg ME (1999). CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. Annu Rev Biochem 68: 821–861.

Sibal L, Agarwal SC, Home PD (2011). Carotid intima-media thickness as a surrogate marker of cardiovascular disease in diabetes. Diabetes Metab Syndr Obes 4: 23-34.

Siddle K (2011). Signalling by insulin and IGF receptors: supporting acts and new players. J Mol Endocrinol 17:47(1): R1-10.

Sikorski RS, Boguski MS, Goebl M, Hieter P (1990). A repeating amino acid motif in CDC23 defines a family of proteins and a new relationship among genes required for mitosis and RNA synthesis. Cell 60: 307–317

Sjostrom L (1973). Fatty acid synthesis de novo in adipose tissue from obese subjects on a hypercaloric high-carbohydrate diet. Scand J Clin Lab Invest 32: 339–349.

Stenzinger A, Kajosch T, Tag C, Porsche A, Welte I, Hofer HW et al. (2005). The novel protein PTPIP51 exhibits tissue- and cell-specific expression. Histochem Cell Biol 123(1): 19-28.

Stenzinger A, Schreiner D, Tag C, Wimmer M et al. (2007). Expression of the novel protein PTPIP51 in rat liver: an immunohistochemical study. Histochem Cell Biol 128(1): 77-84.

Stenzinger A, Schreiner D, Koch P, Hofer HW, Wimmer M (2009). Cell and molecular biology of the novel protein tyrosine-phosphatase-interacting protein 51. Int Rev Cell Mol Biol 275: 183-246.

Teff KL (2008). Visceral nerves: vagal and sympathetic innervation. JPEN J Parentel Enteral Nutr 32(5): 569-571.

Tersigni C, Di Nicuolo F, D'Ippolito S, Veglia M, Castellucci M, Di Simone N (2011). Adipokines: new emerging roles in fertility and reproduction. Obstet Gynecol Surv 66(1): 47-63.

Than A, Ye F, Xue R, Ong JW, Poh CL, Chen P (2011). The crosstalks between adipokines and catecholamines. Mol Cell Endocrinol 332(1-2): 261-270.

Tirosh A, Shai I, Afek A, Dubnov-Raz G, Ayalon N, Gordon B (2011). Adolescent BMI trajectory and risk of diabetes versus coronary disease. N Engl J Med 364(14): 1315-1325.

Vang T, Torgersen KM, Sundvold V, Saxena M, Levy FO, Ska°lhegg BS (2001). Activation of the COOH-terminal Src kinase (Csk) by cAMP-dependent protein kinase inhibits signalling through the T cell receptor. J Exp Med 193, 497–507.

Venable CL, Frevert EU, Kim YB, Fischer BM, Kamatkar S, Neel BG et al. (2000). Overexpression of protein-tyrosine phosphatase-1B in adipocytes inhibits insulin-stimulated phosphoinositide 3-kinase activity without altering glucose transport or Akt/Protein kinase B activation. J Biol Chem 275: 18318–18326.

Wang J, Liu B, Chen X, Su L, Wu P, Wu J, Zhu Z (2011). PTP1B expression contributes to gastric cancer progression. Med Oncol; Epub ahead of print

Wu J, Dent P, Jelinek T, Wolfman A, Weber MJ, Sturgill TW (1993). Inhibition of the EGF-activated MAP kinase signalling pathway by adenosine 3',5'-monophosphate. Science 262: 1065–1069.

Wozniak SE, Gee LL, Wachtel MS, Frezza EE (2009). Adipose tissue: the new endocrine organ? A review article. Dig Dis Sci 54(9): 1847-1856.

Xing L, Culbertson EJ, Wen Y, Robson MC, Franz MG (2011). Impaired laparotomy wound healing in obese rats. Obes Surg 21(12): 1937-1946.

Yu C, Han W, Shi T, Lv B, He Q, Zhang Y et al. (2008). PTPIP51, a novel 14-3-3 binding protein, regulates cell morphology and motility via Raf-ERK pathway. Cell Signal 20: 2208–2220

Zhang X, Odom DT, Koo SH, Conkright MD, Canettieri G, Best J et al. (2005). Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. Proc Natl Acad Sci USA 102: 4459–4464

III. Summary

The thesis aimed to investigate the expression and interaction profile of Protein tyrosine phosphatase interacting protein 51 (PTPIP51) in adipose tissue. Several groups of mice were trained and fed different diets to obtain different levels of insulin resistance. The training was held at 80% of maximum oxygen consumption, as an indicator for aerobe endurance training. The glucose tolerance of each mice was determined at the end of the test period. The expression profile of PTPIP51 in adipose tissue was investigated by immunofluorescence, PCR and Duolink proximity ligation assay.

PTPIP51 was detected in all investigated samples. Its interaction partners PTP1B, 14-3-3β, Insulin receptor (IR) and PKA also were expressed throughout all investigated groups.

The interaction profile of PTPIP51 with its interaction partners differed widely between the groups. PTPIP51 interacts with PTP1B in trained high fat diet animals, normal diet animals and, to a lower extent, high fat diet animals. Interactions between PTPIP51 and 14-3-3β was highest in trained high fat diet animals, whereas it was lowest in high fat diet animals. IR-PTPIP51 interaction was highest in high fat diet animals and lowest in trained standard diet animals. Normal diet and trained high fat diet animals did not show significant differences. The interaction between PTPIP51 and PKA was low in trained standard diet animals, all other groups did not differ significantly in their interaction profile.

In conclusion, this thesis demonstrates the involvement of PTPIP51 in metabolic pathways. The protein seems to act as a switcher between the essential regulatory pathways for lipogenesis and lipolysis in adipocytes.

IV. Zusammenfassung

Die vorliegende Arbeit untersucht die Expression und das Interaktionsprofil des Protein tyrosine phosphatase interacting Proteins 51 (PTPIP51) im Fettgewebe. Hierzu wurden Mäuse in verschiedenen Gruppen trainiert und mit unterschiedlichen Futterzusammenstellungen gefüttert um Unterschiede in der Insulinresistenz zu provozieren. Das Training wurde bei 80% der maximalen Sauerstoffausschöpfung abgehalten, um ein aerobes Ausdauertraining durchzuführen. Am Ende des Tests wurde die Glukosetoleranz der Mäuse bestimmt. Das Fettgewebe wurde mit Hilfe von Immunfluoreszenz, PCR und Duolink proximity ligation assay untersucht.

PTPIP51 wurde in allen untersuchten Tieren nachgewiesen. Auch die Interaktionspartner PTP1B, 14-3-3β, der Insulin Rezeptor (IR) und PKA wurden in allen untersuchten Gruppen exprimiert.

Das Interaktionsprofil zwischen den Gruppen zeigte ausgeprägte Unterschiede. PTPIP51 interagiert mit PTP1B in trainierten fettgefütterten Tieren, in normal gefütterten und, in geringerer Ausprägung, in fettgefütterten Tieren. Die Interaktion zwischen PTPIP51 und 14-3-3β war am stärksten in trainierten fettgefütterten Tieren zu sehen, fettgefütterte Tiere zeigten die wenigsten Interaktionen. IR-PTPIP51-Interaktion war am höchsten in fettgefütterten Tieren und am niedrigsten in trainierten normal gefütterten Tieren. Normal gefütterte und trainierte fettgefütterte Mäuse zeigten keine signifikanten Unterschiede im Interaktionsprofil. Die Interaktion zwischen PTPIP51 und PKA war niedrig in trainierten normal gefütterten Tieren, die anderen Gruppen zeigten keine signifikanten Unterschiede.

Diese Arbeit zeigt die Beteiligung von PTPIP51 in metabolischen Signalwegen. Das Protein scheint in Adipozyten als Vermittler zwischen den essentiellen regulatorischen Signalwegen der Lipogenese und Lipolyse zu agieren.

V. List of publications

A) Original Papers

Viard MJ, Kamm M, Bobrich M, Brobeil A, Petri M, Wimmer M.
PTPIP51 – a multifunctional protein in brain tissue
submitted

Bobrich M, Schwabe SA, Viard M, Kamm M, Brobeil A, Krüger K, Mooren FC, Tag C, Wimmer M. PTPIP51 – Connecting lipolysis and lipogenesis
metabolism, under review

Brobeil A, Bobrich M, Tag C, Wimmer M (2012). PTPIP51 in Protein Interactions: Regulation and In Situ Interacting Partners. Cell Biochem Biophys; Epub ahead of print.

Brobeil A, Bobrich M, Graf M, Kruchten A, Blau W, Rummel M, Oeschger S, Steger K, Wimmer M.
PTPIP51 is phosphorylated by Lyn and c-Src kinases lacking dephosphorylation by PTP1B in acute myeloid leukemia.
Leuk Res 2011 Oct;35(10):1367-1375.

Brobeil A, Bobrich M, Wimmer M.
Protein tyrosine phosphatase interacting protein 51--a jack-of-all-trades protein.
Cell Tissue Res. 2011 May;344(2):189-205.

Bobrich M, Brobeil A, Mooren FC, Krüger K, Steger K, Tag C, Wimmer M.
PTPIP51 interaction with PTP1B and 14-3-3β in adipose tissue of insulin-resistant mice.
Int J Obes (Lond). 2011 Jan 25.

B) Poster presentations

Protein Tyrosine Phosphatase Interacting Protein 51 (PTPIP51) –
a possible switcher between insulin and adrenaline signalling
WORLD DIABETES CONGRESS, DUBAI

Protein Tyrosine Phosphatase Interacting Protein 51 in head and neck squamous cell carcinoma
53rd SYMPOSIUM OF THE SOCIETY FOR HISTOCHEMISTRY, MUNICH
(Co-Author)

PTPIP51 – A possible target for novel Diabetes and obesity therapies
7th ANNUAL MEETING OF THE OLIGONUCLEOTIDE THERAPEUTICS SOCIETY, COPENHAGEN

PTPIP51 – A new marker for ependymoma?
52nd SYMPOSIUM OF THE SOCIETY FOR HISTOCHEMISTRY, PRAGUE

VI. Acknowledgements

First of all, I would like to thank my supervisor and mentor Prof. Dr. Monika Wimmer for her unbelievable help and constant support not only in professional but also in personal matters. She was always there when I needed her and always had time for helpful discussions and reviews of my sometimes imperfect ideas. Prof. Dr. Wimmer also encouraged me to publish papers and to participate in international congresses. It is also her credit that I am where I am now, and I want to thank her for all she has done for me, even though we differed in some points. I hope we will not lose contact, because I really feel honoured that I have had the opportunity to work with such an outstanding person. Hopefully, this work will continue in the future.

Further I would like to thank Prof. Dr. Klaus Steger and his team (Department of Urology and Pediatric Urology, JLU Giessen) for the introductions of methods performed in their laboratory.

Special thanks to Karsten Krüger and Frank-Christoph Mooren for their fair and open cooperation with me and our working group. I will hopefully be able to give them some of the love and support back that they gave me. I am sure we will meet again someday, and I am really looking forward to it.

I would also like to thank the whole team of the Institute of Anatomy and Cell Biology, especially Claudia Tag and Martin Bodenbenner for their very much appreciated technical support and Karola Michael for help with the photographical preparations.

I very much enjoyed to work together with several other doctoral candidates at the Institute of Anatomy and Cell Biology. Max Kamm transcended all my problems and manifold sorrows, I am glad to know him and to spend time with him. Thank you for revision. Jutta Planz was not only a great reviewer of my work but also a good friend. Alexander Brobeil, from the first day of our studies to the last, we kept friends with highs and lows. Thank you for the time together, hopefully we will see again from time to time.

Although they have left the institute, they are not forgotten. I want to thank Stefanie Schwabe for the interesting work together and the review of my thesis. I am glad I found another friend in there. Thanks to Dr. Maxime Viard for a good time and a good work flow. I'm glad to know him, and I'll consult only him in case of severe headache.

Thanks to Tabea Siekmann for the support aside my work in the lab, especially through the hard times in South Africa and after return.

Special thanks to Janika Zinke, who supported me in rough times. Thank you for your very much appreciated help with the layout, and thank you for everything that will be.

Finally my thanks go to my parents, my brother and my sister for their enduring support and love. They were there for me even when I did not realize it. Special thanks to my mom who ensured I am not troubled with other problems.

Der Lebenslauf wurde aus der elektronischen Version der Arbeit entfernt.

The curriculum vitae was removed from the electronic version of the paper.

VIII. Annexes

(1) Bobrich M, Brobeil A, Mooren FC, Krüger K, Steger K, Tag C, Wimmer M (2011). PTPIP51 interaction with PTP1B and 14-3-3β in adipose tissue of insulin-resistant mice. Int J Obes (Lond) 35(11): 1385-1394.

(2) Brobeil A, Bobrich M, Wimmer M (2011a). Protein tyrosine phosphatase interacting protein 51--a jack-of-all-trades protein. Cell Tissue Res 344(2): 189-205.

(3) Bobrich M, Schwabe SA, Brobeil A, Viard M, Kamm M, Mooren FC, Krüger K, Tag C, Wimmer M (2012). PTPIP51 – a new interaction partner of the insulin receptor and PKA in adipose tissue. Metabolism, under review

International Journal of Obesity (2011) 1–10

0307-0565/11
www.nature.com/ijo

ORIGINAL ARTICLE

PTPIP51 interaction with PTP1B and 14-3-3b in adipose tissue of insulin-resistant mice

M Bobrich[1], A Brobeil[1], FC Mooren[2], K Krüger[2], K Steger[3], C Tag[1] and M Wimmer[1]

[1]Institute of Anatomy and Cell Biology, Justus-Liebig-University, Giessen, Germany; [2]Department of Sports Medicine, Justus-Liebig-University, Giessen, Germany and [3]Department of Urology and Paediatric Urology, Justus-Liebig-University Giessen, Giessen, Germany

Objective: We investigated the expression of protein tyrosine phosphatase-interacting protein 51 (PTPIP51) and its interaction with protein tyrosine phosphatase 1B (PTP1B) and 14-3-3b in mice exhibiting insulin resistance and obesity.
Design: A total of 20 mice were included in the study. Eight control animals were fed a normal standard diet, six animals were fed a high-fat diet and six animals were submitted to a treadmill training parallel to the feeding of a high-fat diet. After 10 weeks, a glucose tolerance test was performed and abdominal adipose tissue samples of the animals were collected.
Results: PTPIP51 protein was identified in the adipocytes of all samples. PTPIP51 interacted with PTP1B and with 14-3-3b protein. Compared with untrained mice fed a standard diet, the interaction of PTPIP51 with PTP1B was reduced in high-fat diet-fed animals. The highest interaction of PTPIP51 with 14-3-3b was seen in trained animals on high-fat diet, whereas untrained animals on high-fat diet displayed lowest values.
Conclusion: PTPIP51 is expressed in adipose tissue of humans, rats and mice. Obesity with enhanced insulin resistance resulted in a reduction of PTPIP51 levels in adipocytes and influenced the interactions with PTP1B and 14-3-3b. The interaction of PTPIP51 with PTP1B suggests a regulatory function of PTPIP51 in insulin receptor signal transduction. The interaction of PTPIP51 with 14-3-3b, especially in trained individuals, hints to an involvement of PTPIP51 in the downstream regulation of insulin action.
International Journal of Obesity advance online publication, 25 January 2011; doi:10.1038/ijo.2010.283

Keywords: PTPIP51; PTP1B; 14-3-3b; insulin resistance

Introduction

Adipose tissue is multifunctional. Its main function is its ability to store energy in the form of fatty acids.

The storage of fat is tightly regulated by different signaling pathways. Two of the most important signal transduction ways are the insulin and the protein kinase A (PKA) signaling pathways, which oppose each other's function. Insulin inhibits lipolysis, whereas PKA activates it.[1]

The role of protein tyrosine phosphatases (PTPs) has been described as crucial in insulin signal transduction. PTPs gain importance in insulin-resistant states, as they act as regulators of insulin receptor (IR) signaling.[2–4] In particular, protein tyrosine phosphatase 1B (PTP1B) was found to dephosphorylate the IR and the IR substrate-1.[5,6] It has a major role in insulin resistance and obesity in which its expression is upregulated.[7,8] Mice lacking a functional PTP1B gene are resistant to weight gain on a high-fat diet, and show increased insulin sensitivity in liver and skeletal muscle. PTP1B-null mice do not exhibit increased insulin sensitivity in adipose tissue.[9]

According to Asante-Appiah and Kennedy,[10] PTP1B regulates the response to IR activation by dephosphorylation of IR substrate-1 and other molecules phosphorylated on tyrosine in response to insulin-mediated signaling. Yet, up to now, the exact mechanism of PTP1B in the regulation of the insulin signaling pathway has not been fully established, particularly not in adipose tissue. Nevertheless, PTP1B and its interaction partners might become important new targets for pharmaceutical intervention in insulin-resistant states of diabetes.

Protein tyrosine phosphatase-interacting protein 51 (PTPIP51), a protein with tissue-specific expression, takes part in the regulation of proliferation, differentiation, apoptosis and cell motility.[11] PTPIP51 interacts with PTP1B[11,12] and with several molecules that are located upstream of the

Correspondence: M Bobrich, Institute of Anatomy and Cell Biology, Justus-Liebig-University Giessen, Aulweg 123, 35392 Giessen, Germany.
E-mail: manuel.bobrich@anatomie.med.uni-giessen.de
Received 6 October 2010; revised 22 November 2010; accepted 24 November 2010

mitogen-activated protein kinase pathway, for example, 14-3-3b, a protein that is able to activate the mitogen-activated protein kinase pathway through direct interaction with raf-1.[13] The mitogen-activated protein kinase pathway is crucial for growth and differentiation of adipocytes.[14,15]

We analyzed the expression profile of PTPIP51 in mice fed a standard diet and in mice fed a high-fat diet that induces insulin resistance.[16] As insulin resistance is influenced by physical activity,[17] a third group of animals fed a high-fat diet was submitted to an endurance training protocol. The expression pattern of PTP1B and 14-3-3b, and the analysis of their interaction with PTPIP51, were included in the study.

Our experiments revealed a partial association of PTPIP51 with proteins involved in signaling processes in adipocytes from animals under physiological and pathological conditions.

Materials and methods

Study design

The experiments were performed with Bl6 mice (n ¼ 20). The experiments were approved by the local Animal Care and Use Committee (Gi 20/24 Nr 94 2010).

The animals were kept under standard conditions (12-h light/dark cycle) and fed ad libitum with free access to water. The experiments were run for 10 weeks.

Control group (n ¼ 8). The animals were fed a standard diet (Altromin standard-diet no. 1324, Altromin, Lage, Germany). For nutrient composition, see Table 1.

High-fat diet group (n ¼ 6). The animals were fed a specially assembled high-fat diet containing 45% fat. For nutrient composition of the high-fat diet, see Table 1.

High-fat diet and training group (n ¼ 6). The animals were fed a high-fat diet and submitted to endurance training on a treadmill for 35 min five times a week. The animals were accustomed to the treadmill 1 week before the beginning of the training. The performance of the animals was controlled by measuring VO2 max using a spirometer (Arnfinn Sira, Trondheim, Norway). Running velocity was adapted to 80% of VO2 max.

The insulin resistance was estimated by a glucose tolerance test at the end of the experimental period. The test was performed by an intraperitoneal injection of 2g kg^{-1} body weight of 20% D-Glucose glucose dissolved in sterile 0.9% NaCl solution. Fasting blood glucose levels were determined after 15, 45 and 90 min following application.[18]

After 10 weeks, the animals were killed and the abdominal adipose tissue was frozen in liquid nitrogen pre-cooled isopentan and transferred to −80 1C till further analysis.

Table 1 Energy content of the standard diet and the high-fat diet

Values in kcal per 100 g	Standard diet	High-fat diet
Protein	24	20
Carbohydrates	65	35
Fat	11	45

Quantitative real-time PCR

The amplification of cDNA was carried out in 25 ml reaction volume on the iCycler iQ Real-Time PCR Detection System (Bio-Rad, Munich, Germany). The final reaction tubes contained 100 nM of PTPIP51, PTP1B, 14-3-3b and reference gene b-actin primer, 12.5 ml iQ SYBR Green Supermix (Bio-Rad) and 2 ml of DNA template. The PCR conditions were 94 1C for 3 min followed by 40 cycles for 30 s, 60 1C for 30 s and 72 1C for 1 min. Melting curves were generated for all genes after amplification. Negative controls were included in each run. Pairs of chemically synthesized oligonucleotides were designed using freely available primer design software (Primer3, http://frodo.wi.mit.edu/primer3/). Mouse-specific mRNA templates were used as input sequence. All primers used in this study are listed in Table 2. mRNA expression levels of target genes were normalized to the expression of the housekeeping gene b-actin and quantified using the comparative CT method.[19]

PTPIP51 antibody production

The cDNA sequence encoding amino acids 131–470 was inserted into the BamHI and HindIII sites of the plasmid pQE30 and expressed as His_6-tagged protein in the protease-deficient E. coli expression strain AD202 (araD139DE(argF-lac)169 ompT1000::kan flhD5301 fruA25 relA1 rps150(strR) rbsR22 deoC1). The protein was purified to electrophoretic homogeneity by chromatography on a nickel–agarose column. Immunization of rabbits was performed with 0.5 mg of the purified protein in 0.5 ml RIBI adjuvant followed by booster injections with 0.5 and 0.3 mg on days 14 and 21, respectively. The antiserum was collected on day 28. Monospecific antibodies were prepared following the method described by Olmsted.[20] Briefly, 2 mg of purified antigen were blotted on nitrocellulose after SDS electrophoresis. The protein band was marked using Ponceau solution and had been cut out. After blocking of the membrane strip with 1% low-fat milk powder in phosphate-buffered saline, the membrane was incubated with the antiserum for 1 h followed by extensive washing with Tris-EDTA-buffered saline. The antibodies were eluted with 0.2 M glycine (pH 2.0) for 2 min followed by immediate neutralization with 1 M triethanolamine.

Immunohistochemistry

The primary polyclonal antibody to PTPIP51 was used in 1:1000 dilutions for immunocytochemistry and visualized by Alexa 555 (Molecular Probes, Darmstadt, Germany, cat. no. A21428). Primary monoclonal mouse and goat

Table 2 List of the primers used for quantitative reverse transcriptase PCR experiments

Target	Sequence (5′–3′)	Product length, bp	Exon	Template
PTPIP51	Forward (25 bp), GGTCCGCTCTCATATAGAAGAGAAC Reverse (22 bp), CCTCTCTCTGGCAAAAGGGAAC	70	3–4	510–579
PTP1B	Forward (26 bp), CATCAAGAAAGTACTGCTGGAGATGC Reverse (24 bp), TCCTTCCACTGATCCTGCACTGAC	144	ND	902–1045
14-3-3b	Forward (24 bp), TGACAATCTTGTTAGTGCCTGTCA Reverse (24 bp), AATGTCAAGGTAGAGGGGTGAGAC	175	ND	2132–2306

Abbreviations: ND, not determined; PTP1B, protein tyrosine phosphatase 1B; PTPIP51, protein tyrosine phosphatase-interacting protein 51.

antibodies were used for double staining experiments with 14-3-3b and PTP1B. The reaction of the primary monoclonal mouse antibody was visualized using fluorescein isothiocyanate-donkey-anti-mouse-F(ab)2 secondary antibody (Dianova, Hamburg, Germany, cat. no. 715-096-150). The primary monoclonal goat antibody was visualized using fluorescein isothiocyanate-mouse-anti-goat-IgG secondary antibody (Sigma-Aldrich Chemie GmbH, Steinheim, Germany, cat. no. G2904). Nuclei were displayed by DAPI (4′,6-diamidino-2-phenylindole) staining.

Primary monoclonal mouse antibodies used for double staining experiments were against PTP1B and 14-3-3b (Santa Cruz Biotechnology, Santa Cruz, CA, USA, cat. nos. sc-1718 and sc-25276). The reaction of the primary monoclonal mouse antibody was visualized using Alexa Fluor 488 secondary antibodies (Molecular Probes, Darmstadt, Germany, cat. no. A11001). Nuclei were displayed by DAPI staining.

Five samples of rat adipose tissue and two samples of human visceral adipose tissue were used for immunocytochemistry.

All samples of mouse, human and rat were analyzed and representative samples were documented.

Duolink proximity ligation assay

Interaction of PTPIP51 with either PTP1B or 14-3-3b was detected by the DuoLink proximity ligation assay kit (DPLA; Olink biosciences, Uppsala, Sweden; PLA probe anti-rabbit minus for the detection of the rabbit PTPIP51 antibody, cat. no. 90602; PLA probe anti-mouse plus for the detection of the mouse anti PTP1B or 14-3-3b antibody, cat. no. 90701; Detection Kit 563, cat. no. 90134). The DPLA secondary antibodies only hybridize when the two different PLA probes (probe anti-rabbit minus and probe anti-mouse plus) have bound to proteins in such proximity that they are closer than 40 nm. After ligation, forming a circular template, and amplification step, the fluorophore-coupled testing probe binds the amplified oligonucleotide strands. Addition of the fluorescently labeled oligonucleotides that hybridize to the rolling circle amplification product leads to a point-shaped signal that is visible in fluorescence microscopy.

Methanol-fixed air-dried samples were incubated with blocking agent for 1 h. After washing in phosphate-buffered saline for 10 min, primary antibodies for PTPIP51 and PTP1B, respectively 14-3-3b were applied to the samples. The antibodies were diluted in the blocking agent in a concentration of 1:500 and 1:100, respectively. Incubation was carried out overnight in a pre-heated humidity chamber. Slides were washed three times in phosphate-buffered saline for 10 min. Duolink PLA probes detecting rabbit or mouse antibodies were diluted in the blocking agent in a concentration of 1:5 and applied to the slides following incubation for 2 h in a pre-heated humidity chamber at 37 1C. Washing three times in phosphate-buffered saline for 10 min removed unbound PLA probes. For hybridization of the two Duolink PLA probes, Duolink hybridization stock was diluted in the ratio 1:5 in high-purity water and slides were incubated in a pre-heated humidity chamber for 15 min at 37 1C. The slides were washed in tris-buffered saline-Tween 20 for 1 min under gentle agitation. The samples were incubated in the ligation solution consisting of Duolink Ligation stock (1:5) and Duolink Ligase (1:40) diluted in high-purity water for 90 min at 37 1C. Detection of the amplified probe was done with the Duolink Detection kit. Duolink Detection stock was diluted in the ratio 1:5 in high-purity water and applied for 1 h at 37 1C. Final washing steps were carried out by saline-sodium citrate buffer and 70% ethanol.[21,22]

Analysis of DPLA results

The BlobFinder software from the Centre for Image Analysis Quantification was used for quantification of the detected DPLA signals.[23] Positive reaction dots of the DPLA, indicating the interaction of the analyzed proteins (PTPIP51, PTP1B and 14-3-3b) were counted. Sensitivity and blob threshold were set identically for all probes.

Fluorescence microscopy

The Axioplan 2 fluorescence microscope equipped with Plan-Apochromat objectives (Carl Zeiss, Jena, Germany) was used for photo documentation. For visualization of the secondary antibody, Alexa Fluor 555, an excitation filter with a spectrum of 530–560 nm and an emission filter with a spectrum 572–647 nm were used. Alexa Fluor 488 was visualized by an excitation filter in a range of 460–500 nm and an emission filter in a range of 512–542 nm.

Results

In preliminary experiments, PTPIP51 was shown to be expressed in adipose tissue of different species, namely, mouse, rat and humans. Yet, subcellular localization of PTPIP51 differed within the investigated species (Figure 1). In rat adipocytes, PTPIP51 was concentrated in the perinuclear region and was low in the cytoplasm. In humans and mice, PTPIP51 was distributed within the whole cytoplasm, with higher concentrations in the nuclear region.

Owing to the fact of comparable expression values of PTPIP51 in human and mice adipose tissue, mice were used in this study.

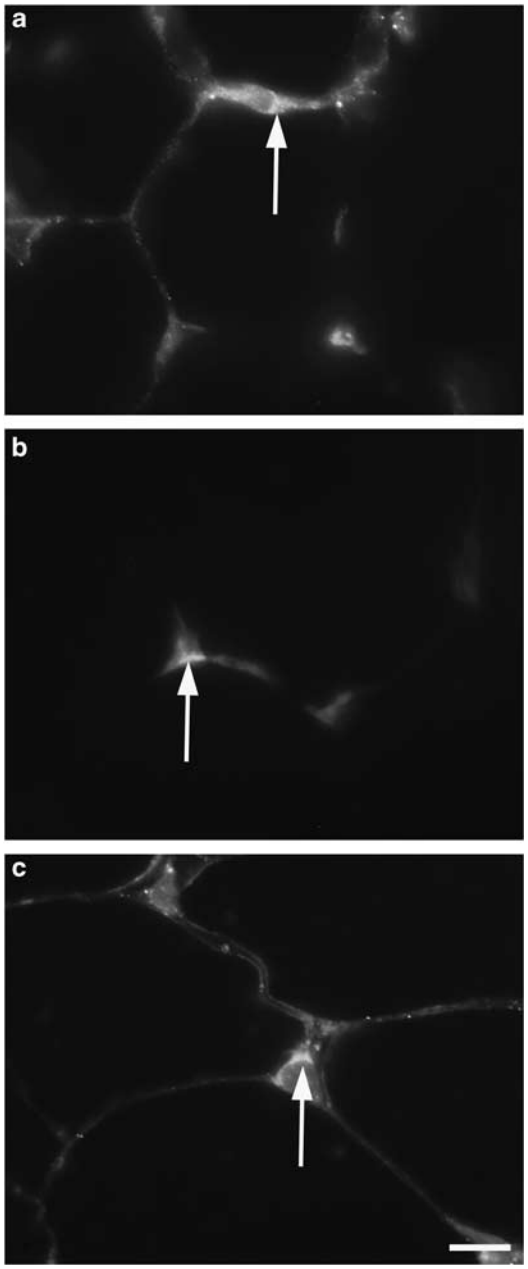


Figure 1 Immunostaining of PTPIP51 in adipose tissue of mouse, rat and human. (a) Mouse adipose tissue; (b) rat adipose tissue; (c) human adipose tissue. Nuclei are stained using DAPI. Arrows, high PTPIP51 concentrations in the perinuclear area. Bar, 10 mm.

Glucose tolerance test and weight gain

After 10 weeks of being fed on high-fat diet or high-fat diet and endurance training protocol, the glucose tolerance was determined. As shown in Table 3, 15 min after glucose load, blood glucose concentrations increased to 130% of basal values in control animals, which almost normalized after 45 min and reached basal values after 90 min.

The high-fat diet-fed animals exhibited a different response to glucose. Mean glucose concentrations were more than doubled at 15 min after glucose load and stayed at higher levels even at 90 min post injection.

High-fat diet-fed and trained animals subjected to the glucose tolerance test displayed glucose values of 180% at 15 min, 158% at 45 min and 132% at 90 min.

Over the experimental period, the weight gain of the animals differed within the three groups. Normal-fed animals had a mean weight gain of 46%, high-fat diet-fed animals gained 132% and the high-fat diet-fed and trained mice gained 128% (Table 4). The weight gain was reflected by increased size of adipocytes basing on the elevated caloric intake. Adipocytes from mice on high-fat diet displayed the highest volumes, whereas adipocytes from control animals were smallest (Figure 2).

PTPIP51 protein and mRNA expression

PTPIP51 protein in adipose tissue sections from mice fed on a control diet, a high-fat diet or a high-fat diet accompanied

Table 3 Average blood glucose concentration after intraperitoneal application of glucose solution

	Before application	At 15 min after application	At 45 min after application	At 90 min after application
Standard diet fed	148.0±21.4	205.25±47.8	163.6±42.5	138.6±24.4
High-fat diet fed	170.2±25.2	403.2±98.0	415.3±70.8	353.3±65.5
High-fat diet fed and trained	153.6±26.6	277.2±64.6	243.2±50.4	202.0±35.2

Table 4 Mean weight of the animals of all three investigated groups at the beginning (week 0) and the ending (week 10) of the test period

	Week 0	Week 10
Standard diet fed	20.1±1.1	29.4±1.7
High-fat diet fed	19.5±1.3	45.3±2.3
High-fat diet fed and trained	18.8±1.3	42.9±4.2

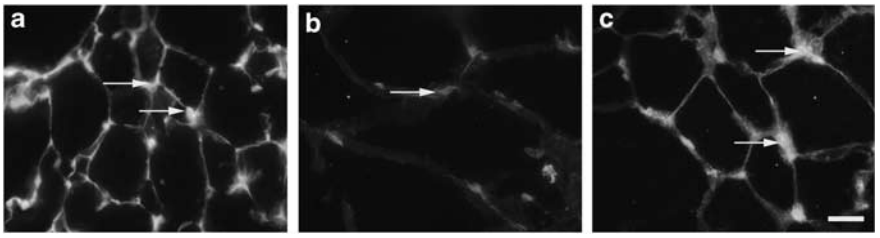


Figure 2 Immunostaining of PTPIP51 in mouse adipose tissue. (a) Adipose tissue of standard-diet-fed animal; (b) adipose tissue of high-fat diet-fed animal; (c) adipose tissue of animal fed on high-fat diet and subjected to training. Nuclei are stained using DAPI. Arrows, high PTPIP51 concentrations in the perinuclear area. Bar, 20 mm.

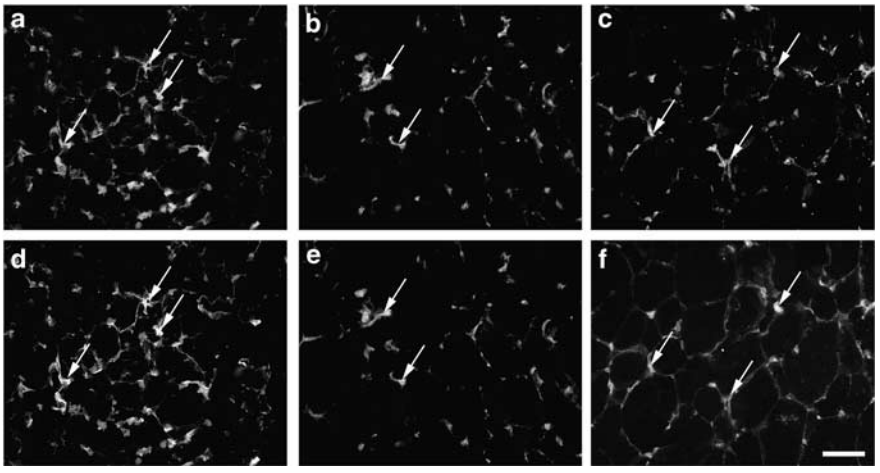


Figure 3 Immunostaining of PTP1B protein (a–c) and PTPIP51 (d–f). (a) PTP1B in adipose tissue of standard-diet-fed animal; (b) PTP1B in adipose tissue of high-fat diet-fed animal; (c) PTP1B in adipose tissue of animal fed on high-fat diet and subjected to training; (d) PTPIP51 in adipose tissue of standard-diet-fed animal; (e) PTPIP51 in adipose tissue of high-fat diet-fed animal; (f) PTPIP51 in adipose tissue of animal fed on high-fat diet and subjected to training. Nuclei are stained using DAPI. Arrows, co-localization of PTP1B and PTPIP51. Bar, 50 mm.

by an endurance treadmill training regimen was identified by a specific polyclonal antibody. Independent of the pre-treatment of the mice, PTPIP51 protein was traceable in adipose tissue of all mice (Figure 2). Adipocytes from high-fat animals displayed a much weaker PTPIP51 reaction when compared with adipocytes from animals fed a standard diet or those fed on a high-fat diet accompanied by a treadmill training (Figure 2). In standard diet animals, PTPIP51 was concentrated around the nucleus. The cytoplasm remained widely unstained, except in the direct proximity of plasma membranes. In adipocytes from high-fat diet-fed animals, PTPIP51 was located around the nucleus and the cytoplasm remained unstained. The distribution of PTPIP51 in animals fed on high-fat diet and subjected to endurance training corresponded to the distribution pattern in adipocytes from standard diet animals.

Highest values of PTPIP51 mRNA were observed in adipose tissue of high-fat diet-fed animals, whereas high-fat diet-fed and trained animals displayed lower amounts of PTPIP51 mRNA. Normal diet animals displayed lowest PTPIP51 mRNA levels (Figure 4a).

PTP1B protein and mRNA expression

As seen by immunostaining experiments, PTP1B protein was expressed in animals of all three groups (Figures 3a–c). The protein was traceable in the whole cytoplasm and co-localized with PTPIP51 mainly in the nuclear region (Figures 3d–f). No difference in the PTP1B protein amount could be assessed.

Highest values of PTP1B mRNA were seen in adipose tissue of high-fat diet-fed animals, whereas standard-diet-fed animals displayed lower amounts of PTP1B mRNA. High-fat diet-fed and trained animals displayed lowest PTP1B mRNA levels (Figure 4b).

Interaction of PTPIP51 and PTP1B

To corroborate a possible interaction of PTPIP51 and PTP1B, a DPLA was applied to adipose tissue sections. The interaction of the two proteins was evidenced by the ligation of the PLA minus oligonucleotide identifying PTPIP51 and PLA plus oligonucleotide identifying PTP1B. Ligated rolling circles were amplified by polymerase activity, with

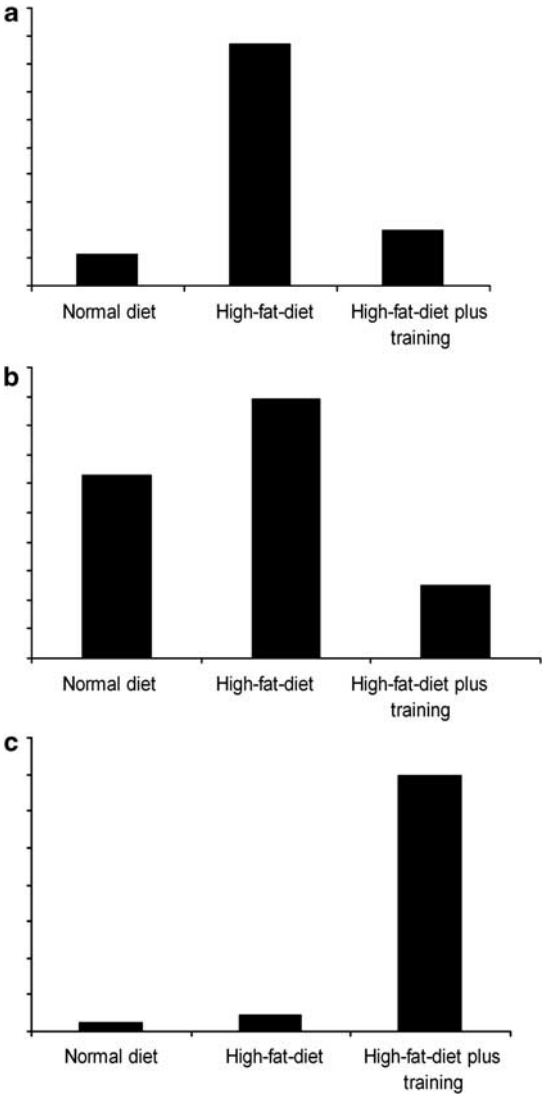


Figure 4 Quantitative real-time PCR analysis of PTPIP51, PTP1B and 14-3-3b in adipose tissue from mice fed a standard diet, high-fat diet or high-fat diet plus training. (a) Normalization approaches on the level of PTPIP51 gene; (b) normalization approaches on the level of PTP1B; (c) normalization approaches on the level of 14-3-3b. Normalizations were made using b-actin as reference gene.

subsequent detection by a fluorophore-coupled testing probe. Each amplification corresponds to a fluorescent dot. In adipocytes of all animals, these hybridized and amplified antibody-linked nucleotide strands were detected, with each dot corresponding to an interaction between PTPIP51 and PTP1B. As shown in Figure 5, PTPIP51 and PTP1B were directly interacting in adipocytes independent from their pre-treatment. Yet, the interaction was partial and the extent differed strongly between the three groups. The highest grade of interaction was observed in the adipocytes derived from animals on standard diet, with values being 1.7-fold higher compared with the interaction in adipocytes of animals fed with high-fat diet. Animals submitted to treadmill training had an intermediate interaction grade (Figure 5).

14-3-3b protein and mRNA expression, and interaction of 14-3-3b with PTPIP51

Immunostaining of 14-3-3b protein in adipocytes displayed its expression in animals of all three experimental groups. 14-3-3b was located within the cytoplasm and co-localized to PTPIP51, especially in the nuclear area (Figure 6).

Highest values of 14-3-3b mRNA were seen in adipose tissue of high-fat diet-fed and trained animals, whereas animals fed on a standard diet or a high-fat diet displayed low amounts of 14-3-3b mRNA (Figure 4c).

Interaction of PTPIP51 and 14-3-3b

To probe a possible interaction of PTPIP51 and 14-3-3b, the DPLA was applied. PTPIP51 and 14-3-3b were partially interacting in all investigated samples, as evidenced by a positive DPLA signal (fluorescence dots). The extent of interaction differed according to the pre-treatment. Highest interaction grade was observed in adipocytes derived from animals submitted to a high-fat diet feeding accompanied by endurance training. The lowest interaction grade was found in adipocytes from high-fat diet-fed animals (Figure 7).

Discussion

PTPIP51 protein is expressed in adipose tissue of human, rat and mouse. On the basis of our results, we conclude that PTPIP51 expression in mice adipose tissue is correlated to the grade of insulin resistance. Fast recovery of blood glucose after glucose load was associated with high PTPIP51 protein levels, as seen in control animals and to a somewhat lower degree in high-fat diet-fed, trained animals. In high-fat diet-fed animals, the recovery of blood glucose concentrations was extensively slowed down.

High-fat diet-fed animals displayed hypertrophic adipocytes, as seen in our experiments and reported before.[24] As suggested by Kadowaki et al.,[25] hypertrophic adipocytes are likely to develop insulin resistance.

Insulin resistance is associated with a reduced tyrosine phosphorylation of the IR.[26] PTP1B is one of the crucial phosphatases responsible for the dephosphorylation of the IR. In our study, PTP1B mRNA was expressed in high levels in adipocytes from high-fat diet-fed animals, whereas high-fat diet-fed and trained mice displayed lowest levels. Differing from the observed RNA levels, PTP1B protein levels were the same in the three experimental groups. Similar results could be found for PTPIP51 mRNA and protein. The mRNA was highest in high-fat diet-fed animals and low in normal diet-fed animals and in animals fed on high-fat diet and subjected to training. The protein levels of PTPIP51 were high in normal diet-fed animals and animals fed on high-fat diet and

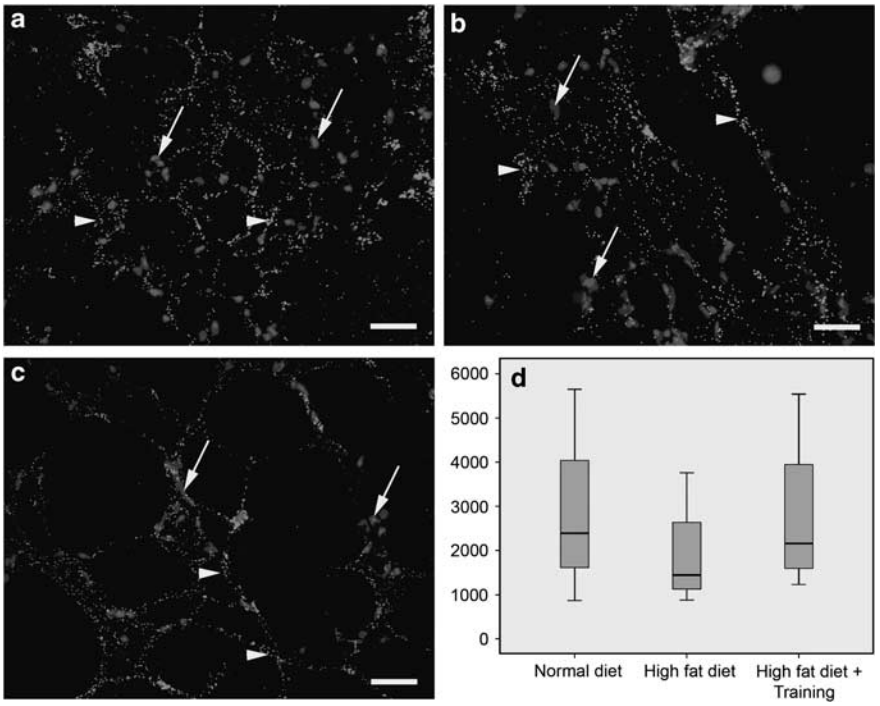


Figure 5 Duolink proximity ligation assay for PTPIP51 and PTP1B in sections of mouse adipose tissue. Rolling circle amplification (RCA) takes place if both proteins are in close proximity. The positive reaction is seen as dots, indicating the position of both interacting proteins. (a) Standard-diet-fed animal; (b) high-fat diet-fed animal; (c) animal fed on high-fat diet and subjected to training; (d) quantitative analysis of the interaction. Ordinate, dots per complete section analysis; arrows, nuclei stained with DAPI; arrowheads, interaction between PTPIP51 and PTP1B. Bars, 50 mm.

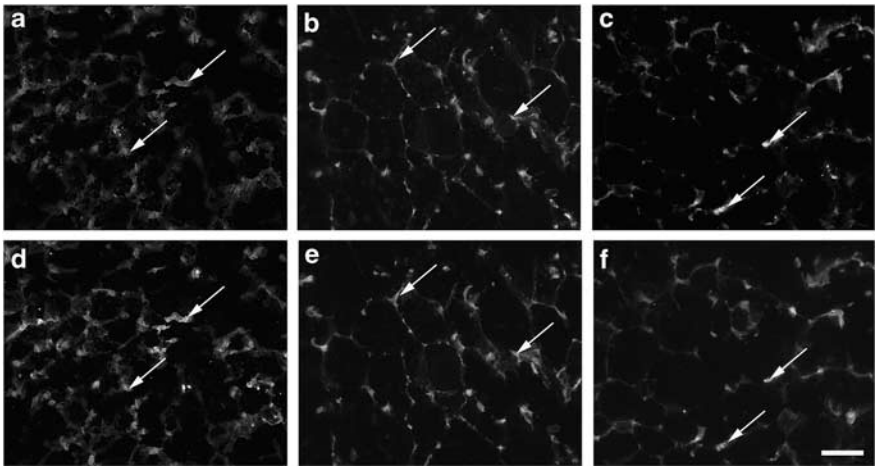


Figure 6 Immunostaining of 14-3-3b (a–c) and PTPIP51 (d–f). (a) 14-3-3b in adipose tissue of standard-diet-fed animal; (b) 14-3-3b in adipose tissue of high-fat diet-fed animal; (c) 14-3-3b in adipose tissue of animal fed on high-fat diet and subjected to training; (d) PTPIP51 in adipose tissue of standard-diet-fed animal; (e) PTPIP51 in adipose tissue of high-fat diet-fed animal; (f) PTPIP51 in adipose tissue of animal fed on high-fat diet and subjected to training. Nuclei are stained using DAPI. Arrows, co-localization of 14-3-3b and PTPIP51. Bar, 50 mm.

subjected to training, but low in high-fat diet-fed animals. These differences in expression levels and protein could be due to different posttranslational modification or stabilization of the mRNA.

Our experiments revealed a co-localization of PTPIP51 with PTP1B. Previous experiments confirmed an interaction of PTPIP51 with PTP1B both in vitro and in vivo.[11] We were also able to verify this interaction in mouse adipocytes by

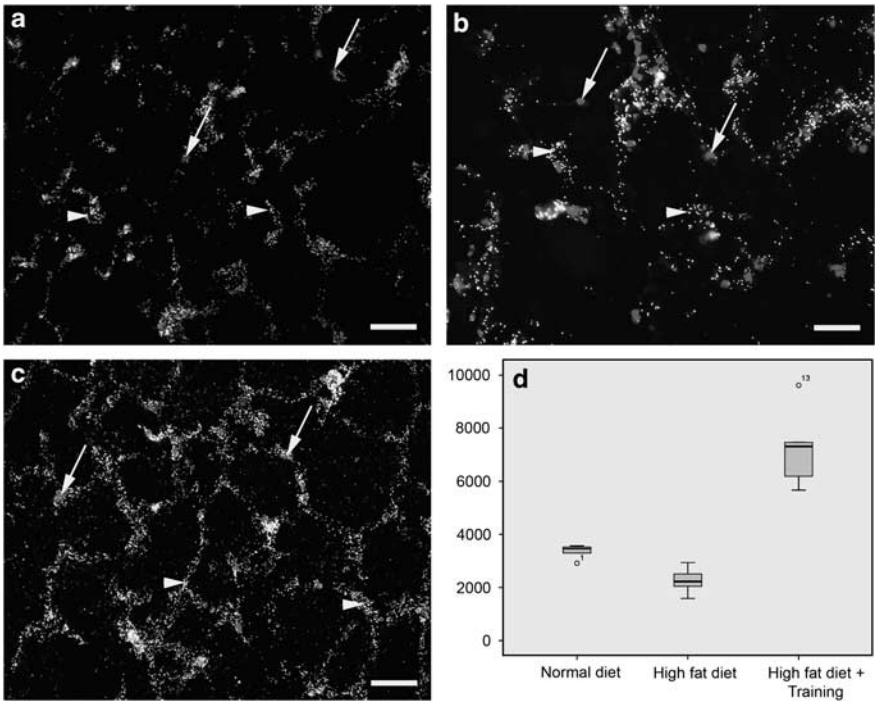


Figure 7 Duolink proximity ligation assay for PTPIP51 and 14-3-3b in sections of mouse adipose tissue. Rolling circle amplification (RCA) takes place if both proteins are in close proximity. The positive reaction is seen as dots, indicating the position of both interacting proteins. (a) Standard-diet-fed animal; (b) high-fat diet-fed animal; (c) animal fed on high-fat diet and subjected to training; (d) quantitative analysis of the interaction. Ordinate, dots per full section analysis. Arrows, nuclei are marked by DAPI. Arrowheads, interaction between PTPIP51 and 14-3-3b. Bars, 50 mm.

the DPLA. Interestingly, this interaction was correlated to the feeding and training status of the mice. High interaction was seen in normal-fed animals and was strongly reduced in high-fat diet-fed animals. Despite their reduced concentration of PTPIP51, interaction values seen for high-fat diet-fed mice submitted to endurance training corresponded to those observed in control animals.

In various tissues, namely, muscle and liver, PTP1B affects insulin signaling by dephosphorylation of the IR.[7,8,26] The role of PTP1B in adipose tissue is less well studied. Overexpression of PTP1B in adipocytes is associated with a decrease in IR- and IR substrate-1-phosphorylation, although the effect of insulin remains evident.[27] Lack of PTP1B in adipocytes leads to increased tyrosine phosphorylation of the IR and, thus, to an increase in IR activity.[28,29]

14-3-3b mediates insulin action in adipocytes[30,31] by modifying the Ras/Raf/MEK/ERK pathway, which is important in adipocyte remodeling and differentiation.[15] Erk-1 activation is observed in insulin signaling in high-fat diet-fed animals.[13] PTPIP51 can affect Raf1 activity and, in consequence, regulate the Ras/Raf/MEK/ERK pathway by its interaction with 14-3-3b.[32] Two conserved protein domains are crucial for the interaction of PTPIP51 and 14-3-3b, namely, conserved region 1 (CR1)-spanning amino acids 43–48 and CR2 spanning amino acids 146–154,[32] leading to the observed hypertrophy of the adipocytes. 14-3-3b mediates effects of the insulin signaling cascade.[13,30,31] Thus, PTPIP51 is able to act as a downstream regulator of the insulin signaling pathway via 14-3-3b.

We observed a strong interaction of PTPIP51 and 14-3-3b in trained high-fat diet-fed animals, suggesting the reversion of insulin resistance observed in animals on high-fat diet by the endurance training. In addition to that, 14-3-3b mRNA levels were very high in animals fed on high-fat diet and subjected to training, whereas normal-diet-fed and high-fat diet-fed animals displayed low 14-3-3b mRNA expression.

The energy status of adipocytes is regulated by PKA.[33] Adrenalin-induced lipolysis works by activation of the PKA signaling cascade.[34] Glucose uptake leads to the activation of the IR and to the reduction of PKA activity, and in consequence to reduced PKA downstream signaling.[35] The GPS 2.1 database identified PTPIP51 as a potential interaction partner of PKA,[36] and PTPIP51 is phosphorylated at its serine 212 residue by PKA in vitro.[11] This phosphorylation site is located in proximity of CR2 of PTPIP51. Phospho-serine/threonine-containing motifs in interaction partners of 14-3-3b can influence the binding of the partners.[35] Taking these facts into account, we hypothesize that PTPIP51 is a shifter between the lipolysis-inducing PKA signaling pathway and the lipolysis-inhibiting insulin signaling pathway. Phosphorylation at the serine 212 residue might trigger the binding of PTPIP51 to 14-3-3b.

Training affects adipose tissue by increasing lypolysis[37,38] and upregulation of cAMP-activated kinase PKA.[38] We observed increased PTPIP51 expression in the adipose tissue of trained, high-fat diet-fed mice compared with untrained high-fat diet-fed mice and an elevated interaction of PTPIP51 with 14-3-3b, suggesting a possible shifts toward higher insulin sensitivity.

Further studies will follow to disclose the complete function of PTPIP51 in diabetes and obesity.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We are grateful to Ms A Erkel and Ms B Fröhlich (Department of Urology and Paediatric Urology, Giessen, Germany) for their excellent technical assistance.

References

1 Choi SM, Tucker DF, Gross DN, Easton RM, Dipilato LM, Dean AS et al. Insulin regulates adipocyte lipolysis via an Akt-independent signalling pathway. Mol Cell Biol 2010; 30: 5009–5020.
2 Kenner KA, Anyanwu E, Olefsky JM, Kusari J. Protein-tyrosine phosphatase 1B is a negative regulator of insulin- and insulin-like growth factor-I-stimulated signalling. J Biol Chem 1996; 271: 19810–19816.
3 Seely BL, Staubs PA, Reichart DR, Berhanu P, Milarski KL, Saltiel AR et al. Protein tyrosine phosphatase 1B interacts with the activated insulin receptor. Diabetes 1996; 45: 1379–1385.
4 Calera MR, Vallega G, Pilch PF. Dynamics of protein-tyrosine phosphatases in rat adipocytes. J Biol Chem 2000; 275: 6308–6312.
5 Salmeen A, Andersen JN, Myers MP, Tonks NK, Barford D. Molecular basis for the dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B. Mol Cell 2000; 6: 1401–1412.
6 Goldstein BJ, Bittner-Kowalczyk A, White MF, Harbeck M. Tyrosine dephosphorylation and deactivation of insulin receptor substrate-1 by protein-tyrosine phosphatase 1B: possible facilitation by the formation of a ternary complex with the Grb2 adaptor protein. J Biol Chem 2000; 275: 4283–4289.
7 McGuire MC, Fields RM, Nyomba BL, Raz I, Bogardus C, Tonks NK et al. Abnormal regulation of protein tyrosine phosphatase activities in skeletal muscle of insulin-resistant humans. Diabetes 1991; 40: 939–942.
8 Ahmad F, Goldstein BJ. Increased abundance of specific skeletal muscle protein-tyrosine phosphatases in a genetic model of insulin-resistant obesity and diabetes mellitus. Metabolism 1995; 44: 1175–1184.
9 Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL et al. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science 1999; 283: 1544–1548.
10 Asante-Appiah E, Kennedy BP. Protein tyrosine phosphatases: the quest for negative regulators of insulin action. Am J Physiol Endocrinol Metab 2003; 284: 663–670.
11 Stenzinger A, Schreiner D, Koch P, Hofer HW, Wimmer M. Cell and molecular biology of the novel protein tyrosine-phosphatase-interacting protein 51. Int Rev Cell Mol Biol 2009; 275: 183–246.
12 Brobeil A, Graf M, Oeschger S, Steger K, Wimmer M. PTPIP51-a myeloid lineage specific protein interacts with PTP1B in neutrophil granulocytes. Blood Cells Mol Dis 2010; 45: 159–168.
13 Bost F, Aouadi M, Caron L, Binétruy B. The role of MAPKs in adipocyte differentiation and obesity. Biochimie 2005; 87: 51–56.
14 Crowe S, Turpin SM, Ke F, Kemp BE, Watt MJ. Metabolic remodeling in adipocytes promotes ciliary neurotrophic factor-mediated fat loss in obesity. Endocrinology 2008; 149: 2546–2556.
15 Capeau J. Insulin signalling: mechanisms altered in insulin resistance. Med Sci (Paris) 2003; 19: 834–839.
16 Storlien LH, Pan DA, Kriketos AD, Baur LA. High-fat-diet-induced insulin resistance. Lessons and implications from animal studies. Ann NY Acad Sci 1993; 683: 82–90.
17 Marques CM, Motta VF, Torres TS, Aguila MB, Mandarim-de-Lacerda CA. Beneficial effects of exercise training (treadmill) on insulin resistance and nonalcoholic fatty liver disease in high-fat fed C57BL/6 mice. Braz J Med Biol Res 2010; 43: 467–475.
18 Bradley RL, Jeon JY, Liu FF, Maratos-Flier E. Voluntary exercise improves insulin sensitivity and adipose tissue inflammation in diet-induced obese mice. Am J Physiol Endocrinol Metab 2008; 295: 586–594.
19 Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402–408.
20 Olmsted JB. Affinity purification of antibodies from diazotized paper blots of heterogeneous protein samples. J Biol Chem 1981; 256: 11955–11957.
21 Söderberg O, Gullberg M, Jarvius M, Ridderstråle K, Leuchowius KJ, Jarvius J et al. Direct observation of individual endogenous protein complexes in situ by proximity ligation. Nat Methods 2006; 3: 995–1000.
22 Gullberg M, Andersson A-C. Visualization and quantification of protein-protein interactions in cells and tissues. Nat Methods 2010; 6: 641–647.
23 Allalou A, Wählby C. BlobFinder, a tool for fluorescence microscopy image cytometry. Comput Methods Programs Biomed 2009; 94: 58–65.
24 Berger JJ, Barnard RJ. Effect of diet on fat cell size and hormone-sensitive lipase activity. J Appl Physiol 1999; 87: 227–232.
25 Kadowaki T, Hara K, Yamauchi T, Terauchi Y, Tobe K, Nagai R. Molecular mechanism of insulin resistance and obesity. Exp Biol Med (Maywood) 2003; 228: 1111–1117.
26 Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM et al. Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol Cell Biol 2000; 20: 5479–5489.
27 Venable CL, Frevert EU, Kim YB, Fischer BM, Kamatkar S, Neel BG et al. Overexpression of protein-tyrosine phosphatase-1B in adipocytes inhibits insulin-stimulated phosphoinositide 3-kinase activity without altering glucose transport or Akt/Protein kinase B activation. J Biol Chem 2000; 275: 18318–18326.
28 Chen C, Zhang Y, Huang C. Berberine inhibits PTP1B activity and mimics insulin action. Biochem Biophys Res Commun 2010; 397: 543–547.
29 Ali MI, Ketsawatsomkron P, Belin de Chantemele EJ, Mintz JD, Muta K, Salet C et al. Deletion of protein tyrosine phosphatase 1b improves peripheral insulin resistance and vascular function in obese, leptin-resistant mice via reduced oxidant tone. Circ Res 2009; 105: 1013–1022.
30 Xiang X, Yuan M, Song Y, Ruderman N, Wen R, Luo Z. 14-3-3 facilitates insulin-stimulated intracellular trafficking of insulin receptor substrate 1. Mol Endocrinol 2002; 3: 552–562.
31 Onuma H, Osawa H, Yamada K, Ogura T, Tanabe F, Granner DK, Makino H. Identification of the insulin-regulated interaction of

phosphodiesterase 3B with 14-3-3beta protein. Diabetes 2002; 51: 3362–3367.
32 Yu C, Han W, Shi T, Lv B, He Q, Zhang Y et al. PTPIP51, a novel 14-3-3 binding protein, regulates cell morphology and motility via Raf-ERK pathway. Cell Signal 2008; 20: 2208–2220.
33 Omar B, Zmuda-Trzebiatowska E, Manganiello V, Göransson O, Degerman E. Regulation of AMP-activated protein kinase by cAMP in adipocytes: roles for phosphodiesterases, protein kinase B, protein kinase A, Epac and lipolysis. Cell Signal 2009; 21: 760–766.
34 Mantovani G, Bondioni S, Alberti L, Gilardini L, Invitti C, Corbetta S et al. Protein kinase A regulatory subunits in human adipose tissue: decreased R2B expression and activity in adipocytes from obese subjects. Diabetes 2009; 58: 620–626.
35 Aitken A. 14-3-3 proteins: a historic overview. Semin Cancer Biol 2006; 16: 162–172.
36 Yu X, Jian R, Xinjiao G, Changjiang J, Longping W, Xuebiao Y. GPS 2.0, a tool to predict kinase-specific phosphorylation sites in hierarchy. Moll Cell Proteomics 2008; 7: 1598–1609.
37 Campbell JE, Fediuc S, Hawke TJ, Riddell MC. Endurance exercise training increases adipose tissue glucocorticoid exposure: adaptations that facilitate lipolysis. Metabolism 2009; 58: 651–660.
38 Nomura S, Kawanami H, Ueda H, Kizaki T, Ohno H, Izawa T. Possible mechanisms by which adipocyte lipolysis is enhanced in exercise-trained rats. Biochem Biophys Res Commun 2002; 295: 236–242.

Cell Tissue Res
DOI 10.1007/s00441-011-1146-1

AT-A-GLANCE ARTICLE

Protein tyrosine phosphatase interacting protein 51—a jack-of-all-trades protein

Alexander Brobeil · Manuel Bobrich · Monika Wimmer

Received: 14 December 2010 / Accepted: 5 February 2011

Abstract Protein tyrosine phosphatase interacting protein 51 (PTPIP51) interacts both in vitro and in vivo with PTP1B, a protein tyrosine phosphatase involved in cellular regulation. PTPIP51 is known to be expressed in many different types of tissues. It is involved in cellular processes such as proliferation, differentiation and apoptosis. Nevertheless, the exact cellular function of PTPIP51 is still unknown. The present review summarizes our current knowledge of the PTPIP51 gene and its mRNA and protein structure.

Keywords PTPIP51 · Gene regulation · mRNA structure · Protein domains · Isoforms

Introduction

About 10 years ago, protein tyrosine phosphatase interacting protein 51 (PTPIP51) was discovered as an interacting partner of protein tyrosine phosphatase 1B (PTP1B; Stenzinger et al. 2009b). Several publications have subsequently dealt with its expression pattern in diverse tissues from various animals. The initial study revealed PTPIP51 to be expressed in epithelial cells of the epidermis, in ciliated cells of the trachea, in the fallopian tubes and in the ependyma of the brain ventricles. Reproductive tissue was also identified as expressing the PTPIP51 protein, PTPIP51 being present in primary spermatocytes with continuous expression throughout differentiation up to the spermatid stage. Nervous tissue and skeletal muscle also show PTPIP51 protein expression, in the latter in a fibre-type-specific manner. Additionally, PTPIP51 has been revealed to be expressed in every cell line investigated, e.g. glucagon-producing cells (INR1G9), human keratinocytes (HaCat), human placental cells (BeWo), acetylcholine-producing cells (NS20Y) and Jurkat T-lymphoma cells (Stenzinger et al. 2005). Rat liver tissue exhibits the PTPIP51 antigen in a subset of hepatocyte nuclei, in cholangiocytes of the bile tract and in non-parenchymal cells, namely Kupffer cells, stellate cells and natural killer cells (Stenzinger et al. 2007).

The expression pattern of PTPIP51 suggests its association with cellular processes such as proliferation and differentiation. Subsequent studies have identified PTPIP51 protein as being involved in apoptosis and malignant transformation (Lv et al. 2006; Koch et al. 2008). This multifunctional character of PTPIP51 protein raises the question as to how such a single protein can fulfill opposing cellular functions. The present review combines the latest experimental knowledge with theoretical database information and computational calculations regarding the PTPIP51 gene and its mRNA and protein structure and gives possible explanations for the multitude of functions performed by PTPIP51 within the processes of proliferation, differentiation and apoptosis.

Present experimental data concerning PTPIP51

Following the basic description of PTPIP51 expression by Stenzinger and co-workers (2005), subsequent studies aimed to create a more detailed picture of its expression correlated to cellular function. Investigations of HaCat cells

This paper is dedicated to Prof. Dr. Drs. mult. h.c. Andreas Oksche on the occasion of his 85th birthday.

A. Brobeil · M. Bobrich · M. Wimmer (✉)
Institute of Anatomy and Cell Biology, Justus-Liebig-University, 35392 Giessen, Germany
e-mail: Monika.Wimmer@anatomie.med.uni-giessen.de

Published online: 03 March 2011

treated with epidermal growth factor (EGF), transforming growth factor β, retinoic acid (RA) and 1,25-dihydroxyvitamin D3 (vitamin D3) provided the first data concerning the functional involvement of PTPIP51 in protein translation (Stenzinger et al. 2006). PTPIP51 expression was found to be influenced by these growth factors. HaCat cells initially displayed a decrease in PTPIP51-positive cells treated with a low concentration of EGF (10^{-9} M) followed by a step-wise up-regulation with higher concentrations of EGF (10^{-7} M). The differentiation-inducing vitamin D3 stimulated PTPIP51 protein expression and augmented the number of PTPIP51-positive HaCat cells. RA had no effect on the number of PTPIP51-positive cells when used at physiological concentrations. Exposure of cells to a supraphysiological concentration of RA (10^{-5} M) resulted in a dramatic increase of PTPIP51-positive cells. Based on these findings, Barop and co-workers (2009) probed cultured human muscle cells for PTPIP51 protein hoping to corroborate the hypothesis that PTPIP51 is involved in differentiation processes. When differentiating into multinucleated myotubes, cultured myoblasts displayed an up-regulation of PTPIP51 mRNA expression. Exposure of the differentiated myotubes to proliferation-promoting conditions resulted in a sharp drop in PTPIP51 mRNA expression. Nevertheless, in both studies, the investigated cell lines always displayed a basic expression of PTPIP51 during the proliferation state.

The haematopoietic system is characterized by a high turnover of cells, which subsequently undergo terminal differentiation. In healthy bone marrow trephine specimens, PTPIP51 is neither associated with proliferating cells nor with apoptotic cells. PTPIP51 protein is restricted to the myeloid lineage. Furthermore, mature neutrophil granulocytes of bone marrow trephine specimens, umbilical cord blood and peripheral venous blood contain large amounts of PTPIP51 protein (Brobeil et al. 2010). Tyrosine kinases mediate the signals of various haematopoietic cytokines (Miranda and Johnson 2007). The signal transduction is controlled by distinct protein tyrosine phosphatases such as PTP1B (Heinonen and Tremblay 2006; Barreda et al. 2004). As PTPIP51 and PTP1B interact in neutrophil granulocytes of umbilical cord blood and peripheral venous blood, a signal transducing function of PTPIP51 can be assumed (Brobeil et al. 2010). Moreover, Bobrich and co-workers have confirmed the interaction of PTPIP51 and PTP1B in mice adipose tissue (Bobrich et al. 2011).

Apart from this, the PTPIP51 protein, if overexpressed in HEK293T and in HeLa cells, is connected to the apoptotic machinery (Lv et al. 2006). Hallmarks of apoptosis are divided into morphological and biochemical alterations. Cell shrinkage, chromatin condensation and cell fragmentation into small vesicles are typical morphological characteristics for apoptotic cells. In biochemical terms, apoptosis is characterized by DNA degradation through endogenous DNases, the activation of caspases and the depolarization of the mitochondrial membrane with the subsequent release of pro-apoptotic factors (Saraste and Pulkki 2000; Ly et al. 2003). These processes have also been observed when PTPIP51 is overexpressed in HEK293T cells. After transfection, a significant increase in the active caspase 3 and its substrate poly ADP ribose polymerase is detected (Lv et al. 2006). Moreover, the membrane potential of the mitochondria is reduced and cytochrome c is released. Thus, PTPIP51-transfected cells with subsequent overexpression display all the features of apoptosis. Interestingly, PTPIP51 protein contains a transmembrane domain (TMD) that is essential for its translocation to mitochondria and the induction of apoptosis. This association of PTPIP51 with apoptotic processes has also been observed in vivo; however, a restriction solely to apoptotic functions has been excluded (Stenzinger et al. 2009a; Maerker et al. 2008).

Apoptotic cell death is crucial for the normal development of various organs such as the retina (Linden et al. 2005). The screening of embryonic tissue samples at various stages of murine eye development has stressed the importance of PTPIP51 expression during the maturation of this organ (Maerker et al. 2008). Apoptotic cells labelled by the TdT-mediated dUTP-biotin nick-end labelling assay display no exclusive association with the PTPIP51 protein. On the contrary, not all PTPIP51-positive cells undergo apoptosis.

During retinal differentiation, PTPIP51 expression is down-regulated by the ciliary neurotophic factor (CNTF; Roger et al. 2007). CNTF has neuroprotective effects and can prevent neurons from degenerating in diseases such as Huntington's (de Almeida et al. 2001). Hence, PTPIP51 down-regulation mediated through CNTF is probably a regulatory mechanism to adjust the pro-apoptotic effect of PTPIP51 in a cell-specific manner contributing to the differentiation process.

Summarizing these findings, we postulate that the PTPIP51 protein is regulated through growth factors and cytokines that thereby adapt the expression of PTPIP51 to cellular requirements such as proliferation, differentiation or apoptosis.

In a systematic morphological study, the PTPIP51 protein was found to be restricted to specific areas and nuclei of the mouse brain. Neurons were labeled with antibodies against the PTPIP51 antigen, whereas glial cells remained unstained (Koch et al. 2009b). In malignant transformation such as glioblastoma, however, glial cells started to express PTPIP51 (M. Petri, P. Koch, A. Stenzinger, K. Kuchelmeister, U. Nestler, K. Steger, M. Viard, M. Wimmer, in preparation).

Such a correlation of PTPIP51 protein expression and malignant transformation was also confirmed for various

skin carcinomas such as basal cell carcinomas (BCC), squamous cell carcinomas (SCC), Bowen's disease and keratoacanthomas (Koch et al. 2008). Malignant cells of BCC and SCC showed a co-localization of PTPIP51 and β-catenin. Dysregulated β-catenin signalling was found within various cancer types such as colonic cancer, melanoma and prostate cancer (Morin 1999). PTPIP51 expression in colonic cancer and melanoma is being confirmed by ongoing studies in our laboratory. In addition, breast cancer samples of ductal and lobular origin display a variable expression pattern of PTPIP51. A recent study has shed light on PTPIP51 expression in prostate cancer (Koch et al. 2009a; see also below). In addition to occurring within solid tumours, the PTPIP51 protein has also been traced in a malignant transformed myeloid cell in acute myeloid leukemia (AML). PTPIP51 expression is not correlated with the subtype of AML as classified by the French-American-British system (A. Brobeil, M. Bobrich, M. Graf, A. Kruchten, W. Blau, M. Rummel, S. Oeschger, K. Steger, M. Wimmer, in preparation).

However, the exact cellular function of PTPIP51 is still unknown. Recent studies on the impact of PTPIP51 on signalling pathways have identified PTPIP51 as being involved in the mitogen-activated protein kinase (MAPK) pathway (Yu et al. 2008; Bobrich et al. 2011). Yu and co-workers (2008) have shown that PTPIP51 is capable of increasing ERK activation by interacting with Raf-1 through 14-3-3 proteins. Consequently, the cells display an elongated cell morphology and increased cell migration, adhesion and spreading. The down-regulation of PTPIP51 abrogates these effects. Apart from its effect on cellular morphology and cell migration, PTPIP51 interacts with 14-3-3beta in adipocytes of specifically fed and trained mice, as verified in vivo by duolink proximity ligation assay (DPLA). The punctate pattern of the hybridized and amplified antibody-linked nucleotide strands for PTPIP51 and the investigated interaction partner is only apparent when both proteins are in closest proximity. Strikingly, the high-fat diet trained mice display the highest grade of interaction compared with high-fat diet mice or control mice. Recent studies (to be published) have revealed an up-regulation of the insulin receptor mRNA in high-fat diet trained mice. 14-3-3 proteins are known to mediate the insulin signal (Xiang et al. 2002; Onuma et al. 2002). Under these conditions, PTPIP51 expression is up-regulated because of the increased demand for the linker partner. Furthermore, PTPIP51 acts as a cross-linking molecule within the c-Kit signalling pathway in cells of AML bone marrow trephine biopsy specimens (A. Brobeil, M. Bobrich, M. Graf, A. Kruchten, W. Blau, M. Rummel, S. Oeschger, K. Steger, M. Wimmer, in preparation). Two Src kinases, namely c-Src and Lyn, phosphorylate PTPIP51 at its tyrosine residue 176. In AML blasts, the entire PTPIP51 protein is nearly completely phosphorylated at tyrosine 176. Probing AML samples by DPLA has shown that PTPIP51 has no effect on MAPK pathway regulation. The phosphorylation of critical tyrosine residues might be a regulatory mechanism controlling the effect of PTPIP51 on the MAPK pathway (see below).

These experimental data stress the functional involvement of PTPIP51 in proliferation, differentiation, apoptosis and malignant transformation. In the following sections, the experimental findings are discussed in relation to theoretical considerations gained by database searches and computational calculations, thus extending the knowledge of the function of PTPIP51.

PTPIP51 gene: structure and mechanisms of regulation

The PTPIP51 gene is located on the minus strand of human chromosome 15 (15q15.1, position 41,028,086-41,047,458) encompassing 13 exons and having a length of 19,373 base pairs (bp). Up to now, the nomenclature for the PTPIP51 gene has been inconsistent. The gene belongs to the family with sequence similarity 82 (FAM82). The gene family is subdivided into three groups, named alphabetically with A, B and C. PTPIP51 is a member of subclass C, wherefore the PTPIP51 gene is named FAM82C. Alternatively, the FAM82 members are categorized solely into groups A and B. In this case, the PTPIP51 gene can be identified as FAM82A2. This nomenclature of the PTPIP51 gene was established in the 17th revision of the human genome in March 2006. Prior to that, the PTPIP51 gene and its protein were known as FLJ10579.

Apart from this basic information, little is known about the PTPIP51 gene and its regulation. A recent study has provided critical new insights into the regulation of the PTPIP51 gene by investigating the methylation status of the gene promoters (Koch et al 2009a). The investigated CpG-rich promoter region is located on intron 2 at position 793 to 1112 (Fig. 1b). Another predicted CpG-rich island is located ahead of exon 1 (Fig. 1a). This island is probably the main initiation site for transcription attributable to the full transcription of the PTPIP51 gene. Figure 1c, d gives an overview of the promoter location and predicted transcription factors (Transcription Element Search System, http://www.cbil.upenn.edu/cgi-bin/tess/tess).

DNA methylation stabilizes the chromatin structure and is crucial to epigenetic gene regulation in mammals (Shames et al. 2007). Gene silencing is achieved by the methylation of CpG-rich islands associated with gene promoters thereby inhibiting the binding of relevant transcription factors. Demethylation leads to the opposite effect: cells display increased transcription and translation of demethylated genes and their products (Ellis et al. 2009).

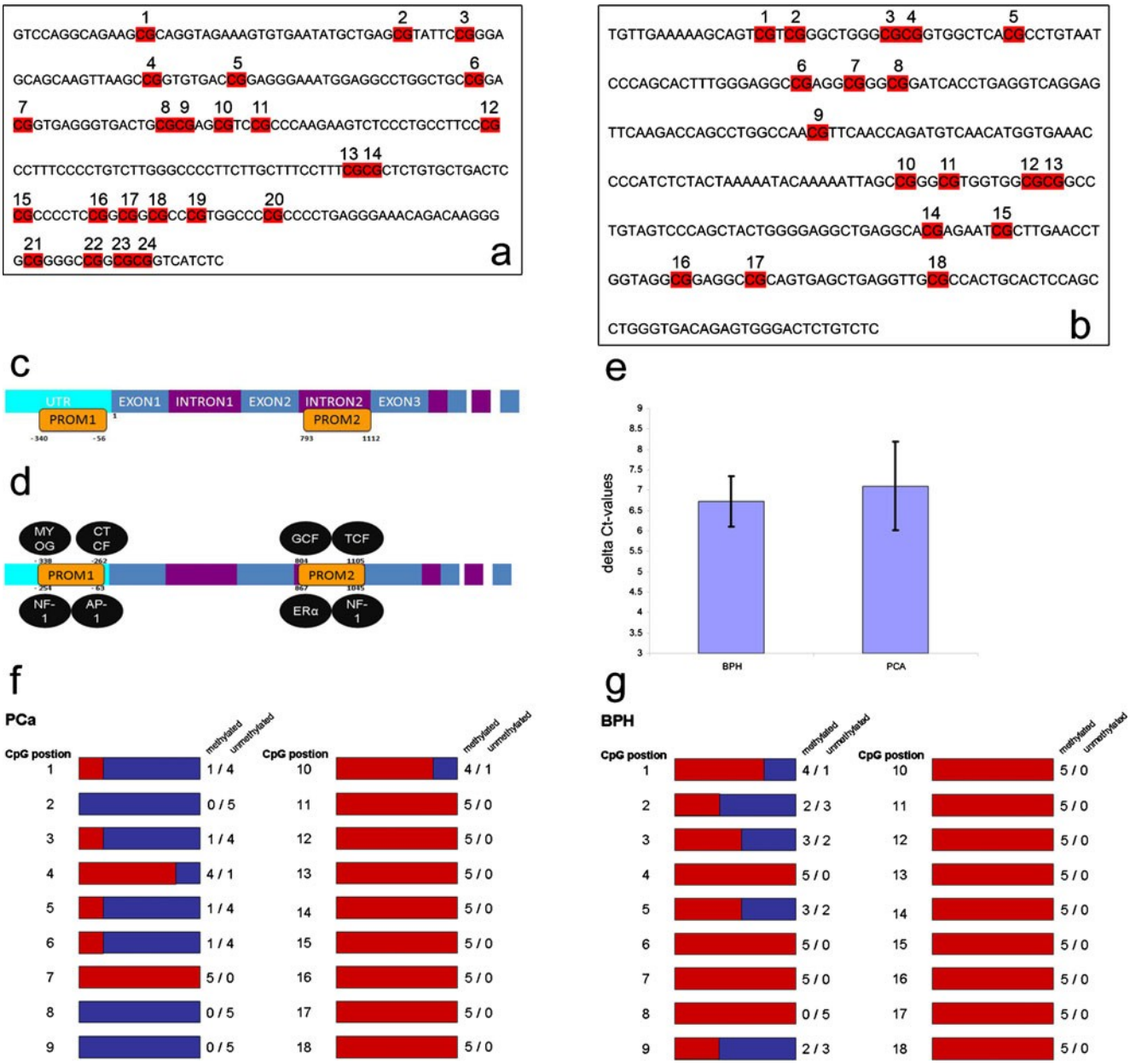


Fig. 1 The protein tyrosine phosphatase interacting protein 51 (PTPIP51) gene has two CpG-rich islands, which resemble possible promoter regions. These CpG-rich islands can be methylated and silenced, preventing transcription. a Potential PTPIP51 promoter region located in front of exon 1. b PTPIP51 promoter sequence including the investigated CpG-rich island. c Location of the two promoters (PROM1, PROM2) of the PTPIP51 gene. d Calculated transcription factors binding to the promoters (MYOG myogenin, CTCF CCCTC-binding factor [zinc finger protein], NF-1 nuclear factor 1, GCF GC-binding factor, ERα oestrogen receptor alpha, AP-1 activator protein 1, TCF T-cell factor). e Quantitative reverse transcription with the polymerase chain reaction of samples of benign prostate hyperplasia (BPH) and prostate carcinoma (PCA). f Promoter methylation of the promoter region 2 of the PTPIP51 gene in prostate cancer (PCa). g Promoter methylation of the promoter region 2 of the PTPIP51 gene in benign prostate hyperplasia (BPH)

Koch and co-workers (2009a) have linked the overexpression of the PTPIP51 protein to the methylation status of the PTPIP51 gene in prostate cancer. In benign prostate hyperplasia, eighteen investigated CpG-rich regions are hypermethylated compared with their methylation status in prostate cancer (Fig. 1f, g).

Glial cells from mouse brain samples show no transcription of the PTPIP51 protein (Koch et al. 2009b). Interestingly, in glioblastoma, malignant transformed glial cells exhibit PTPIP51 expression. Two changes are known to occur with regard to DNA methylation status during carcinogenesis: genome-wide hypomethylation and dysre-

gulation of the methylation status at distinct CpG-rich promoters (Shames et al. 2007). Thus, promoter methylation might be one mechanism of PTPIP51 gene regulation. Subsequent studies are needed to correlate the promoter methylation status to diseased and healthy tissue.

As genes are regulated multifactorially, we performed database searches for other regulatory mechanisms. The cAMP-response element binding protein (CREB) target gene database (http://natural.salk.edu/CREB/) added evidence that the PTPIP51 gene has a cAMP-response element (CRE) with the full site sequence (TGACGTCA). Preliminary experiments revealing the presence of a CRE were performed. Using a chromatin immunoprecipitation-on-chip (ChIP-chip), CREB and phosphorylated CREB were shown to bind to the promoter region of the PTPIP51 gene. Two values were provided by the study: the confidence level of the binding (P-value) and the ratio of ChIP signal over control genomic DNA (binding ratio). The recommended cut-off values for ChIP-positive gene were $P<0.001$ and a binding ratio of >2. The obtained values were highly significant except for islet cells, according to the recommended cut-off values. Furthermore, CREB and phosphorylated CREB binding was shown to increase with exposing time to the protein kinase A activator forskolin in the HEK293T cell line (Zhang et al. 2005). Various signalling pathways were shown to activate CREB by phosphorylation at serine 133. In addition to protein kinase A and the Ca^{2+}/calmodulin-dependent protein kinases, the MAPK pathway is capable of activating CREB by phosphorylation mediated through p90 ribosomal S6 kinase (p90RSK; Shaywitz and Greenberg 1999; McCubrey et al. 2000). The archetype of the MAPK pathway activating receptor is the EGF receptor (EFGR). Activation of the EGFR by its main ligand, viz. EGF, leads to cell proliferation paralleled by the activation of the MAPK pathway and CREB. Immortalized human keratinocyte cancer cells (HaCat) exposed to EGF show a concentration-dependent up-regulation of PTPIP51 expression (Stenzinger et al. 2006). Subsequent in vivo studies are crucial to ascertain PTPIP51 gene regulation by a CRE but the theoretical information related to experiments hints at the presence of a CRE near its gene locus.

Moreover, glioblastoma cells express a mutant of the EGFR. This EGFR variant III enhances the tumourigenic potential by activating and sustaining mitogenic, anti-apoptotic and pro-invasive signalling pathways (Gan et al. 2009). The constitutive signal might enhance CREB phosphorylation with the subsequent up-regulation of PTPIP51. This suggests the existence of an additional mechanism for PTPIP51 expression in transformed glioma cells.

Roger et al. (2007) have identified CNTF as down-regulating PTPIP51 gene expression in retinal explants. CREB activation is reduced in retinal explants of wild-type mice subjected to CNTF and brain-derived neurotrophic factor (Azadi et al. 2007). Thus, decreased PTPIP51 expression correlates with lower amounts of active CREB.

In summary, the PTPIP51 gene is assumed to be regulated by at least two mechanisms: on the one hand, the methylation and demethylation of critical CpG-rich residues in the promoter regions of the PTPIP51 gene and, on the other hand, the CRE-controlled transcription.

PTPIP51 mRNA: structure, post-transcriptional processing and protein translation

The mRNA sequence (National Center for Biotechnology Information [NCBI] Reference Sequence database accession code NM_018145) consists in 2251 bp distributed over 13 exons, whereby exon 1 is a non-coding exon. Exon 1 forms the 5′ untranslated region (5′-UTR) of PTPIP51 mRNA.

Exon 13 encompasses 707 bp but only the first 51 bp encode for amino acids 454 to 470 of the PTPIP51 protein. The triplet on exon 13 at position 1596 resembles the stop-codon-related sequence UAA and terminates protein synthesis.

For the initiation of transcription, an AUG triplet is required with a specific surrounding sequence. The optimal consensus sequence has been identified as follows: GCCRCCaugG (R stands for purine bases). Positions -3 and +4 play key roles for the strength of initiation (Kozak 2005). Strikingly, the first AUG triplet is located on exon 1, which does not code for the PTPIP51 protein. The surrounding bases are in accordance with the optimal consensus sequence for the initiation of translation. Analysis of the full mRNA sequence of PTPIP51 by an open reading frame (ORF) finder (http://www.bot.uni-heidelberg.de/sms/javascript/orf_find.html) identifies five ORFs by using AUG as the exclusive start codon, reading frame 3 on the direct strand and a minimum ORF length of four codons. The first ORF is located at position 45 to 47 of the PTPIP51 mRNA. The coding mRNA sequence ends after nine codons with a stop codon at position 72 to 74. The following ORF from base 186 to base 1598 encompasses the coding sequence for the PTPIP51 full-length protein (Fig. 2a). Hence, the upstream ORF (upORF) might serve as a regulator for the translation of the PTPIP51 protein. This mechanism has been observed for several mRNAs (Kozak 2005). The PTPIP51 mRNA structure including upORF can also be traced in other mammals and thus evolutionary conservation can be assumed. PTPIP51 mRNAs of mouse and rat exhibit an upORF that differs in its localization compared with the human PTPIP51 mRNA. The upORF overlaps the protein coding ORF resulting in a low level of PTPIP51 protein translation

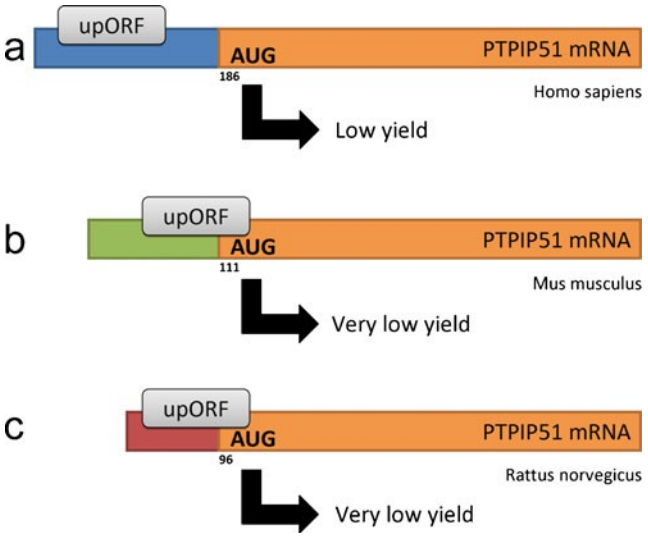


Fig. 2 PTPIP51 mRNA exhibits a regulatory upstream open reading frame (upORF). The upORF is conserved between the three investigated species. a Location of the upORF in human PTPIP51 mRNA. b The upORF of rats shows an overlap with the main open reading frame (ORF). c PTPIP51 mRNA of mice exhibit an overlapping upORF with the main ORF

(Fig. 2b, c). Such strict regulation of mRNA translation is mainly observed for growth factors and other proto-oncogenes (Kotechov 2008). The expression of the PTPIP51 protein and mRNA in human skeletal muscle cell lines is differentiation-dependent. Undifferentiated myoblasts show both low mRNA and protein levels of PTPIP51. Differentiation into multinucleated myotubes results in a linear increase of PTPIP51 protein levels proceeded by an intense increase of PTPIP51 mRNA expression (Barop et al. 2009). UpORF repression of translation is unlikely. However, in cases of reaching a critical protein level that could induce apoptosis, upORF might sustain the mRNA/protein ratio by limiting the translation of PTPIP51 mRNA. Interestingly, the transcript starting at the alternative promoter of the PTPIP51 gene lacks exon 1 and thus the upORF. This leads to the translation of the full-length protein without any control of the protein expression by an upORF. The observed overexpression of the PTPIP51 protein in prostate cancer corroborates this hypothesis. In benign prostate hyperplasia and in prostate cancer, PTPIP51 mRNA has been observed to be expressed in the same amounts by quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) experiments (Fig. 1e). Compared with benign prostate hyperplasia, the alternative promoter is found to be hypomethylated in prostate cancer (Fig. 1f, g; Koch et al. 2009a). DNA and promoter methylation is one reason for switching from the core-promoter to an alternative promoter (Davuluri et al. 2008). The new transcript lacks the upORF resulting in an overexpression of PTPIP51. Thus, we hypothesize that promoter switching and the multiple start codons are a cancer-dependent mechanism for the dysregulation of both PTPIP51 mRNA transcription and translation leading to independent growth and proliferation.

The full translation of PTPIP51 mRNA results in a protein product of 52 kDa. This protein corresponds to the full-length form of PTPIP51. As listed in Table 1, immunoblotting of various tissues displays different PTPIP51 levels with isoforms of variable molecular weights. Hitherto, no study has analysed the underlying mechanism of PTPIP51 isoform synthesis and no experimental data have verified the existence of isoforms at the mRNA level. Experimental findings are limited to Western blot analyses. Nevertheless, two theoretical mechanisms explaining the expression of PTPIP51 protein isoforms are discussed here. The molecular weights obtained by Western blot analyses have been correlated to hypothetical database information and computational calculations: one probable mechanism is the use of different initiation-sites on PTPIP51 mRNA for translation. The scanning of the coding ORF for internal start codons has led to six additional AUG triplets being found at positions 375 to 377, 573 to 575, 804 to 806, 1044 to 1046, 1275 to 1277 and 1506 to 1508 corresponding to exon 3, 4, 5, 6, 9 and 12, respectively. These alternative initiation codons result in expected molecular weights of 45 kDa, 38 kDa and 30 kDa (ProteinCalculator v3.3, http://www.scripps.edu/~cdputnam/protcalc.html; Stenzinger et al. 2005). The underlying mechanism is called leaky scanning. Optimal sequences border the AUG triplet and are recognized by ribosomes as translational initiation site (TIS). AUG triplets lacking this consensus sequence are reduced in their ability to start translation. Thus, a subset of ribosomes misses the start codon and continues scanning downstream until it recognizes an alternative AUG triplet. This mechanism allows the ribosomes to detect different TIS within the ORF leading to distinct isoforms of the protein. The resulting products are truncated at the N-terminal end (Kochetov 2008).

The first and second alternative AUG triplets marking the start of PTPIP51 protein translation are not in accordance with the optimal consensus. In contrast, the third alternative start codon is bordered by an adenosine at position -3 and a guanine at position +4. This is an optimal context for translation initiation. Thus, PTPIP51 isoforms with molecular weights lower than 30 kDa are unlikely to be translated exclusively by the leaky scanning mechanism.

Immunoblot analyses corroborate the expression of PTPIP51 isoforms with molecular weights of 30, 45, 52 kDa, e.g. in isolated neutrophil granulocytes of peripheral venous blood (Table 1). The 38-kDa isoform is known and assigned under Q96TC7-2 in the Uniprot database. Moreover, the 45-kDa and 30-kDa isoform are listed in the NCBI

Table 1 Molecular weights of PTPIP51 isoforms revealed by immunoblotting

Tissue	Origin	PTPIP51 isforms [kDa]
Acute myeloid leukmia	Human	52, 38.25,13
Embryonic tissue		12day: 75,60,30
		13day: 100,75,60,48,35,30
		14day: 100,75,60,48,35,30
Adult eye	Human	28
Brain	Mouse	Hippocampus: 45,38,37,35
		Cortex: 150,65,52,48,33,30,27
		Cerebellum: 150,120,100,75,52,48,35,33,30,27
Skeletal muscle	Rat	40,25
Skeletal muscle cell line	Human	0day: 60,48
		13day: 80,60,48
		13day + 10: 60-48
Trachea	Rat	65,36
Jurkat	Human	75,52,48,38
HaCat	Human	120,100,68,52,48,38,35,33,25,17,
Blood	Human	65,52,45,38
Testis	Rat	48
Sperm	Human	37
Placenta	Human	52,45,38,35,28
Spinal cord	Human	40
Liver	Tat	75,52,33,27

protein database under accession numbers BAG53446 and BAB15298, respectively.

Nevertheless, some of the observed isoforms cannot be explained by leaky scanning suggesting a second mechanism: alternative splicing is another possibility for augmenting the number of PTPIP51 isoforms. Proteomic diversity in multicellular eukaryotes is based mainly on alternative splicing (Nilsen and Gravely 2010). Recent studies report that almost 95%–100% of pre-mRNAs transcribed from multi-exon genes are alternatively spliced (Nilsen and Graveley 2010). PTPIP51 mRNA exhibits 13 exons and is therefore highly likely to be alternatively spliced. Alternative splicing occurs in a developmentally, differentiation and even signal-transduction-dependent manner (Sánchez 2008; Calarco et al. 2009; Lynch 2007). Up to now, experimental data corroborating the presence of alternatively spliced transcripts are missing. However, the theoretical alternative splicing prediction (ASPic, http://t.caspur.it/ASPIC/index.php, Bonizzoni et al. 2005) web-tool calculates 24 alternative spliced mRNAs for PTPIP51 (Fig. 3a). As has been shown recently, the prediction is reliable, e.g. the predicted alternatively spliced transcripts of the mitochondrial transcription factor A have all been corroborated by RT-PCR (De Virgilio et al. 2011). In the alternative splicing and transcript diversity (ASTD) database (http://www.ebi.ac.uk/astd/main.html) of the European Bioinformatics Institute (EBI) a splice variant of PTPIP51 has been assigned the identification number TRAN00000049919. The splice variant codes for 107 amino acids (aas) of the C-terminus of PTPIP51 (PEPT00000039018). This variant is also a predicted spliced product listed by the ASPic web-tool. Hence, the observed alternatively spliced variant expression and the predicted spliced variants support the hypothesis that PTPIP51 mRNA is alternatively spliced. In addition, PTPIP51 isoforms are also expressed depending on the differentiation status and origin of the cell. In human neutrophil granulocytes, PTPIP51 is expressed with molecular weights of 30, 38, 45 and 52 kDa. As shown by qRT-PCR with exon-specific primers for PTPIP51 mRNA detection, PTPIP51 exons are expressed in similar amounts making alternative splicing unlikely (Fig. 3b). Thus, in neutrophil granulocytes, the leaky scanning mechanism is probably responsible for PTPIP51 isoform production. To confirm this, qRT-PCR experiments covering all exons are needed. Furthermore, the assembled cDNA has to be sequenced. Other tissues exhibit a heterogeneous mix of PTPIP51 isoforms with variable molecular weights (Table 1). Here, alternative splicing might play an important role in the expression of specific PTPIP51 isoforms for the maintenance of its various cellular functions. Further qRT-PCR experiments covering all exons of PTPIP51 might shed light on alternative splicing processes concerning PTPIP51 mRNA.

These findings indicate that PTPIP51 protein translation is strictly controlled, by the inhibition of overexpression, in order to prevent subsequent cell dysfunction. Alternative AUG triplets serving as start codons enable the expression

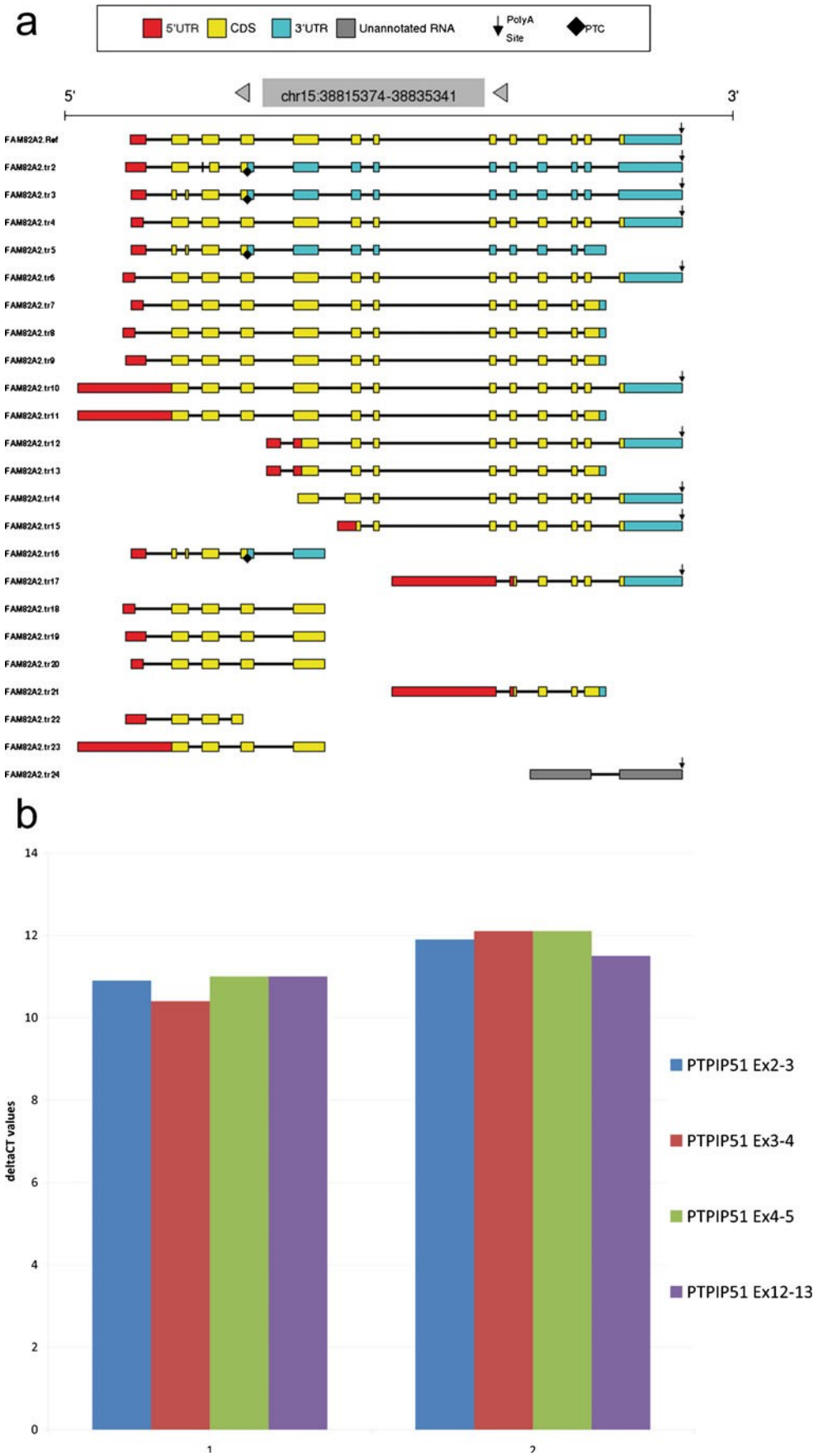


Fig. 3 PTPIP51 mRNA consists in 13 exons, which are likely to be processed by alternative splicing. a The alternative splicing prediction server (ASPic, http://t.caspur.it/ASPIC/index.php, Bonizzoni et al. 2005) calculated 24 possible splice variants (UTR untranslated region, CDS coding sequence, PTC premature termination codon, chr chromosome). The overview illustrates their structures (image source: http://t.caspur.it/ASPIC/tmp/trans_list874_job1221.png) b qRT-PCR of isolated neutrophil granulocytes by using exon-specific primer. 1 = sample 1; 2 = sample 2

of various isoforms. Alternative splicing is a second mechanism used to enhance the cellular adapted expression of PTPIP51 isoforms. We hypothesize that both mechanisms can occur singularly or in combination, depending on the investigated tissue or cells.

PTPIP51 protein: isoforms exhibit functional adapted domains

As shown by various studies, the PTPIP51 protein is associated with proliferation, differentiation and apoptosis (Barop et al. 2009; Koch et al. 2008; Lv et al. 2006). To date, the protein structure of PTPIP51 is unclear. Therefore, a predicted model of PTPIP51 protein structure has been generated by the I-TASSER server (http://zhanglab.ccmb.med.umich.edu/I-TASSER/; Roy et al. 2010). The predicted protein structure and surface are presented in Fig. 4a, b.

The PTPIP51 protein belongs to a family of microtubule-associated proteins called regulators of microtubule dynamics (RMD). The human family consists in three members, which are consecutively numbered. The alternative name for PTPIP51 is human RMD-3 (hRMD-3; Uniprot accession no. Q96TC7). Oishi and co-workers (2007) have cosedimented PTPIP51 associated to taxol-stabilized microtubules. In interphase cells, PTPIP51 protein is found within defined spots throughout the cytoplasm, whereas in mitotic cells, PTPIP51 is translocated to the spindle apparatus (own observations; Oishi et al. 2007 supplement). As indicated in Fig. 5a, the sequences of RMD-1 and PTPIP51 both show conserved regions. The tetratricopeptide repeat (TPR) domains of RMD-1 marked by a line spanning the relevant protein sequence (Fig. 5a) are also present within the PTPIP51 protein. Furthermore, PTPIP51 is known to encompass a TPR-like domain spanning aa303 to aa447. Leaky scanning has found all PTPIP51 isoforms containing the TPR-like domain. An example of this is shown for the full-length protein and the 38-kDa isoform in Fig. 5b-e. A subset of alternatively spliced isofoms of PTPIP51 lacks this domain. First, the TPR domain is associated with the cell cycle (Sikorsky et al. 1990). The two microtubule-associated proteins PTPIP51 and RMD-1 are recruited to the spindle apparatus during mitosis and RMD-1 is known to be crucial for normal kinetochore binding of spindle microtubules (Oishi et al. 2007). Interestingly, the proteins most important for mitosis contain several TPR domains (Goebl and Yanagida 1991). Moreover, PTPIP51 protein has been found to interact with the kinetochore protein Nuf2 in vivo by DPLA (A. Brobeil, M. Bobrich, M. Wimmer, in preparation). The human Nuf2 (hNuf2) protein has been shown to be associated with the outer kinetochore plate. Additional short interfering RNA experiments have corroborated the stabilizing function in microtubule attachment to the kinetochore. hNuf2 is a coiled-coil protein (DeLuca et al. 2002) and acts as a linker between the plus end of the microtubules and the kinetochore. Various coiled-coil proteins are associated with the kinetochore, such as mitosin, centromere protein F or centromere/kinetochore protein ZW10 (Rose and Meier 2004). Thus, PTPIP51 is highly likely to form a complex with hNuf2 during mitosis. We can therefore assume that PTPIP51 is involved in mitotic processes such as the regulation of the spindle apparatus and accurate chromosome segregation.

The centrosome is an essential part for the formation of the mitotic spindle apparatus. In addition, the centrosome is the microtubule-organization centre of the cell and nucleates the microtubules (Rieder et al. 2001). The centrosome is formed by two centrioles, which are sourrounded by the pericentriolar matrix (PCM). Human ninein (hNinein) is one component of the PCM and possibly stabilizes the linkage between the microtubules and the centrioles (Delgehyr et al. 2005). In a yeast two-hybrid screen, CGI-99 (alternatively C14orf166) has been identified as an interaction partner of hNinein, which is associated with the centrosome. Interestingly, CGI-99 is able to inhibit the phosphorylation of

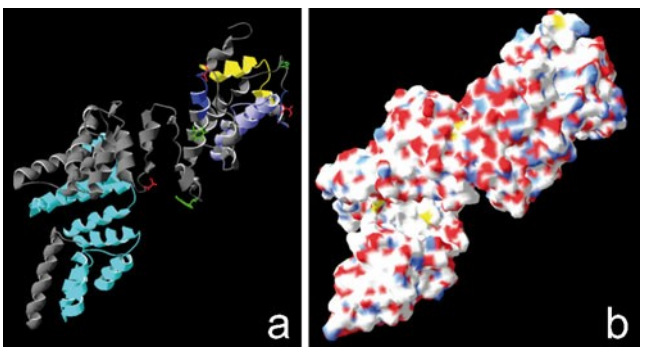
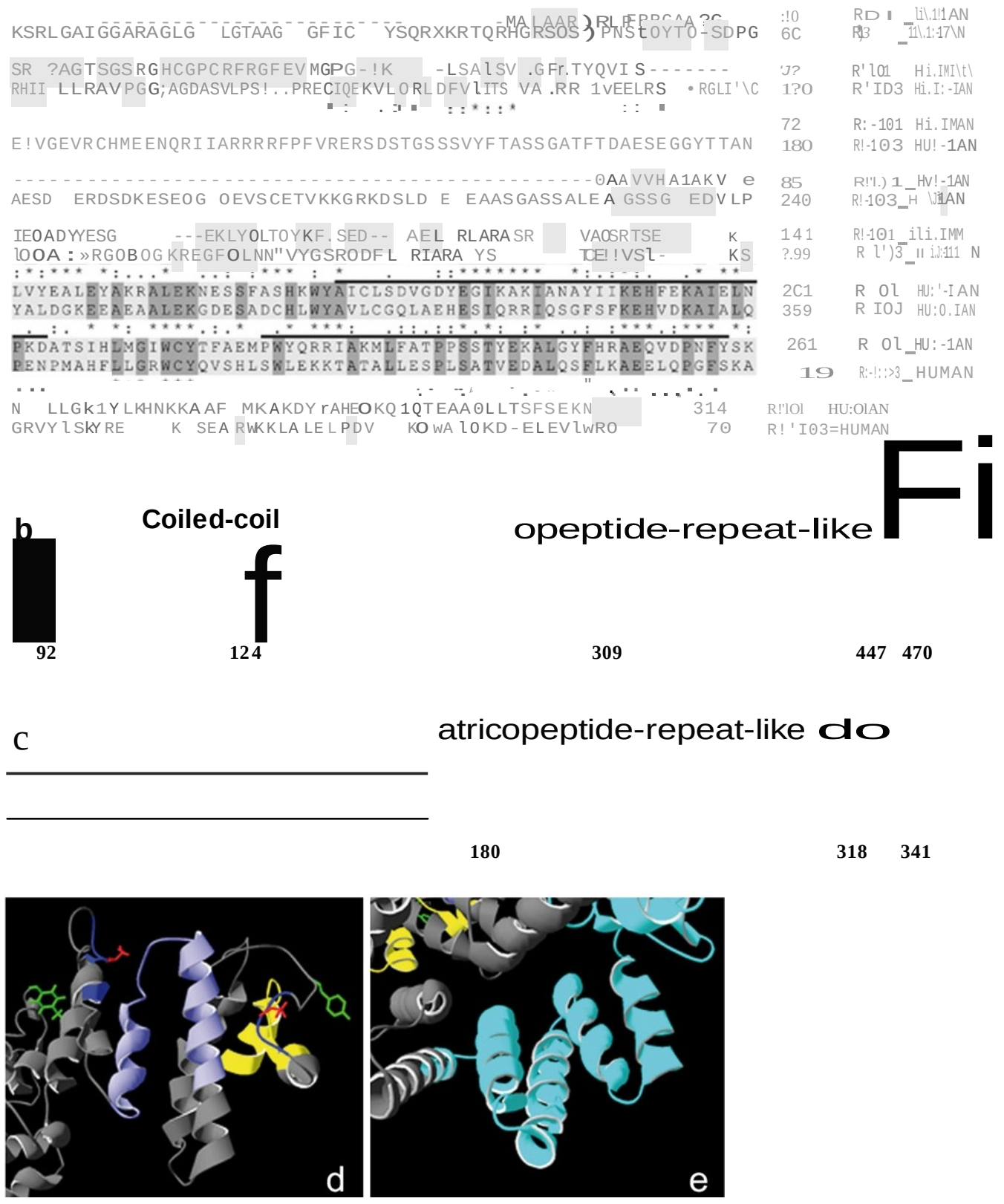


Fig. 4 The tertiary protein structure of PTPIP51 is unknown. The I-Tasser server (http://zhanglab.ccmb.med.umich.edu/I-TASSER/; Roy et al. 2010) predicts a possible structure of the PTPIP51 protein. a Ribbon structure of PTPIP51 (yellow transmembrane domain, purple coiled-coil, blue conserved domains 1 and 2, cyan tetratricopeptide-like domain, red known serine phosphorylation residues, green known tyrosine phosphorylation residues). b Calculated surface of the PTPIP51 protein model

Fig. 5 PTPIP51 is also known as a regulator of microtubule dynamics 3 (RMD-3) and belongs to a new family of microtubule-associated proteins. PTPIP51 and RMD-1 exhibit sequence homologies. Both proteins contain a tetratricopeptide-like domain and PTPIP51 possesses an additional coiled-coil domain. a Sequence alignment of PTPIP51 and RMD-1. b PTPIP51 full-length protein (52 kDa) with its coiled-coiled and tetratricopeptide-like domains. c The PTPIP51 38-kDa isoform exclusively exhibits the tetratricopeptide-like domain. d Tertiary structure of PTPIP51 coiled-coil domain. e Tertiary structure of PTPIP51 tetratricopeptide-like domain


a
b
Coiled-coil
opeptide-repeat-like
92
124
309
447
470
c
atricopeptide-repeat-like
180
318
341
d
e

hNinein mediated by glycogen synthase kinase 3 beta and therefore influences the nucleation of the microtubules (Howng et al. 2004). The direct interaction of PTPIP51 and CGI-99 has been revealed by co-immunoprecipitation experiments and in vivo by DPLA (A. Brobeil, M. Bobrich, M. Wimmer, in preparation). Furthermore, during mitosis, PTPIP51 is also located at the spindle body suggesting the formation of a complex with CGI-99 that regulates hNinein phosphorylation. CGI-99 is strikingly overexpressed in various brain tumours such as astrocytomas (Hwong et al. 2004). Data obtained in our laboratory confirm that PTPIP51 is expressed in malignantly transformed glial cells (M. Petri, P. Koch, A. Stenzinger, K. Kuchelmeister, U. Nestler, K. Steger, M. Viard, M. Wimmer, in preparation). Ongoing studies are examining the interaction of PTPIP51 with CGI-99 in astrocytoma and the impact resulting from this interaction for malignant growth.

In addition to this mitotic function, TPR-domain-containing proteins are involved in processes such as protein transport, neurotransmitter release, phosphate turnover and splicing (Blatch and Lässle 1999). Vesicle trafficking is functionally associated with coiled-coil proteins (Gillingham and Munro 2003). PTPIP51 possesses such a coiled-coil domain.

The transport and release of the neurohormones vasopressin and oxytocin are hallmarks of the function of the posterior pituitary. Both hormones are produced in hypothalamic magnocellular neurons (Russel and Leng 2000). Expression studies in the mouse brain have shown PTPIP51 to be expressed in hypothalamic magnocellular neurons with their fibres extending to the neurohypophysis. Furthermore, PTPIP51 is co-localized with the hormone vasopressin and its carrier protein neurophysin II (Fotheringham et al. 1991; Koch et al. 2009b). Here, PTPIP51 probably mediates vesicle transport and terminal release of the hormones by association with the microtubule transport system.

PTPIP51 can also act as a signalling molecule in a distinct signalling pathway. Interacting with Raf-1 mediated by proteins of the 14-3-3 family, PTPIP51 influences the

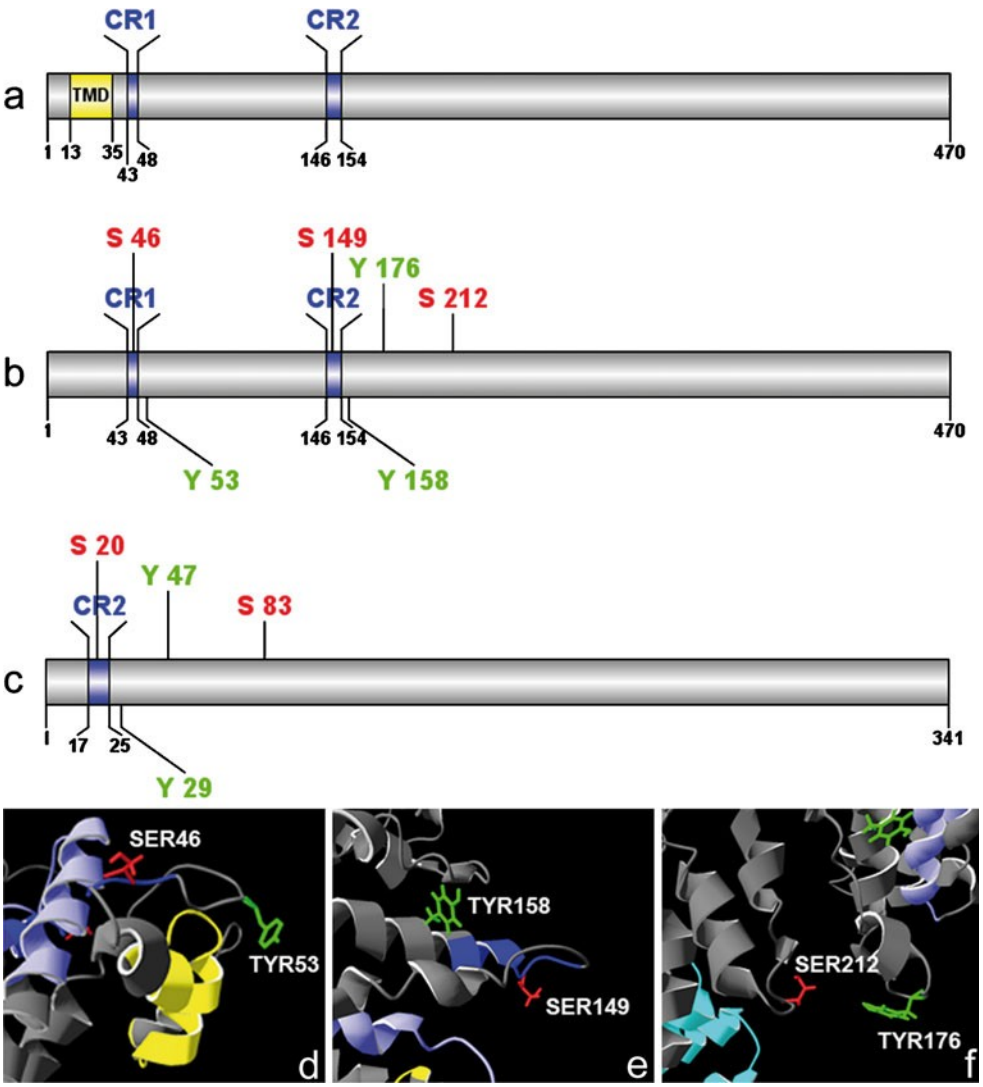


Fig. 6 PTPIP51 has two binding sites for 14-3-3 proteins, named conserved regions 1 and 2 (CR1, CR2), each of which is capable of binding to 14-3-3. A transmembrane domain (TMD) is located in close proximity to the conserved regions of PTPIP51. These domains are surrounded by serine and tyrosine phosphorylation sites. The serine residues aa46 and aa149 are critical for 14-3-3 binding to PTPIP51. The tyrosine residues aa53 and aa158 are probable regulation sites inhibiting the interaction of PTPIP51 and 14-3-3. a Localization of the TMD and the two conserved regions of PTPIP51. b Serine phosphorylation sites (red) and tyrosine phosphorylation sites (green) and their relationship to the conserved regions (PTPIP51 full-length protein). c Serine phosphorylation sites (red) and tyrosine phosphorylation sites (green) and their relationship to the conserved regions (PTPIP51 38-kDa isoform). d Tertiary structure of CR1 and its serine aa46 and tyrosine 53 residues. e Tertiary structure of CR2 and its serine aa149 and tyrosine aa158 residues. f Tertiary structure of serine aa212 and tyrosine aa176 residues

activity of the MAPK pathway. The interactions with the 14-3-3 proteins take place at two defined sites of PTPPI51. These sites, named conserved regions (CR) 1 and 2, span aas43 to 48 and aas 146 to 154. Notably, the presence of only one conserved region is sufficient for binding to 14-3-3 with subsequent Raf-1 modulation (Jin et al. 2004; Yu et al. 2008). Figure 6a, d, e illustrates the localization of the two conserved regions of the PTPIP51 protein. Two recent studies have added evidence that PTPIP51 is involved in the MAPK pathway (Bobrich et al. 2011; M. Petri, P. Koch, A. Stenzinger, K. Kuchelmeister, U. Nestler, K. Steger, M. Viard, M. Wimmer, in preparation). In contrast, AML blasts display no interaction of PTPIP51 and Raf-1 (A. Brobeil, M. Bobrich, M. Graf, A. Kruchten, W. Blau, M. Rummel, S. Oeschger, K. Steger, M. Wimmer, in preparation). This may be attributable to the observed high phosphorylation status of PTPIP51 in AML blasts. PTP1B interacts with PTPIP51 in normal neutrophil granulocytes (Brobeil et al. 2010). The interaction is abolished in AML blasts leading to the high phosphorylation status of PTPIP51. PTP1B

Table 2 Tyrosine kinases phosphorylating PTPIP51 (Abl V-abl Abelson murine leukemia viral oncogene homolog 1, Abl2 Abelson-related gene protein, EGFR epidermal growth factor receptor, FGFR fibroblast growth factor receptor, IGF insulin-like growth factor 1 receptor, InsR insulin receptor, JAK Janus kinase, Fyn proto-oncogene tyrosine protein, HCK haematopoietic cell kinase, Lyn Src-family tyrosine kinase, Lck lymphocyte-specific protein tyrosine kinase, Scr Rous sarcoma nonreceptor tyrosine kinase, BLK B lymphocyte kinase, brk breast tumor kinase, Syk spleen tyrosine kinase, ZAP70 zeta-chain-associated protein kinase 70, PDGFR platelet-derived growth factor receptor, KIT CD117)

Position	Kinase	Peptide	Experimental data
53	Abl	SLPNSLDYTQTSDPG	No
158	Abl	STGSSSVYFTASSGA	No
176	Abl	DAESEGGYTTANAES	No
53	Abl2	SLPNSLDYTQTSDPG	No
53	EGFR	SLPNSLDYTQTSDPG	No
158	EGFR	STGSSSVYFTASSGA	No
176	EGFR	DAESEGGYTTANAES	No
53	FGFR3	SLPNSLDYTQTSDPG	No
53	IGF1R	SLPNSLDYTQTSDPG	No
158	IGF1R	STGSSSVYFTASSGA	No
176	InsR	DAESEGGYTTANAES	No
53	JAK1	SLPNSLDYTQTSDPG	No
176	JAK1	DAESEGGYTTANAES	No
53	JAK2	SLPNSLDYTQTSDPG	No
176	JAK2	DAESEGGYTTANAES	No
53	JAK3	SLPNSLDYTQTSDPG	No
158	Fyn	STGSSSVYFTASSGA	No
176	Fyn	DAESEGGYTTANAES	No
53	Yes	SLPNSLDYTQTSDPG	No
300	Yes	EVSEKKSYALDGKEE	No
158	HCK	STGSSSVYFTASSGA	No
158	Lyn	STGSSSVYFTASSGA	Yes
176	Lyn	DAESEGGYTTANAES	Yes
158	Lck	STGSSSVYFTASSGA	No
176	Lck	DAESEGGYTTANAES	No
158	Src	STGSSSVYFTASSGA	Yes
176	Src	DAESEGGYTTANAES	Yes
158	BLK	STGSSSVYFTASSGA	No
176	BLK	DAESEGGYTTANAES	No
176	Brk	DAESEGGYTTANAES	No
53	Syk	SLPNSLDYTQTSDPG	No
176	Syk	DAESEGGYTTANAES	No
53	ZAP70	SLPNSLDYTQTSDPG	No
158	ZAP70	STGSSSVYFTASSGA	No
176	ZAP70	DAESEGGYTTANAES	No
158	PDGFRa	STGSSSVYFTASSGA	No
176	PDGFRa	DAESEGGYTTANAES	No
158	KIT	STGSSSVYFTASSGA	Yes

function is double-edged in cancer. It can act both as a tumour suppressor and as a tumour promoter dependent on the cancer type (Lessard et al. 2010). As shown in Fig. 6b, d-f, PTPIP51 possesses serine and tyrosine phosphorylation sites. The majority of kinases involved in their phosphorylation are still unknown. Serine residues 46 and 212 have been demonstrated to be phosphorylated during cell cycle progression and mitosis (Cantin et al. 2008; Daub et al. 2008; Dephoure et al. 2008). Furthermore, PTPIP51 is phosphorylated by c-Src at the tyrosine 176 residue (A. Brobeil, M. Bobrich, M. Wimmer, in preparation). In addition, A. Brobeil, M. Bobrich, M. Graf, A. Kruchten, W. Blau, M. Rummel, S. Oeschger, K. Steger, M. Wimmer (in preparation) have identified Lyn, another member of the Src family kinases (SFK) and revealed that it interacts with PTPIP51 in human AML blasts by phosphorylating tyrosine 176. Minor phosphorylations by SFK or other kinases have been observed for tyrosine residues 53 and 158. The phosphorylation is reversed by the T-cell protein tyrosine phosphatase (Stenzinger et al. 2009b). Computational predictions of PTPIP51-interacting kinases for the phosphorylation at tyrosine residues 53, 158 and 176 and at serine residues 46, 149 and 212 are listed in Tables 2, 3. Strikingly, except for serine residue 212, the serine residues are located in the two conserved regions (Fig. 6b, S46 and S149). The serine residues aa46 and aa149 have to be phosphorylated and are critical for 14-3-3 binding (Yu et al. 2008). Using the group-based prediction system 2.1 (GPS 2.1; http://gps.biocuckoo.org/; Xue et al. 2008), protein kinase C (PKC) has been identified to mediate the phosphorylation at the serine residues of CR1 and CR2. The two serine residues of the conserved regions are surrounded by two tyrosine residues, viz. aa53 and aa158. An additional protein domain is located in close proximity to the CR1, namely a TMD (Fig. 6b, d), which is part of the mitochondrial apoptotic system. Apoptosis is initiated by the localization of the PTPIP51 full-length protein to the mitochondria (Lv et al. 2006). The 38-kDa isoform lacks this TMD and is therefore incapable of inducing apoptosis (Lv et al. 2006). Nevertheless, the CR2 is located at the truncated N-terminus of the 38-kDa isoform promoting 14-3-3 binding with subsequent Raf-1 activity modulation (Yu et al. 2008; for a representation, see Fig. 6c). Bound 14-3-3 protein caps this TMD structure (Fig. 6d). This capping could be a mechanism to prevent uncontrolled entry into apoptosis during the cell cycle. According to the phosphorylation atlas of mitotic proteins, PTPIP51 is phosphorylated at serine residues aa49 and aa212 (Dephoure et al. 2008). A similar regulatory mechanism is well established for BAD, a pro-apoptotic protein of the Bcl-2 family. 14-3-3 proteins separate BAD from the mitochondrial membrane. Moreover, BAD and PTPIP51 are both controlled by the same kinase p90RSK phosphorylating the critical serine residue 46 in PTPIP51 (Tan et al. 2000; see Table 3). Figure 7 illustrates the hypothetical involvement of PTPIP51 in signalling pathways maintaining cell survival.

The c-Abl proto-oncoprotein is a member of the Src family of non-receptor tyrosine kinases. c-Abl is a ubiquitously expressed protein that localizes both to the nucleus and the cytoplasm where it plays distinctly different roles (Shaul and Ben-Yehoyada 2005). Expression of c-Abl in the cytoplasm results in cell proliferation and survival. Cytoplasmic c-Abl is involved in the response to growth factor stimulation by promoting DNA synthesis, F-actin assembly and receptor trafficking (Sirvent et al. 2008). In contrast, activated nuclear c-Abl induces apoptosis after genotoxic stress. The molecular mechanisms that transport c-Abl to the nucleus have been studied by Yoshida (2007a). 14-3-3 proteins sequester c-Abl in the cytosol of normal cells. Genotoxic stress leads to the phosphorylation of 14-3-

Table 3 Serine/threonine kinases phosphorylating PTPIP51 (PK protein kinase, RSK ribosomal s6 kinase family, IPL1 Aurora protein kinase)

Position	Kinase	Peptide	Experimntal data
46	PKA	QRHGRSQSLPNSLDY	No
149	PKA	FPFVRERSDSTGSSS	No
212	PKA	VKMGRKDSLDLEEEA	Yes
46	PKC	QRHGRSQSLPNSLDY	No
149	PKC	FPFVRERSDSTGSSS	No
46	p70RSK	QRHGRSQSLPNSLDY	No
149	p70RSK	FPFVRERSDSTGSSS	No
212	p70RSK	VKMGRKDSLDLEEEA	No
46	IPL1-yeast	QRHGRSQSLPNSLDY	No
212	IPL1-yeast	VKMGRKDSLDLEEEA	No
46	RSK1	QRHGRSQSLPNSLDY	No
149	RSK1	FPFVRERSDSTGSSS	No
212	RSK1	VKMGRKDSLDLEEEA	No
46	RSK2	QRHGRSQSLPNSLDY	No

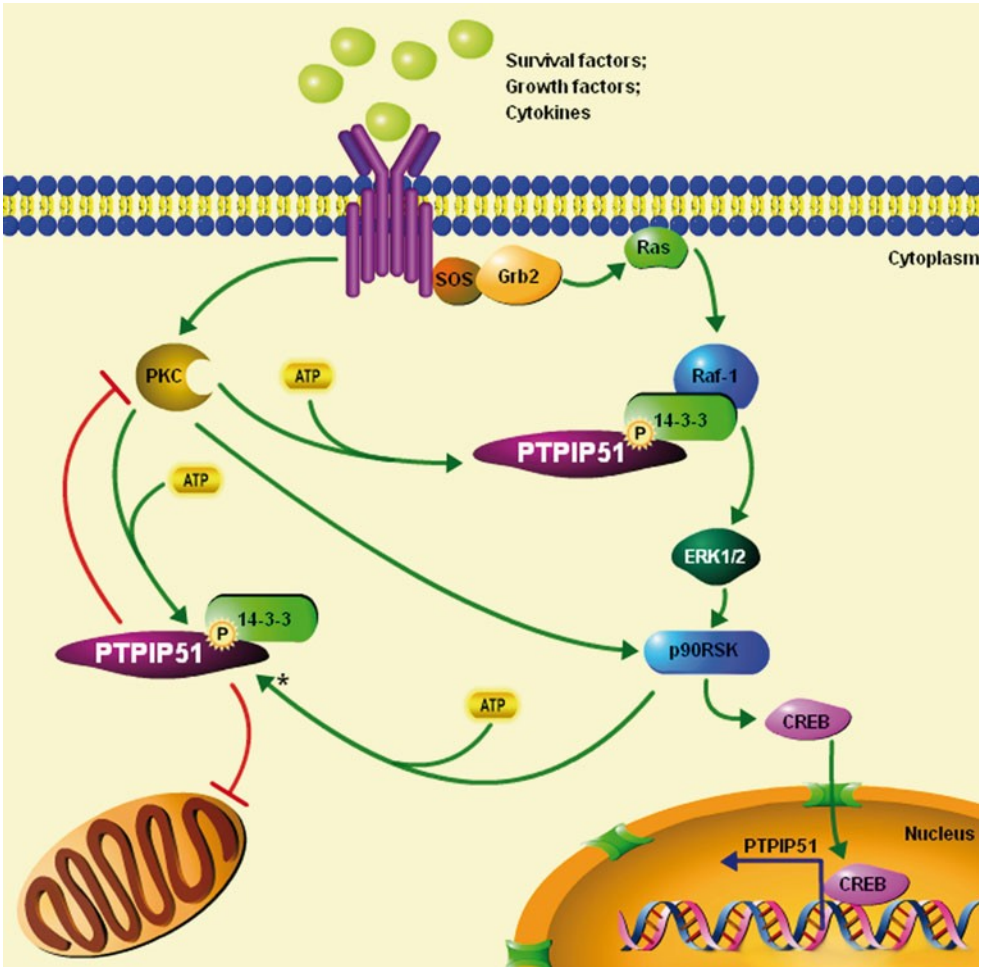


Fig. 7 Cell survival is mediated through distinct signalling pathways, namely the mitogen-activated protein kinase (MAPK) pathway and protein kinase C (PKC) signalling. In both pathways, PTPIP51 can interact with 14-3-3 and thus modify the two pathways. A necessary prerequisite is the phosphorylation of PTPIP51 by PKC. This results in the inhibition of the translocation of PTPIP51 to the mitochondrial membrane and thus in the inhibition of apoptosis (pathway left). The star indicates the computational calculation of the interaction (CREB cAMP-response element binding protein, ERK1/2, p90RSK, SOS, Grb2, Ras, Raf-1 members of the MAPK pathway). The diagram was created by using the Pathwaybuilder online tool at www.proteinlounge.com

3 by c-jun N-terminal kinase. Thus, c-Abl is able to enter the nucleus and induce apoptosis (Yoshida 2007a).

A possible downstream substrate of the c-Abl tyrosine kinase is PKCdelta. DNA damage is followed by the activation of c-Abl, which then phosphorylates PKCdelta, thereby inducing apoptosis (Yoshida 2007b). Not only DNA damage but also endoplasmic reticulum (ER) stress can be followed by apoptosis. ER stress leads to the interaction of PKCdelta and c-Abl. The PKCdelta-Abl complex conveys the apoptotic signal to the mitochondria (Qi and Mochly-Rosen 2008). Earlier studies by Lv et al. (2006) and Stenzinger et al. (2009b) have revealed that

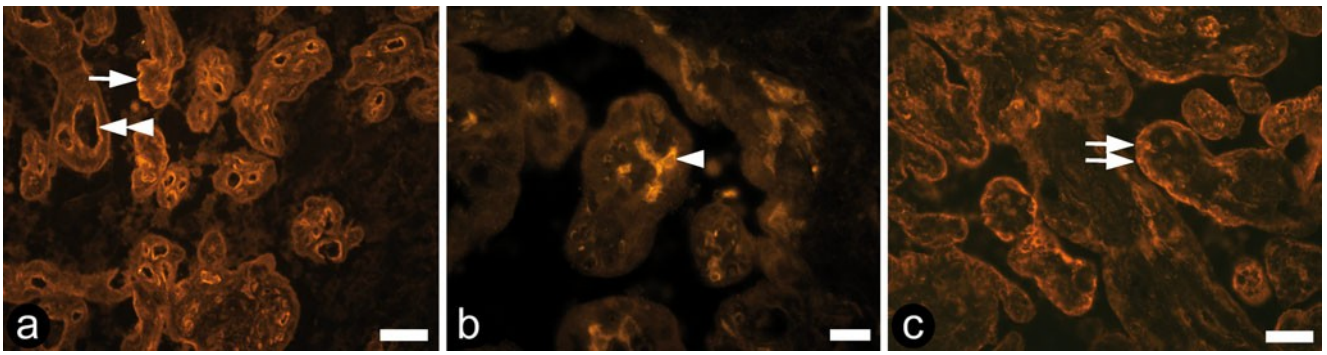


Fig. 8 Localization of PTPIP51 protein containing the N-terminus, sequence aas114-129 or the C-terminus in placenta. a The N-terminal-containing isoform of PTPIP51 is restricted to the syncytial knots (arrow) and endothelial cells lining the blood vessels (double arrow head). b The isoforms of PTPIP51 including the sequence aas114-129 are exclusively expressed in the cytotrophoblast (arrow). c The syncytiotrophoblast reacted positively for the C-terminal sequence of PTPIP51 (double arrow). Bars 20 μm

PTPIP51 plays a pivotal role in apoptosis. GPS 2.1 (http://gps.biocuckoo.org/) has shown PTPIP51 to be a substrate of c-Abl phoshorylating tyrosine residues 53 and 176. The close proximity of serine residue 46 to the tyrosine residue 53 suggests that both have a regulatory function. Recent cell culture experiments have revealed that tyrosine phosphorylation is critical for the regulation of 14-3-3 binding to PTPIP51. In HaCat cells, phosphatases have been blocked by exposing cells to pervanadate. As a consequence, tyrosine residues of PTPIP51 are hyperphosphorylated. Subsequent interaction quantification has revealed a significant decrease of PTPIP51 and 14-3-3beta interaction. Therefore, we conclude that tyrosine phosphorylation is an important regulatory mechanism for 14-3-3 binding to PTPIP51 (A. Brobeil, M. Bobrich, M. Wimmer, in preparation). The interaction of Bcr with 14-3-3 proteins is comparably regulated. The adaptor proteins 14-3-3 bind to specific phosphoserine motifs of Bcr. Phosphorylation of tyrosine residues on Bcr inhibit the binding of 14-3-3 proteins. In consequence, their ability to form a complex with Raf-1 is inhibited with respect to modulating Raf-1 activity (Peters and Smithgall 1999). PTPIP51 also modulates Raf-1 activity through 14-3-3 adaptor molecules (Yu et al. 2008). This mechanism is probably responsible for the c-Abl-induced dissociation of the 14-3-3 proteins from the CR1, rendering the TMD accessible and leading to protein translocation to the mitochondria and the induction of apoptosis. In addition, serine/threonine protein phosphatases (PPs) such as PP1, PP2A and PP2B enable BAD to interact with Bcl-X_L and initiate cell death (Klumpp and Krieglstein 2002). These protein phosphatases might also desphosphorylate PTPIP51 at its serine 46 residue leading to the dissociation of the 14-3-3 proteins with subsequent apoptosis. By regulating the serine phosphorylation by PKC, the PTPIP51/14-3-3 complex probably inhibits PKC activity, which is reflected in the alternative name of 14-3-3 proteins, namely kinase C inhibitor proteins. 14-3-3 proteins have a motif exhibiting similarity to the pseudo-substrate site on PKC and might inhibit kinase activity by blocking the active site (Robinson et al. 1994).

The important functions of PTPIP51 are reflected by its expression in many different tissues with diverse subcellular localizations, probably determined by its local function. Brobeil and co-workers (2010) have used peptide-specific antibodies to detect sequences of PTPIP51 comprising the C-terminus, N-terminus and an amino acid sequence spanning aa114 to 129. The subcellular distributions of PTPIP51 isoforms are given for the human placenta in Fig. 8. The human placenta exhibits an isoform-specific localization of PTPIP51 (Fig. 8a-c). The N-terminal containing PTPIP51 is restricted to a small subset of syncytiothrophoblast cells lining the syncytial knots (Fig. 8a, arrow). Endothelial cells also express PTPIP51 containing this peptide sequence (Fig. 8a, double arrowhead). All other cells remain negative for the N-terminal peptide sequence (Fig. 8a). Only the cytotrophoblast stains positively for the PTPIP51 including aas114-129 (Fig. 8b, arrowhead). All cells of the syncytiotrophoblast express PTPIP51 isoforms that include the C-terminal sequence (Fig. 8c, double arrow).

As PTPIP51 takes part in many cellular processes, it is present in almost every cell compartment. The leaky scanning mechanism provides an effective tool to generate multiple isoforms delivered to different compartments (Kochetov 2008). For example, use of the second AUG triplet within the main ORF results in the lack of the N-terminal-located mitochondrial target sequence and, therefore, the protein cannot be translocated to the mitochondria.

Concluding remarks

PTPIP51 is a multifunctional protein with an important impact on major cellular functions such as proliferation, differentiation and apoptosis, thereby being a real functional jack–of-all-trades protein. The strict regulation of the PTPIP51 gene and its mRNA indicates that PTPIP51 is probably a potent oncogene, the dysregulation of which might result in the onset of cancer. Further studies are needed to reveal the detailed functions of PTPIP51 in the integration of distinct signalling networks.

Acknowledgements We thank the native speaker Mr. Phillip Grant (Department of Differential Psychology and Personality Research, Justus Liebig University, Gießen) for excellent linguistic revision.

References

Azadi S, Johnson LE, Paquet-Durand F, Perez MT, Zhang Y, Ekström PA, Van Veen T (2007) CNTF + BDNF treatment and neuroprotective pathways in the rd1 mouse retina. Brain Res 1129:116–129

Barop J, Sauer H, Steger K, Wimmer M (2009) Differentiation-dependent PTPIP51 expression in human skeletal muscle cell culture. J Histochem Cytochem 57:425–435

Barreda DR, Hanington PC, Belosevic M (2004) Regulation of myeloid development and function by colony stimulating factors. Dev Comp Immunol 28:509–554

Blatch GL, Lässle M (1999) The tetratricopeptide repeat: a structural motif mediating protein-protein interactions. Bioessays 21:932–939

Bobrich M, Brobeil A, Mooren FC, Krüger K, Steger K, Wimmer M (2011) PTPIP51 interaction with PTP1B and 14-3-3beta in adipose tissue of insulin resistant mice. Int J Obes (Lond) [Epub ahead of print]

Bonizzoni P, Rizzi R, Pesole G (2005) ASPIC: a novel method to predict the exon-intron structure of a gene that is optimally compatible to a set of transcript sequences. BMC Bioinform 6:244

Brobeil A, Graf M, Oeschger S, Steger K, Wimmer M (2010) PTPIP51-a myeloid lineage specific protein interacts with PTP1B in neutrophil granulocytes. Blood Cells Mol Dis 45:159–168

Calarco JA, Superina S, O'Hanlon D, Gabut M, Raj B, Pan Q, Skalska U, Clarke L, Gelinas D, Van der Kooy D, Zhen M, Ciruna B, Blencowe BJ (2009) Regulation of vertebrate nervous system alternative splicing and development by an SR-related protein. Cell 138:898–910

Cantin GT, Yi W, Lu B, Park SK, Xu T, Lee JD, Yates JR 3rd (2008) Combining protein-based IMAC, peptide-based IMAC, and MudPIT for efficient phosphoproteomic analysis. J Proteome Res 7:1346–1351

Daub H, Olsen JV, Bairlein M, Gnad F, Oppermann FS, Körner R, Greff Z, Kéri G, Stemmann O, Mann M (2008) Kinase-selective enrichment enables quantitative phosphoproteomics of the kinome across the cell cycle. Mol Cell 31:438–448

Davuluri RV, Suzuki Y, Sugano S, Plass C, Huang TH (2008) The functional consequences of alternative promoter use in mammalian genomes. Trends Genet 24:167–177

De Almeida LP, Zala D, Aebischer P, Déglon N (2001) Neuroprotective effect of a CNTF-expressing lentiviral vector in the quinolinic acid rat model of Huntington's disease. Neurobiol Dis 8:433–446

Delgehyr N, Sillibourne J, Bornens M (2005) Microtubule nucleation and anchoring at the centrosome are independent processes linked by ninein function. J Cell Sci 118:1565–1575

DeLuca JG, Moree B, Hickey JM, Kilmartin JV, Salmon ED (2002) hNuf2 inhibition blocks stable kinetochore-microtubule attachment and induces mitotic cell death in HeLa cells. J Cell Biol 159:549–555

Dephoure N, Zhou C, Villén J, Beausoleil SA, Bakalarski CE, Elledge SJ, Gygi SP (2008) A quantitative atlas of mitotic phosphorylation. Proc Natl Acad Sci USA 105:10762–10767

De Virgilio C, Pousis C, Bruno S, Gadaleta G (2011) New isoforms of human mitochondrial transcription factor A detected in normal and tumoral cells. Mitochondrion 11:287–295

Ellis L, Atadja PW, Johnstone RW (2009) Epigenetics in cancer: targeting chromatin modifications. Mol Cancer Ther 8:1409–1420

Fotheringham AP, Davidson YS, Davies I, Morris JA (1991) Age-associated changes in neuroaxonal transport in the hypothalamo-neurohypophysial system of the mouse. Mech Ageing Dev 60:113–121

Gan HK, Kaye AH, Luwor RB (2009) The EGFRvIII variant in glioblastoma multiforme. J Clin Neurosci 16:748–754

Gillingham AK, Munro S (2003) Long coiled-coil proteins and membrane traffic. Biochim Biophys Acta 1641:71–85

Goebl M, Yanagida M (1991) The TPR snap helix: a novel protein repeat motif from mitosis to transcription. Trends Biochem Sci 16:173–177

Heinonen KM, Tremblay ML (2006) Protein tyrosine phosphatase 1B in hematopoiesis. Cell Cycle 5:1053–1056

Howng SL, Hsu HC, Cheng TS, Lee YL, Chang LK, Lu PJ, Hong YR (2004) A novel ninein-interaction protein, CGI-99, blocks ninein phosphorylation by GSK3beta and is highly expressed in brain tumors. FEBS Lett 566:162–168

Jin J, Smith FD, Stark C, Wells CD, Fawcett JP, Kulkarni S, Metalnikov P, O'Donnell P, Taylor P, Taylor L, Zougman A, Woodgett JR, Langeberg LK, Scott JD, Pawson T (2004) Proteomic, functional, and domain-based analysis of in vivo 14-3-3 binding proteins involved in cytoskeletal regulation and cellular organization. Curr Biol 14:1436–1450

Klumpp S, Krieglstein J (2002) Serine/threonine protein phosphatases in apoptosis. Curr Opin Pharmacol 2:458–462

Koch P, Stenzinger A, Viard M, Märker D, Mayser P, Nilles M, Schreiner D, Steger K, Wimmer M (2008) The novel protein PTPIP51 is expressed in human keratinocyte carcinomas and their surrounding stroma. J Cell Mol Med 12:2083–2095

Koch P, Petri M, Paradowska A, Stenzinger A, Sturm K, Steger K, Wimmer M (2009a) PTPIP51 mRNA and protein expression in tissue microarrays and promoter methylation of benign prostate hyperplasia and prostate carcinoma. Prostate 69:1751–1762

Koch P, Viard M, Stenzinger A, Brobeil A, Tag C, Steger K, Wimmer M (2009b) Expression profile of PTPIP51 in mouse brain. J Comp Neurol 517:892–905

Kochetov AV (2008) Alternative translation start sites and hidden coding potential of eukaryotic mRNAs. Bioessays 30:683–691

Kozak M (2005) Regulation of translation via mRNA structure in prokaryotes and eukaryotes. Gene 361:13–37

Lessard L, Stuible M, Tremblay ML (2010) The two faces of PTP1B in cancer. Biochim Biophys Acta 1804:613–619

Linden R, Martins RA, Silveira MS (2005) Control of programmed cell death by neurotransmitters and neuropeptides in the developing mammalian retina. Prog Retin Eye Res 24:457–491

Lv BF, Yu CF, Chen YY, Lu Y, Guo JH, Song QS, Ma DL, Shi TP, Wang L (2006) Protein tyrosine phosphatase interacting protein 51 (PTPIP51) is a novel mitochondria protein with an N-terminal mitochondrial targeting sequence and induces apoptosis. Apoptosis 11:1489–1501

Ly JD, Grubb DR, Lawen A (2003) The mitochondrial membrane potential (deltapsi(m)) in apoptosis; an update. Apoptosis 8:115–128

Lynch KW (2007) Regulation of alternative splicing by signal transduction pathways. Adv Exp Med Biol 623:161–174

Maerker D, Stenzinger A, Schreiner D, Tag C, Wimmer M (2008) Expression of PTPIP51 during mouse eye development. Histochem Cell Biol 129:345–356

McCubrey JA, May WS, Duronio V, Mufson A (2000) Serine/threonine phosphorylation in cytokine signal transduction. Leukemia 14:9–21

Miranda MB, Johnson DE (2007) Signal transduction pathways that contribute to myeloid differentiation. Leukemia 21:1363–1377

Morin PJ (1999) Beta-catenin signaling and cancer. Bioessays 21:1021–1030

Nilsen TW, Graveley BR (2010) Expansion of the eukaryotic proteome by alternative splicing. Nature 463:457–463

Oishi K, Okano H, Sawa H (2007) RMD-1, a novel microtubule-associated protein, functions in chromosome segregation in Caenorhabditis elegans. J Cell Biol 179:1149–1162 [Erratum in: J Cell Biol (2009) 186:629]

Onuma H, Osawa H, Yamada K, Ogura T, Tanabe F, Granner DK, Makino H (2002) Identification of the insulin-regulated interaction of phosphodiesterase 3B with 14-3-3 beta protein. Diabetes 51:3362–3367

Peters KL, Smithgall TE (1999) Tyrosine phosphorylation enhances the SH2 domain-binding activity of Bcr and inhibits Bcr interaction with 14-3-3 proteins. Cell Signal 11:507–514

Qi X, Mochly-Rosen D (2008) The PKCdelta-Abl complex communicates ER stress to the mitochondria—an essential step in subsequent apoptosis. J Cell Sci 121:804–813

Rieder CL, Faruki S, Khodjakov A (2001) The centrosome in vertebrates: more than a microtubule-organizing center. Trends Cell Biol 11:413–419

Robinson K, Jones D, Patel Y, Martin H, Madrazo J, Martin S, Howell S, Elmore M, Finnen MJ, Aitken A (1994) Mechanism of inhibition of protein kinase C by 14-3-3 isoforms. 14-3-3 isoforms do not have phospholipase A2 activity. Biochem J 299:853–861

Roger J, Goureau O, Sahel JA, Guillonneau X (2007) Use of suppression subtractive hybridization to identify genes regulated by ciliary neurotrophic factor in postnatal retinal explants. Mol Vis 13:206–219

Rose A, Meier I (2004) Scaffolds, levers, rods and springs: diverse cellular functions of long coiled-coil proteins. Cell Mol Life Sci 61:1996–2009

Roy A, Kucukural A, Zhang Y (2010) I-TASSER: a unified platform for automated protein structure and function prediction. Nat Protoc 5:725–738

Russell JA, Leng G (2000) Veni, vidi, vici: the neurohypophysis in the twentieth century. Exp Physiol 85:1–6

Sánchez L (2008) Sex-determining mechanisms in insects. Int J Dev Biol 52:837–856

Saraste A, Pulkki K (2000) Morphologic and biochemical hallmarks of apoptosis. Cardiovasc Res 45:528–537

Shames DS, Minna JD, Gazdar AF (2007) DNA methylation in health, disease, and cancer. Curr Mol Med 7:85–102

Shaul Y, Ben-Yehoyada M (2005) Role of c-Abl in the DNA damage stress response. Cell Res 15:33–35

Shaywitz AJ, Greenberg ME (1999) CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. Annu Rev Biochem 68:821–861

Sikorski RS, Boguski MS, Goebl M, Hieter P (1990) A repeating amino acid motif in CDC23 defines a family of proteins and a new relationship among genes required for mitosis and RNA synthesis. Cell 60:307–317

Sirvent A, Benistant C, Roche S (2008) Cytoplasmic signalling by the c-Abl tyrosine kinase in normal and cancer cells. Biol Cell 100:617–631

Stenzinger A, Kajosch T, Tag C, Porsche A, Welte I, Hofer HW, Steger K, Wimmer M (2005) The novel protein PTPIP51 exhibits tissue- and cell-specific expression. Histochem Cell Biol 123:19–28

Stenzinger A, Schreiner D, Pfeiffer T, Tag C, Hofer HW, Wimmer M (2006) Epidermal growth factor-, transforming growth factor-beta-, retinoic acid- and 1, 25-dihydroxyvitamin D3-regulated expression of the novel protein PTPIP51 in keratinocytes. Cells Tissues Organs 184:76–87

Stenzinger A, Schreiner D, Tag C, Wimmer M (2007) Expression of the novel protein PTPIP51 in rat liver: an immunohistochemical study. Histochem Cell Biol 128:77–84

Stenzinger A, Märker D, Koch P, Hoffmann J, Baal N, Steger K, Wimmer M (2009a) Protein tyrosine phosphatase interacting protein 51 (PTPIP51) mRNA expression and localization and its in vitro interacting partner protein tyrosine phosphatase 1B (PTP1B) in human placenta of the first, second, and third trimester. J Histochem Cytochem 57:143–153

Stenzinger A, Schreiner D, Koch P, Hofer HW, Wimmer M (2009b) Cell and molecular biology of the novel protein tyrosine-phosphatase-interacting protein 51. Int Rev Cell Mol Biol 275:183–246

Tan Y, Demeter MR, Ruan H, Comb MJ (2000) BAD Ser-155 phosphorylation regulates BAD/Bcl-XL interaction and cell survival. J Biol Chem 275:25865–25869

Xiang X, Yuan M, Song Y, Ruderman N, Wen R, Luo Z (2002) 14-3-3 facilitates insulin-stimulated intracellular trafficking of insulin receptor substrate 1. Mol Endocrinol 3:552–562

Xue Y, Ren J, Gao X, Jin C, Wen L, Yao X (2008) GPS 2.0, a tool to predict kinase-specific phosphorylation sites in hierarchy. Mol Cell Proteomics 7:1598–1608

Yoshida K (2007a) Regulation for nuclear targeting of the Abl tyrosine kinase in response to DNA damage. Adv Exp Med Biol 604:155–165

Yoshida K (2007b) PKCdelta signaling: mechanisms of DNA damage response and apoptosis. Cell Signal 19:892–901

Yu C, Han W, Shi T, Lv B, He Q, Zhang Y, Li T, Zhang Y, Song Q, Wang L, Ma D (2008) PTPIP51, a novel 14-3-3 binding protein, regulates cell morphology and motility via Raf-ERK pathway. Cell Signal 20:2208–2220

Zhang X, Odom DT, Koo SH, Conkright MD, Canettieri G, Best J, Chen H, Jenner R, Herbolsheimer E, Jacobsen E, Kadam S, Ecker JR, Emerson B, Hogenesch JB, Unterman T, Young RA, Montminy M (2005) Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. Proc Natl Acad Sci USA 102:4459–4464

PTPIP51 – a new interaction partner of the insulin receptor and PKA in adipose tissue

Bobrich M[1], Schwabe SA[1], Brobeil A[1], Viard M[2], Kamm M[1], Mooren FC[3], Krüger K[3], Tag C[1], Wimmer M[1]

[1]Institute of Anatomy and Cell Biology, Justus Liebig University, Giessen, Germany

[2]Department of Neurology, Justus Liebig University Giessen, Germany

[3]Department of Sports Medicine, Justus Liebig University, Giessen, Germany

Corresponding author:

Manuel Bobrich

Institute of Anatomy and Cell Biology

Justus-Liebig-University Giessen

Aulweg 123

35392 Giessen

Germany

Tel.: +49.(0)641.99.47012

Fax: +49.(0)641.99.47009

Page heading: PTPIP51 interaction in adipose tissue

Abstract: 225
Manuscript: 2326
References: 25
Tables: 2
Figures: 5

There is no conflict of interest to declare.

Abstract

Aims: Our previous experiments revealed an association of PTPIP51 (Protein Tyrosine Phosphatase interacting protein 51) with the insulin signalling pathway through PTP1B and 14-3-3beta. We aimed to clarify the role of PTPIP51 in adipocyte metabolism.

Methods: Four groups of ten black six mice each were used. Two groups were fed a standard diet; two groups were fed a high fat diet. After four weeks of adaptation, two groups (one high fat diet and one standard diet) were submitted to endurance training, while the remaining two groups served as untrained control groups. After ten weeks, we measured the insulin resistance of every mouse using a glucose tolerance test. Adipose tissue samples were analyzed by immunofluorescence and Duolink proximity ligation assay to verify and quantify interactions of PTPIP51 with either insulin receptor (IR) or cAMP activated protein kinase A (PKA).

Results: PTPIP51 and the IR respectively PKA were colocalized in all groups. Standard diet animals that were submitted to endurance training showed low PTPIP51-IR- and PTPIP51-PKA-interaction. The interaction levels of both the IR and PKA were associated to the grade of insulin sensitivity, feeding and training status of the animals.

Conclusion: PTPIP51 might serve as a linking protein in adipocyte metabolism by connecting the IR-triggered lipogenesis with the PKA-dependent lipolysis. PTPIP51 interacts with both proteins, therefore being a potential gateway for the cooperation of both pathways.

Keywords

Diabetes, Obesity, insulin resistance, adipocyte

Abbreviations

cAMP activated Protein Kinase A (PKA); Duolink Proximity Ligation Assay (DPLA); High fat diet (HFD); High fat diet and training (HFDT); Hormone sensitive lipase (HSL); Insulin receptor (IR); Mitogen Activated Protein Kinase (MAPK); Phosphodiesterase-3B (PDE-3B); Protein Tyrosine Phosphatase Interacting Protein 51 (PTPIP51); Standard diet (SD); Standard diet and training (SDT).

Introduction

Obesity abets several life style diseases such as diabetes type II, atherosclerosis or impaired wound healing. These adverse effects can be avoided or, if already present, reduced by physical activity. Physical activity increases sympathetic activity and blood catecholamine levels. Adaption to periodic physical exercise is well-studied. Exercise training and increased catecholamine levels affect adipocyte metabolism, on the one hand by influencing insulin secretion [1] and on the other hand by additionally channelling the adipocyte metabolism towards lipolysis [2]. This effect is mediated by beta2-adrenergic receptors which activate the intracellular adenylyl-cyclase. Adenylyl-cyclase transforms ATP into cyclic AMP (cAMP), which in turn increases the activity level of cAMP dependent protein kinase A (PKA) [3, 4]. Protein kinase A thereupon initiates lipolysis [4].

The most potent antagonist of catecholamines on adipose tissue is insulin. Activation of the insulin receptor results in decreased lipolysis, increased lipogenesis and uptake of fatty acids.

Lipolysis is decreased by insulin receptor induced phosphodiesterase-3B (PDE-3B) and 14-3-3beta dependent reduction of PKA activity [3, 5]. Furthermore, the insulin receptor activates several lipogenic enzymes by tyrosine phosphorylation and promotes GLUT-4 recruitment to the cell membrane [6].

Protein Tyrosine Phosphatase Interacting Protein 51 (PTPIP51) is expressed in different organs with a multitude of functions [7, 8]. As previously shown for mouse adipose tissue, PTPIP51 acts as an effector in insulin signalling. PTPIP51 expression was correlated to the insulin resistance of the animals [9], according to the expression of its interaction partner Protein Tyrosine Phosphatase 1B (PTP1B). In addition to that, PTPIP51 is connected to the lipolytic MAPK pathway by its

interaction with raf-1 mediated by 14-3-3beta [7]. 14-3-3beta also interacts with phosphodiesterase 3B, resulting in reduced intracellular cAMP levels and decreased PKA activity [9]. As 14-3-3beta and PTP1B are important for adipocyte metabolism control, the role of its interaction partner PTPIP51 needs to be disclosed.

The interaction of PTPIP51 with the insulin receptor and PKA was investigated in adipose tissue from animals kept under different defined experimental conditions to clarify the role of PTPIP51 in adipocyte metabolism.

Material and Methods

Study design

The experiments were performed with male Bl6 mice (n=40) and approved by the local Animal Care and Use Committee (Gi 20/24 Nr.94/2010). The animals were kept under standard conditions (12 hr light / dark cycle) with no access to running wheels. They were fed ad libitum with free access to water. The animals were aged ten weeks at the beginning of the training. The experiments were run for 14 weeks, with a four week period of fat feeding followed by a ten week training period.

Control group (SD): The animals were fed a standard diet (Altromin standard-diet #1324, Altromin, Lage, Germany).

Endurance training control group (SDT): The animals were fed a standard diet (Altromin standard-diet #1324, Altromin, Lage, Germany) and trained for a period of ten weeks. The endurance training was performed on a motorized treadmill for 35 min/day at 12 m/min, at 12 % grade, 5 times per week, for ten weeks. Running speed was 0.27 m/s ± 0.05 m/s corresponding to 80 % of maximal oxygen consumption.

High-fat-diet group (HFD): The animals were fed a high-fat-diet containing 45% fat for 14 weeks of the experimental period.

High-fat-diet, training group (HFDT): The animals were fed a high-fat-diet during the complete experimental time period. The endurance training period started four weeks after the beginning of high fat diet feeding. The endurance training was performed on a motorized treadmill for 35 min/day at 12 m/min, at 12 % grade, five times per week, for ten weeks. Running speed was 0.27 m/s ± 0.05 m/s corresponding to 80 % of maximal oxygen consumption.

For diet composition see table 1, for mean body weights see table 2.

Values in kcal/100g	Standard diet	High fat diet
Protein	24	20
Carbohydrates	65	35
Fat	11	45

Table 1: Energy content of standard diet and high fat diet.

	Basic value (10 weeks of age)	Beginning of training (14 weeks of age)	End of test period (24 weeks of age)
Normal diet (ND)	23.22+- 2.41	23.16 ± 1.03	27.74 ± 0.96
Normal diet and training (NDT)	22.81+- 3.11	22.79 ± 0.77	26.54 ± 1.15
High fat diet (HFD)	23.67+- 3.02	40.37 ± 2.97	50.91 ± 2.56
High fat diet and training (HFDT)	23.44+-2.88	42.22 ± 3.18	49.23 ± 3.43

Table 2: Mean body weight in grams with standard deviation.

After 14 weeks the animals were sacrificed in the morning between 10 and 12 o'clock and the abdominal adipose tissue was frozen in liquid nitrogen precooled isopentan and transferred to -80°C till further analysis. See also Bobrich et al. [9]. A glucose tolerance test was performed to determine the Insulin sensitivity of the mice [9, 10, 11]. For testing the glucose tolerance of the animals they were injected intraperitoneally with 2g of 20% D-Glucose glucose dissolved in sterile 0.9% NaCl solution per kg body weight. The blood was collected and glucose concentration was

determined. The test was performed in the morning; last feeding of the animals took place the previous day.

PTPIP51 Antibody production

An antibody against a defined peptide sequence at the C-terminus of PTPIP51 (sequence: IQKDLEELEVILRD, exon 13) was produced (BioLux, Stuttgart, Germany). Identity and purity of the synthesized peptide were approved by ESI-MS and UV-analysis. Rabbits were immunized with the KLH-conjugated peptide. The specificity of the antibody was tested by ELISA and Western blot. Preabsorption experiments were performed [9, 12].

Immunofluorescence

Immunofluorescence staining was performed according to a standard protocol [9]. The primary PTPIP51 antibody to the C-Terminus was used in 1:800 dilutions and visualized by Alexa 555 (Molecular Probes, Darmstadt, Germany, Cat.-# A21428). Primary monoclonal mouse antibodies were used for double staining experiments with PKA (ab58187, Abcam plc, 330 Cambridge Science Park, Cambridge, CB4 0FL) and the beta-subunit of the IR (clone CT-3, Millipore, 28820 Single Oak Drive, Temecula, CA 92590). To avoid unspecific binding of the primary mouse antibodies, samples were preincubated with biotin-coupled anti-mouse-antibodies for one hour. The reaction of the primary monoclonal mouse antibody was visualized using Alexa fluor 488 secondary antibodies (Molecular Probes, Darmstadt, Germany, Cat.-# A11001).

Confocal laser scanning microscopy

198 Confocal images of cells were obtained with a Leica confocal laser scanning
199 microscope (CLSM, 5 TCS SP2, Leica, Bensheim, Germany). Confocal images of
200 Alexa Fluor 555 fluorescence were acquired using 6 Plan-Apochromat ×40/1.4 oil
201 objective, 548 nm excitation wavelengths (helium-neon laser) and a 560–585 nm
202 band-pass filter. The pinhole diameter was set to yield optical sections of 1 Airy unit.
203 For the detection of Alexa Fluor 488, we used a Plan-Apochromat ×40/1.4 oil
204 objective, the 488 nm excitation wavelength of an argon laser, and a 505–530 nm
205 band-pass filter. The pinhole diameter was set to yield optical sections of 1 Airy unit.
206 Confocal images of To-Pro-3 fluorescence (Molecular probes, Cat.# T3605) (nuclear
207 staining) were acquired using Plan-Apochromat ×40/1.4 oil objective, 633 nm
208 excitation wavelengths (helium-neon laser), the 650–670 nm bandpass filter. The
209 pinhole diameter was set to yield optical sections of 1 Airy unit. Acquired DIC and
210 confocal images were analyzed and combined using the LCS software (Leica
211 Confocal Software).
212 Acquired images were subsequently processed by ImageJ (v1.43m; Rasband, W.S.,
213 ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA,
214 http://imagej.nih.gov/ij/, 1997-2011) using an iterative deconvolution plug-in by Bob
215 Dougherty (http://www.optinav.com/imagej.html, Iterative Deconvolution). Options
216 were set for all confocal acquired images as follows: 8 numbers of iteration and 2.0
217 pixels of LP filter diameter. Point spread function was calculated for each channel
218 separately by the ImageJ plug-in created by Bob Dougherty
219 (http://www.optinav.com/imagej.html, Diffraction Limit PSF).

220

221 Intensity correlation analysis

Intensity correlation analysis (ICA) was carried out using ImageJ (v1.43m; Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2011) and an appropriate plug-in for ICA included in the plug-in package of the Wright cell imaging facility [13] (http://www.uhnres.utoronto.ca/facilities/wcif/fdownload.html).

Duolink proximity ligation assay (DPLA)

Interaction of PTPIP51 with either PKA or beta-subunit of the IR was detected by the proximity ligation assay kit DuoLink (Olink biosciences, Uppsala, Sweden, PLA probe anti-rabbit minus for the detection of the rabbit PTPIP51 antibody, Cat.# 90602; PLA probe anti-mouse plus for the detection of the mouse anti PKA or IR antibody, Cat.# 90701; Detection Kit 563, Cat.# 90134).

The DPLA was performed according to the manufacturer's protocol; negative controls were included [9, 14, 15]. For principle of the DPLA see figure 1.

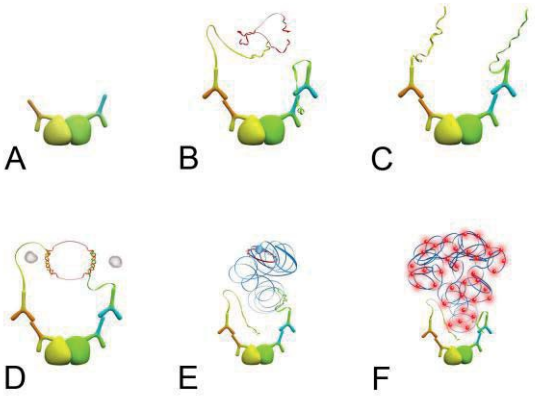


Figure 1

Fluorescence microscopy

The Axioplan 2 fluorescence microscope equipped with Plan-Apochromat objectives (Carl Zeiss Jena, Germany) was used for photo documentation. For visualization of

241 the DPLA an excitation filter with a spectrum of 530-560nm and an emission filter
242 with a spectrum of 572-647nm were used.
243
244 Analysis of Duolink proximity ligation assay results
245 The Duolink Image Tool (Olink biosciences, Uppsala, Sweden) was used for
246 quantification of the detected DPLA signals. All analyzed sections were investigated
247 with equal light intensity and shutter speed. Sensitivity of the software scan and blob
248 threshold were set identically for all probes. The positive reaction dots were counted
249 automatically.
250
251 Database research
252 We accomplished a database research using the GPS 2.1 database [16] to identify
253 kinases within adipocyte metabolism that possess the ability to phosphorylate
254 PTPIP51.
255
256
257
258
259
260
261
262

Results

Glucose tolerance test

The glucose tolerance was determined by a standard glucose tolerance test at the end of the experimental period. SD and SDT animals displayed a slight increase of blood glucose levels 30 minutes after application of glucose which was almost normalized after 60 minutes.

In HFD animals, blood glucose levels strongly increased from the basal level of 1mg/dl to 4 mg/dl 60 minutes after application. Blood glucose levels did not return to normal values within 120 minutes.

In HFDT animals, blood glucose levels increased to 2.7 mg/dl 30 minutes after application and almost returned to normal values after 120 minutes (Fig. 2).

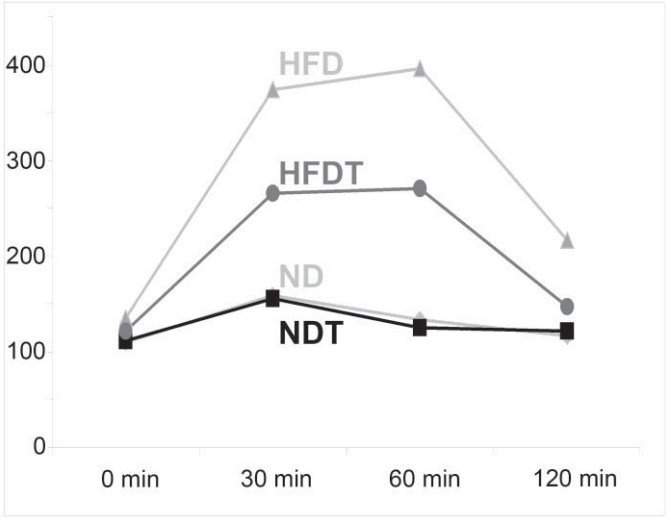


Figure 2

PTPIP51 protein is colocalized with the insulin receptor (IR) and protein kinase A (PKA)

PTPIP51 localization in adipose tissue did not differ between the four experimental set ups. PTPIP51 immunoreactivity was detected within the cytoplasm and at the plasma membrane (Fig. 3).

The IR revealed a similar expression pattern when compared to PTPIP51. In the cytoplasm, the immune reaction of the IR antibody was lower compared to PTPIP51. The computed data of the intensity correlation analysis is displayed in figure 3B. Co-localization is displayed in yellow to orange and non-co-localized parts are shown in blue. Most colocalization was detected at the plasma membrane.

PKA is also co-localized with PTPIP51, especially concerning its expression in the cytoplasm. The computed data of the intensity correlation analysis is displayed in figure 3D. Co-localization is displayed in yellow to orange and non-co-localized parts are shown in blue. Most colocalization was detected within the cytoplasm.

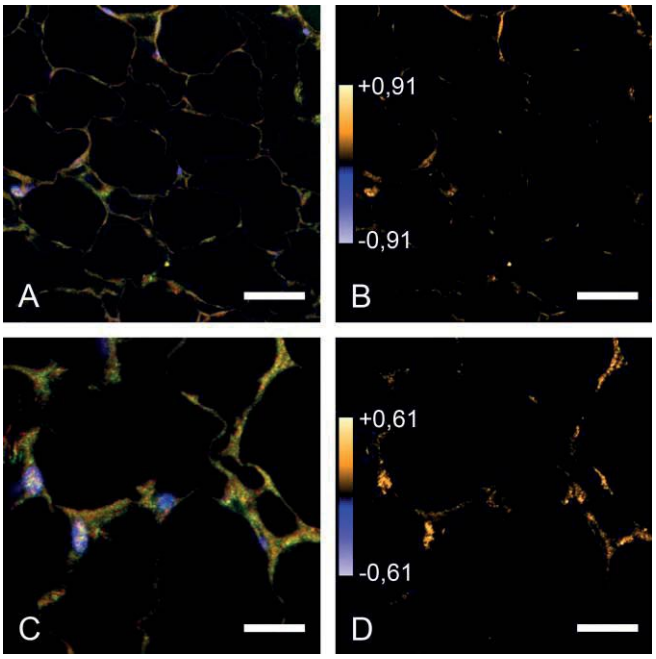


Figure 3

PTPIP51 and IR interact and the interaction depends on the feeding status

The Duolink proximity ligation assay (DPLA) was performed to detect interactions between PTPIP51 and the beta-subunit of the IR. The interaction of PTPIP51 and IR was verified as seen in figure 4, where each dot indicates the interaction of both proteins. This interaction of PTPIP51 and the IR was found in adipocytes of animals from all experimental groups. Specifity of the DPLA was tested (Fig. 4).

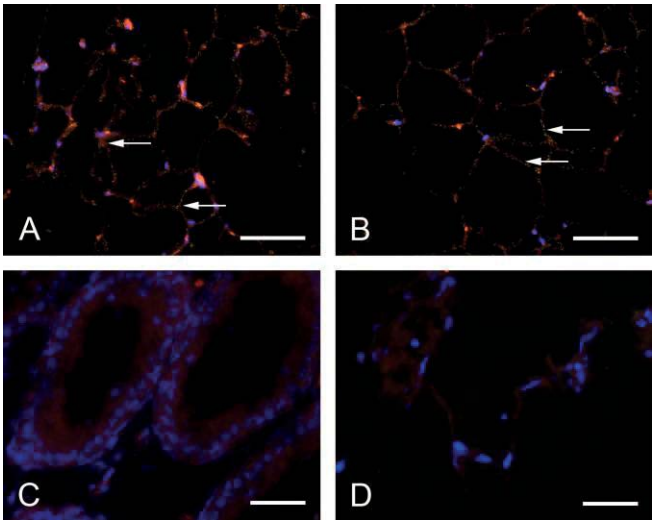


Figure 4

The grade of interaction in adipose samples from SD animals corresponds to the level in HFDT animals. A decrease in the interaction was detected in samples from SDT animals. HFD animals displayed the highest interaction level (Fig. 5A).

Interaction between PTPIP51 and PKA displayed a dependency on training status

The Duolink proximity ligation assay was performed to detect interactions between PTPIP51 and PKA. The assumption of an interaction of PTPIP51 and PKA based on the database research was verified (Fig. 4). PTPIP51 and PKA were interacting in adipocytes of animals from all experimental groups.

The Interaction levels of SD, HFD and HFDT mice did not show significant differences. The interaction levels seen in SDT mice were decreased (Fig. 5B).

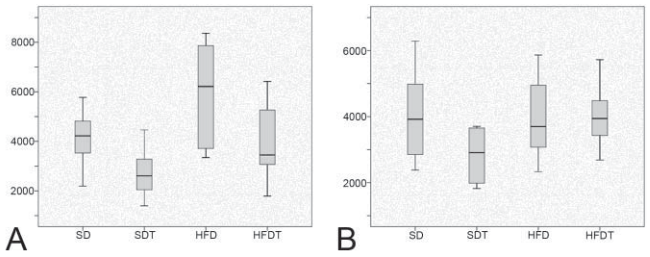


Figure 5

Discussion

To sum up the results, adipocytes from mice subjected to different diets and activity levels displayed a connection between PTPIP51 and the insulin receptor respectively PKA. Animals of the four experimental groups displayed differences in their glucose resistance. The glucose tolerance test revealed a glucose intolerant status in HFD and HFDT animals, whereas the glucose tolerance of HFDT animals improved compared to HFD animals.

Immunofluorescence data displayed colocalization of PTPIP51 with IR and PKA suggesting an interaction between PTPIP51 and IR respectively PKA. Basing on these facts, we performed a GPS 2.1 database research. Search results displayed PTPIP51 to be phosphorylated at tyrosine 176 by the insulin receptor and at serine 46 by PKA [16]. These two phosphorylation sites are possible regulatory sites for the interaction of PTPIP51 with 14-3-3beta, thus being able to influence the MAPK pathway by interaction with raf-1 [17]. Phosphorylation of Serine 46 in PTPIP51 results in augmented interaction with raf-1 via 14-3-3 and thus in increased ERK1/2 phosphorylation, promoting the MAPK pathway [12, 17]. The MAPK pathway is suspected to possess two opposite functions in adipocytes. Depending on the activator, ERK1/2 conveys either adipocyte growth or lipolysis [18]. PKA is known to induce lipolysis through the MAPK pathway [18, 19].

The insulin receptor induces tyrosine phosphorylation of PTPIP51 at tyrosine 176, which leads to reduced interaction of PTPIP51 with 14-3-3beta and raf-1 [20], thereby decreasing the MAPK pathway activity.

Analysis of the PTPIP51 interaction profile of adipocytes displayed high interaction levels with the insulin receptor in HFD animals. Interaction levels were reduced by 30% in SD and HFDT and by 50% in SDT animals, compared to HFD animals.

High fat feeding increases the lipogenic activity in adipose tissue [21, 22]. This is consistent with our observation of high interaction levels in HFD animals. Training leads to reduced adipocyte size [23]. The reduced PTPIP51-IR interaction in trained groups compared to the corresponding untrained groups suggests that PTPIP51 is especially involved in processes of insulin induced lipogenesis or in its antilipolytic action.

Interaction levels between PTPIP51 and PKA did not show significant differences in SD, HFD and HFDT animals. In NDT animals, interaction was reduced.

The low interaction in NDT animals seems to be controversial. But, as PKA is known to also interact with hormone-sensitive lipase (HSL), a change of the interaction partner of PKA away from PTPIP51 under these preconditions is assumable. The interaction between PKA and HSL is a potent promoter for lipolysis in adipocytes. The activity of both PKA and HSL are increased in endurance trained animals [24, 25].

Our experiments show that PTPIP51 could mediate between lipogenesis and lipolysis by switching between the lipogenic insulin pathway and the lipolytic PKA pathway. Further experiments need to be conducted in order to determine the magnitude in which PTPIP51 engages in adipocyte metabolism.

Acknowledgements

We are grateful to Ms Silvia Tietz (Department of Neurology, Justus-Liebig-University Giessen, Germany) for her excellent technical assistance.

Disclosure statement

There is no conflict of interest.

Author contribution

Stefanie Schwabe, Alexander Brobeil, Maxime Viard, Max Kamm and Claudia Tag performed experiments and revised the manuscript.

Prof. Mooren, Dr. Krüger and Prof. Wimmer revised the manuscript and designed the study.

Manuel Bobrich designed the study, performed experiments, wrote and revised the manuscript.

Dedication

This publication is dedicated to Mr. Hans Werner Hofer who promoted the research on PTPIP51 with all his energy. He died much too early († 18.05.2011).

References

1. Teff KL. Visceral nerves: vagal and sympathetic innervation. JPEN J Parentel Enteral Nutr 2008;32(5):569-71.

2. Leskinen T, Rinnankoski-Tuikka R, Rintala M et al. Differences in muscle and adipose tissue gene expression and cardio-metabolic risk factors in the members of physical activity discordant twin pairs. PLoS One 2010;5(9):e12609.

3. Lafontan M. Advances in adipose tissue metabolism. Int J Obes (Lond) 2008;32:S39-51.

4. Mantovani G, Bondioni S, Alberti L et al. Protein kinase A regulatory subunits in human adipose tissue: decreased R2B expression and activity in adipocytes from obese subjects. Diabetes 2009;58(3):620-6.

5. Aitken A. 14-3-3 proteins: a historic overview. Semin Cancer Biol 2006;16(3):162-72.

6. Kersten S. Mechanisms of nutritional and hormonal regulation of lipogenesis. EMBO Rep 2001;2(4):282-6.

7. Koch P, Viard M, Stenzinger A et al. Expression profile of PTPIP51 in mouse brain. J Comp Neurol 2009;517(6):892–905.

410 8. Stenzinger A, Schreiner D, Koch P et al. Cell and molecular biology of the novel
411 protein tyrosine-phosphatase-interacting protein 51. Int Rev Cell Mol Biol
412 2009;275:183-246.
413
414 9. Bobrich M, Brobeil A, Mooren FC et al. PTPIP51 interaction with PTP1B and 14-3-
415 3beta in adipose tissue of Insulin-resistant mice. Int J Obes (Lond).
416 2011;doi:10.1038/ijo.2010.283
417
418 10. Fukuda S, Ohta T, Sakata S et al. Pharmacological profiles of a novel protein
419 tyrosine phosphatase 1B inhibitor, JTT-551. Diabetes Obes Metab 2010;12(4):299-
420 306.
421
422 11. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose
423 tolerance testing: comparison with the euglycemic Insulin clamp. Diabetes Care
424 1999;22(9):1462-70.
425
426 12. Brobeil A, Graf M, Oeschger S, et al. PTPIP51-a myeloid lineage specific protein
427 interacts with PTP1B in neutrophil granulocytes. Blood Cells Mol Dis 2010;45(2):159-
428 68.
429
430 13. Li Q, Lau A, Morris TJ et al. A syntaxin 1, Galpha(o), and N-4 type calcium
431 channel complex at a presynaptic nerve terminal: analysis by quantitative 5
432 immunocolocalization. J Neurosci 2004;24:4070-81.
433
434 14. Gullberg M, Andersson AC. Visualization and quantification of protein-protein
435 interactions in cells and tissues. Nat Methods 2010;6:641–7.

15. Söderberg O, Gullberg M, Jarvius M. Direct observation of individual endogenous protein complexes in situ by proximity ligation. Nat Methods 2006;3:995–1000.

16. Yu X, Jian R, Xinjiao G et al. GPS 2.0, a tool to predict kinase-specific phosphorylation sites in hierarchy. Moll Cell Proteomics 2008;7:1598–609.

17. Yu C, Han W, Shi T et al. PTPIP51, a novel 14-3-3 binding protein, regulates cell morphology and motility via Raf-ERK pathway. Cell Signal 2008;20(12):2208-20

18. Gehart H, Kumpf S, Ittner A, et al. MAPK signalling in cellular metabolism: stress or wellness? EMBO Rep 2010;11(11):834-40.

19. Carmen GY, Víctor SM. Signalling mechanisms regulating lipolysis. Cell Signal 2006;18(4):401-8.

20. Brobeil A, Bobrich M, Wimmer M. Protein tyrosine phosphatase interacting protein 51--a jack-of-all-trades protein. Cell Tissue Res. 2011;344(2):189-205.

21. Berger JJ, Barnard RJ. Effect of diet on fat cell size and hormone-sensitive lipase activity. J Appl Physiol 1999;87(1):227-32

22. Kadowaki T, Hara K, Yamauchi T et al. Molecular mechanism of Insulin resistance and obesity. Exp Biol Med (Maywood) 2003;228(10):1111-7.

461 23. Peres SB, de Moraes SM, Costa CE et al. Endurance exercise training increases
462 Insulin responsiveness in isolated adipocytes through IRS/PI3-kinase/Akt pathway. J
463 Appl Physiol 2005;98(3):1037-43
464
465 24. Campbell JE, Fediuc S, Hawke TJ et al. Endurance exercise training increases
466 adipose tissue glucocorticoid exposure: adaptations that facilitate lipolysis.
467 Metabolism 2009;58(5):651-60.
468
469 25. Collins S, Cao W, Robidoux J. Learning new tricks from old dogs: beta-
470 adrenergic Receptors teach new lessons on firing up adipose tissue metabolism. Mol
471 Endocrinol 2004;18(9):2123-31.
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486

Figure legends

Fig 1: Principle of the Duolink Proximity Ligation Assay. A) Binding of primary antibodies to PTPIP51 and the IR, resp. PKA; B) Binding of secondary antibodies, PLA probe PLUS and PLA probe MINUS; C) Connector oligos only hibridize if both proteins are closer than 40nm; D) Ligation of the complex forms a circular template; E) Rolling circle amplification; F) Amplification yields a some hundred-fold replication, added fluorophore labelled probes highlight the reaction, resulting in a point-shaped signal.

Fig 2: Glucose tolerance test: Average of blood glucose concentrations in mg/dl after intraperitoneal application of glucose solution. Blood glucose levels were determined 0, 30, 60 and 120 minutes after application. Glucose tolerance is inversely related to Insulin resistance.
SD standard diet group (n=10), SDT standard diet and training group (n=10), HFD high fat diet group (n=10), HFDT high fat diet and training group (n=10).

Fig 3: Immunostaining of PTPIP51, insulin receptor and PKA in adipocytes of normal controls.
A) Immunostaining of PTPIP51 and the insulin receptor; B) Intensity correlation of PTPIP51 and the insulin receptor, high correlation is displayed in yellow; C) Immunostaining of PTPIP51 and PKA; D) Intensity correlation of PTPIP51 and the insulin receptor, high correlation is displayed in yellow; Bar (A): 60µm; Bar (C): 15µm

Fig 4: Duolink proximity ligation assay to detect interactions between PTPIP51 and the insulin receptor (IR) resp. PKA

A) Interaction between PTPIP51 and the IR; B) Interaction between PTPIP51 and PKA; C) Negative control, PTPIP51 and the IR in testis, no interaction known; D) Negative control, DPLA without primary antibodies in adipose tissue; Nuclei are marked by DAPI staining; Arrows: positive reactions; Bars (A, B): 50µm; Bars (C, D): 20µm

Fig 5: Semiquantitative analysis of the interaction between PTPIP51 and its interaction with IR and with PKA.

Every section was subdivided into rectangles of the same size and dots were counted. The results were averaged for each section.

A) Semiquantitative evaluation of PTPIP51 interaction with IR;

B) Semiquantitative evaluation of PTPIP51 interaction with PKA.

SD: standard diet group; SDT: standard diet and training group; HFD: high fat diet group; HFDT: high fat diet and training group.