***PTEN* targeting: the search for novel insulin sensitisers provides new insight into obesity research**

Francesco Beguinot

Published online: 29 November 2006
© Springer-Verlag 2006

Abbreviations

PI3K	phosphatidylinositol 3-kinase
PIP ₃	phosphatidylinositol 3,4,5-trisphosphate
PTEN	phosphatase and tensin homologue
SH2	SRC homology domain 2
SHIP	SH2-containing inositol 5-phosphatase

Breakthrough information in one field of science is often generated by studies in other areas. This has been the case for the phosphatase and tensin homologue (PTEN) and its identification as a drug discovery target for type 2 diabetes. In 1996, the susceptibility gene for Cowden syndrome (now known as *PTEN*) was mapped to human chromosome 10q22–q23 [1]. Cowden syndrome is an autosomal dominant disorder characterised by multiple hamartomas, which affect derivatives of all three germ layers, and increased risks of breast, thyroid and endometrial cancers [2]. Germline mutations in the tumour-suppressor gene *PTEN* have been found in 80% of patients affected by Cowden syndrome [3]. *PTEN* encodes a dual-function lipid and protein phosphatase that represents a major 3-phosphatase in the phosphatidylinositol 3-kinase (PI3K)/Akt signalling system [4, 5]. *PTEN* also represents the first phosphatase gene to be implicated in the aetiology of an inherited cancer syndrome. A number of disorders associ-

ated with germline *PTEN* mutations have been subsequently recognised.

Following insulin exposure, the PI3K-dependent increase in levels of phosphatidylinositol 3,4,5-trisphosphate (PIP₃) results in the membrane localisation and/or activation of a number of proteins that possess a pleckstrin homology (PH) domain [6]. One such protein is the serine/threonine kinase known as phosphoinositide-dependent kinase 1, which phosphorylates and activates several downstream kinases, including AKT1–3 [7] (Fig. 1). In addition, the protein kinase mammalian target of rapamycin (mTOR), which forms a complex with the regulatory protein rictor, has recently been identified as phosphoinositide-dependent kinase 2, which also phosphorylates AKT [8]. These PIP₃-dependent events induce important metabolic responses, including glucose transport, lipid and glycogen synthesis, modulation of gene expression, cell proliferation and inhibition of apoptosis. The PI3K pathway mediates the effects of insulin [9] and leptin [10] on satiety. As these mechanisms are relevant to cell metabolism, it was postulated that PIP₃ breakdown may also affect the physiology of insulin action, leading to greater focus on the function of PTEN in insulin signalling. Indeed, a number of studies found that, in addition to its tumour-suppressor function, PTEN serves as a major regulator of PI3K signalling in a number of cells. Much of this work was prompted by the finding that DAF18, the *Caenorhabditis elegans* PTEN homologue, downregulates insulin receptor-like signalling in the worm [11]. Insulin signalling

F. Beguinot (✉)
Department of Cellular and Molecular Biology and Pathology,
University of Naples 'Federico II',
5 via Sergio Pansini,
Naples 80131, Italy
e-mail: beguino@unina.it

F. Beguinot
C.N.R. Institute of Experimental Endocrinology and Oncology,
University of Naples 'Federico II',
5 via Sergio Pansini,
Naples 80131, Italy

Fig. 1 Molecular events following insulin activation of PI3K. Insulin-induced IRS-PI3K association activates PI3K, causing phosphorylation of phosphatidylinositol 4,5-bisphosphate [PI(4,5)P₂], increased levels of PIP₃, and recruitment/activation of PI3K-dependent molecules, including the serine/threonine kinase phosphatidylinositol

dependent kinase 1 (PDK1). PDK-1 is a major regulator of a number of downstream effectors eliciting the wide spectrum of insulin biological responses in sensitive tissues. The 3-phosphatase PTEN and the 5-phosphatase SHIP2 attenuate these effects by dephosphorylating PIP₃. SGK: serum/glucocorticoid regulated kinase

is also negatively regulated by PTEN in *Drosophila melanogaster* [12].

Studies on mice with PTEN ablation in liver [13, 14], adipose [15] or skeletal muscle tissues [16] have investigated the effect of PTEN on whole-body glucose homeostasis. In general, this work has provided further support for a role for PTEN in insulin sensitivity and metabolic regulation (Table 1). Fasting plasma glucose and insulin levels were reduced, and glucose tolerance was enhanced, in mice with liver-specific PTEN ablation. However, these mice exhibited a marked hepatomegaly and liver steatosis, suggesting fat redistribution from other tissues to liver. In addition, all the *PTEN* knock-out mice developed liver tumours by 18 months of age. While excess tumour occurrence was not evident in mice heterozygous for the *PTEN* gene, these mice showed no improved insulin sensitivity, suggesting that partial inhibition of liver PTEN activity may not represent an effective approach to insulin resistance in type 2 diabetes. Fat-specific PTEN ablation also lowered fasting plasma insulin and improved glucose tolerance in mice, with a significant improvement in whole-body sensitivity to insulin. In contrast, skeletal muscle-specific *PTEN* knock-out mice exhibited little metabolic abnormality. However, when subjected to a high-

fat diet, these animals showed protection against the development of hyperglycaemia and abnormalities in glucose tolerance. Cell proliferation in fat and muscle was not

Table 1 Effect of *PTEN* perturbation on glucose homeostasis in the mouse

Genetic perturbation	Metabolic phenotype	Reference
Liver-specific ablation	Reduced fasting plasma insulin, enhanced glucose tolerance and insulin sensitivity	[13, 14]
Fat-specific ablation	Reduced fasting plasma insulin, enhanced glucose tolerance and insulin sensitivity	[15]
Muscle-specific ablation	Protection from insulin-resistance and diabetes when exposed to high-fat diet	[16]
Ubiquitous haploinsufficiency	Fasting hypoglycaemia, enhanced glucose tolerance and insulin sensitivity	[17]

affected by loss of PTEN, supporting the possibility that targeted inhibition of PTEN function in these tissues may improve insulin sensitivity in a safe and effective manner.

In contrast with these studies, the work by Wong et al. in this issue of *Diabetologia* has defined the effect of a partial systemic reduction of PTEN on the regulation of insulin sensitivity in intact mice [17]. These studies were conducted in *PTEN* haploinsufficient animals with only a 15–50% reduction in tissue levels of PTEN. The haploinsufficiency did however greatly increase insulin sensitivity, thus demonstrating that small systemic changes in the levels of PTEN may improve insulin action. Based on previous studies in the tissue-specific *PTEN* knock-out mice, this is an unexpected finding, as mice heterozygous for the *PTEN* gene in liver do not show improved insulin sensitivity. The pro-apoptotic effect of PTEN does raise concerns regarding the safety of PTEN targeting as a means of improving insulin action in chronic disorders such as type 2 diabetes. Even so, studies on PTEN biology have provided proof-of-concept evidence for the potential of inhibiting PtdIns(3,4,5)P₃ phosphatases for enhancing insulin sensitivity in insulin-resistant states.

These advances have opened new and exciting avenues of investigation into the regulation of food intake and peripheral energy metabolism, and have increased interest regarding another family of lipid phosphatases, the SRC homology domain 2 (SH2)-containing inositol 5-phosphatases (SHIPs). Of these, SHIP2 (encoded by the gene *Inpp1l*) is highly expressed in skeletal muscle and brain [18]. Unlike PTEN, which acts on both PtdIns(3,4)P₂ and PIP₃, SHIP2 catalyses only PIP₃ hydrolysis. Wong et al. [17] point out that SHIP2 may not have a physiological role in regulating glucose homeostasis, consistent with the concept that PTEN and not SHIP2 is the major phosphatase involved in desensitising insulin signalling along the PI3K/Akt pathway. Unexpectedly, mice engineered to be null for *Inpp1l* were resistant to obesity induced by a high-fat diet [18]. Indeed, *Inpp1l* knock-out mice respond to a high-fat diet by increasing energy expenditure and preferentially eliminating the excess fat mass. Given the high level of *Inpp1l* expression in brain, it has been proposed that central mechanisms regulating food intake and energy metabolism, including insulin signalling, might be affected by reduced lipid 5'-phosphatase activity. Thus, compared with PTEN, SHIP2 exhibits greater potential as a drug target in obesity and diabetes. In vitro and in vivo investigations indicate possible efficacy in these disorders and, in contrast to *PTEN* knock-out mice, *Inpp1l*-null mice feature no increase in tumour occurrence after more than a year of survival. The exciting perspectives offered by SHIP2 targeting are tempered by difficulties in predicting the consequences of in vivo exposure to inhibitors of SHIP2 catalytic activity, and in identifying inhibitors of potential pharmacological interest.

As noted by Sherlock Holmes, Sir Arthur Conan Doyle's famous detective, when one follows two separate chains of thought, points of intersection eventually emerge which should approximate the truth. Studies on an inherited cancer syndrome and the pathogenesis of type 2 diabetes seem to follow this maxim. Interestingly, in this case, approximation to the truth seems to point towards a third disorder: obesity.

References

1. Nelen MR, Padberg GW, Peeters EAJ et al (1996) Localization of the gene for Cowden disease to 10q22–23. *Nat Genet* 13:114–116
2. Eng C (2000) Will the real Cowden syndrome please stand up: revised diagnostic criteria. *J Med Genet* 37:828–830
3. Liaw D, Marsh DJ, Li J et al (1997) Germline mutations of the *PTEN* gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet* 16:64–67
4. Li D-M, Sun H (1997) TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor B. *Cancer Res* 57:2124–2129
5. Maehama T, Dixon JE (1998) The tumor suppressor PTEN/MMAC1 dephosphorylates the lipid second messenger phosphoinositol 3,4,5-triphosphate. *J Biol Chem* 273:13375–13378
6. Vanhaesebroeck B, Alessi DR (2000) The PI3K-PDK1 connection: more than just a road to PKB. *Biochem J* 346:561–576
7. Mora A, Komander D, van Aalten DM, Alessi DR (2004) PDK1, the master regulator of AGC kinase signal transduction. *Semin Cell Dev Biol* 15:161–170
8. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 307:1098–1101
9. Carnevali JB, Ribeiro EB, Araujo EP et al (2003) Selective impairment of insulin signalling in the hypothalamus of obese Zucker rats. *Diabetologia* 46:1629–1640
10. Zhao AZ, Huan JN, Gupta S, Pal R, Sahu A (2002) A phosphatidylinositol 3-kinase phosphodiesterase 3B-cyclic AMP pathway in hypothalamic action of leptin on feeding. *Nat Neurosci* 5:727–728
11. Ogg S, Ruvkun G (1998) The *C. elegans* PTEN homolog DAF-18 acts in the insulin receptor-like metabolic signalling pathway. *Mol Cell* 2:887–893
12. Goberdhan DC, Paricio N, Goodman EC, Mlodnick M, Wilson C (1999) *Drosophila* tumor suppressor *PTEN* controls cell size and number by antagonizing the Chico/PI3-kinase signalling pathway. *Genes Dev* 13:3244–3258
13. Horie Y, Suzuki A, Kataoka E et al (2004) Hepatocyte-specific PTEN deficiency results in steatohepatitis and hepatocellular carcinomas. *J Clin Invest* 113:1774–2087
14. Stiles B, Wang Y, Stahl A et al (2004) Liver-specific deletion of negative regulator PTEN results in fatty liver and insulin hypersensitivity. *Proc Natl Acad Sci USA* 101:2082–2087
15. Kurlawalla-Martinez C, Stiles B, Wang Y et al (2005) Insulin hypersensitivity and resistance to streptozotocin-induced diabetes in mice lacking PTEN in adipose tissue. *Mol Cell Biol* 25:2498–2510
16. Wijesekara N, Konrad D, Eweida M et al (2005) Muscle-specific PTEN deletion protects against insulin resistance and diabetes. *Mol Cell Biol* 25:1135–1145
17. Wong JT, Kim PTW, Peacock JW (2006) *PTEN* (phosphatase and tensin homologue gene) haploinsufficiency promotes insulin hypersensitivity. *Diabetologia* DOI 10.1007/s00125-006-0531-x
18. Sleeman MW, Wortley KM, Lai KM et al (2005) Absence of the lipid phosphatase SHIP2 confers resistance to dietary obesity. *Nat Med* 11:199–205