Chapter 8
The PTEN and Myotubularin Phosphoinositide 3-Phosphatases: Linking Lipid Signalling to Human Disease

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Abstract Two classes of lipid phosphatases selectively dephosphorylate the 3 position of the inositol ring of phosphoinositide signaling molecules: the PTEN and the Myotubularin families. PTEN dephosphorylates PtdIns(3,4,5)P_3, acting in direct opposition to the Class I PI3K enzymes in the regulation of cell growth, proliferation and polarity and is an important tumor suppressor. Although there are several PTEN-related proteins encoded by the human genome, none of these appear to fulfill the same functions. In contrast, the Myotubularins dephosphorylate both PtdIns(3)P and PtdIns(3,5)P_2, making them antagonists of the Class II and Class III PI 3-kinases and regulators of membrane traffic. Both phosphatase groups were originally identified through their causal mutation in human disease. Mutations in specific myotubularins result in myotubular myopathy and Charcot-Marie-Tooth peripheral neuropathy; and loss of PTEN function through mutation and other mechanisms is evident in as many as a third of all human tumors. This chapter will discuss these two classes of phosphatases, covering what is known about their biochemistry, their functions at the cellular and whole body level and their influence on human health.

Keywords PTEN · Myotubularin · Phosphoinositide · Phosphatase · PI 3-kinase

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8.1 Introduction

Phosphoinositides are second messengers that relay extracellular signals to initiate cellular signaling cascades. They are derived from the precursor phosphatidylinositol (PtdIns), which can be transiently phosphorylated at the D3, D4 or D5 position of the inositol head group. Seven phosphoinositide species have been currently identified, each with unique subcellular localization patterns and distinct roles in cellular signaling pathways. Generation of phosphoinositides phosphorylated at the D3 position of the inositol head group is a critical component in phosphoinositide metabolism, and in the coordination of cellular responses required for appropriate physiological development. Phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3) levels are low in quiescent cells but increase transiently in response to agonist stimulation. Agonist-induced activation of the class I phosphatidylinositol 3-kinase (PI3K) results in the generation of PtdIns(3,4,5)P_3 at the plasma membrane through the phosphorylation of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) at the D3 position of its inositol head group. PtdIns(3,4,5)P_3 directs numerous cellular processes, including cell proliferation, growth, survival, cell polarity and migration. Phosphatidylinositol 3-phosphate (PtdIns(3)P) is constitutively generated at the site of early endosomes by the class III PI3K (Vps34) or by the class II PI3K. PtdIns(3)P regulates endosomal fusion and motility; receptor sorting and recycling; and vesicle trafficking. Phosphatidylinositol 3,5-bisphosphate (PtdIns(3,5)P_2) is generated by phosphorylation of PtdIns(3)P by the PIKfyve kinase on early and/or late endosomes and regulates endosomal sorting and endomembrane homeostasis. The downstream cellular effects of 3-phosphorylated phosphoinositides are transmitted via the recruitment of specific phosphoinositide-binding proteins. This occurs either through interaction of the phosphoinositide inositol head group with basic amino acid residues or alternatively via interaction with discrete phosphoinositide-binding domains, for example pleckstrin homology (PH) domains. Dysregulation of 3-phosphoinositide metabolism leads to the disruption of cellular function and the development of disease. Therefore, their levels are tightly regulated by the activity of the phosphoinositide kinases, which generate them; and the activity of phosphoinositide 3-phosphatases, which selectively remove the phosphate group at the D3 position of their inositol head-groups. The phosphoinositide 3-phosphatases include PTEN and its related homologs; and the multiple members of the myotubularin (MTM) family. These enzymes share a highly conserved phosphatase domain, containing a CX_5R catalytic motif, and both will be described within this chapter. While *in vitro* kinetic analyses have demonstrated enzyme activity of PTEN and MTMs toward membrane-bound phosphoinositides and soluble inositol phosphates, membrane-bound phosphoinositides are recognized as their preferred physiological substrates. Therefore, at the functional level, the 3-phosphatases act preferentially at membrane microdomains at the plasma membrane or on intracellular organelles such as early endosomes.

PTEN is a recognized tumor suppressor gene, which is frequently mutated at the 10q23 chromosomal locus in both spontaneous cancers and hereditary cancer predisposition syndromes. PtdIns(3,4,5)P_3 is the recognized physiological substrate

of PTEN, generating PtdIns(4,5)P_2. Therefore, PTEN directly antagonizes the Class I PI3K at the plasma membrane, and thereby regulates cell proliferation, survival, cell cycle progression, cell polarity, migration, invasion, embryonic development, immune function, insulin signaling and glucose metabolism. The myotubularins are a large family consisting of 9 catalytically active and 7 catalytically inactive family members. Heterodimeric interaction between active and inactive members of the myotubularin family regulates catalytic activity and/or sub-cellular protein localization of the active family members. Myotubularins hydrolyze PtdIns(3)P and PtdIns(3,5)P_2, to generate PtdIns and PtdIns(5)P respectively. Therefore, MTMs antagonize Class II and III PI3Ks, thereby regulating phosphoinositide-dependent endosomal membrane homeostasis. Mutations in various members of the myotubularin family are associated with human disease, including the peripheral neuropathy Charcot-Marie Tooth disease; and mytobular or centronuclear myopathies. Dephosphorylation of phosphoinositides at the D3 position of the inositol head-group is also a function of Sac1, which has been extensively described in Chap. 7, and will not be further described here. The following chapter will discuss the prominent features of these two families of 3-phosphoinositide phosphatase enzymes, focusing on current studies that enhance our knowledge of how the loss of function of these proteins contributes to human diseases.

8.2 PTEN

PTEN/MMAC/TEP1 (phosphatase and tensin homolog deleted on chromosome ten/mutated in multiple advanced cancers/TGFβ-regulated and epithelial cell-enriched phosphatase) is a tumor suppressor that is frequently mutated in sporadic human cancers and also in the inherited autosomal dominant cancer predisposition syndromes, Cowden disease, Lhermitte-Duclos disease, Bannayan-Zonana syndrome, and Proteus and Proteus-like syndromes (Yin and Shen 2008). These syndromes are characterized by developmental disorders, including neurological abnormalities, multiple hamartomas, and an associated increased risk of cancer development in later life, including breast, thyroid, and endometrial cancers (Liaw et al. 1997; Marsh et al. 1997; Tsuchiya et al. 1998). Other PTEN-like phosphatases have been identified in humans including the Trans-membrane Phosphatase with Tensin homology (TPTE), and the TPTE and PTEN homologous inositol lipid phosphatase (TPIP/TPTE2). However, these enzymes appear to be expressed predominantly in the testis, and although their functions are poorly defined, they seem quite distinct from those of PTEN, reviewed in (Sasaki et al. 2009).

PTEN shares sequence homology with the protein tyrosine phosphatase family and initial reports identified PTEN as a dual-specificity protein phosphatase (Li and Sun 1997; Myers et al. 1997). Subsequent studies using recombinant PTEN identified its 3-phosphatase activity toward PtdIns(3,4,5)P_3, PtdIns(3,4)P_2, PtdIns(3)P, and Ins(1,3,4,5)P_4 (Maehama and Dixon 1998). Whilst constitutive elevation of both PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 have been identified in PTEN-null cells (Haas-Kogan et al. 1998; Taylor et al. 2000b), there is evidence to indicate that

PtdIns(3,4,5)P_3 is the major physiological target of PTEN. Firstly, a H93A PTEN mutant selectively reduces PTEN activity toward PtdIns(3,4,5)P_3, but not PtdIns(3,4)P_2 (Lee et al. 1999). Furthermore, the catalytic efficiency of PTEN for PtdIns(3,4,5)P_3 as a substrate is 200-fold greater than that for PtdIns(3,4)P_2 (McConnachie et al. 2003). Therefore, in the physiological context, it is likely PTEN is a phosphoinositide phosphatase that preferentially hydrolyzes the D3-position phosphate from PtdIns(3,4,5)P_3, to generate PtdIns(4,5)P_2, and thereby directly antagonizes phosphoinositide 3-kinase (PI3K) signaling and attenuates Akt activation to regulate cell survival and proliferation (Salmena et al. 2008).

8.2.1 PTEN Structure

Several domains and motifs have been identified in PTEN that contribute to its activity, stability or localization (Fig. 8.1). PTEN contains two major domains, which associate across an extensive interface through hydrogen bonding (Lee et al. 1999; Li et al. 1997; Steck et al. 1997). Within the amino-terminal domain is the catalytic phosphatase domain, that contains a conserved CX_5R catalytic motif; and also an extreme amino-terminal PtdIns(4,5)P_2—binding motif (Walker et al. 2004; Campbell et al. 2003; Iijima et al. 2004). The carboxyl-terminal domain contains a calcium-independent phospholipid binding C2 domain that regulates its plasma membrane localization; two PEST (proline, glutamic acid, serine, threonine) sequences, and a PDZ (Post synaptic density protein, Drosophila disc large tumor suppressor, zonula occludens-1 protein)-binding domain, that mediates the interaction with several binding partners and can affect PTEN protein stability (Salmena et al. 2008). Examination of PTEN's crystal structure reveals the presence of a more enlarged catalytic pocket, in comparison to protein tyrosine phosphatases, which also have a CX_5R catalytic motif, facilitating the association of PtdIns(3,4,5)P_3 with particular basic catalytic residues (Lee et al. 1999). The amino-terminal PtdIns(4,5)P_2—binding motif, in addition to the C2 domain, regulates the transient association of PTEN from the cytosol to the plasma membrane, positioning the phosphatase for maximal access to its membrane-bound phosphoinositide substrate.

8.2.2 Regulation of PTEN

PTEN can be regulated both at the level of transcription, or by post-translational modification. Indeed, regulation of PTEN at the transcriptional level plays a prominent role in those cancers or cases of Cowden disease in which mutation of *PTEN* is absent, but PTEN expression is lost. Naturally occurring alternative splice variants of *PTEN* have been identified in both normal and cancerous tissue (Sharrard and Maitland 2000). However, in identified *PTEN* mutation-negative cases of Cowden disease or sporadic breast cancers, alternative splice variants of *PTEN* are found to associate with decreased transcription of full-length *PTEN* (Sarquis et al. 2006; Agrawal and Eng 2006). Epigenetic silencing of the *PTEN* promoter through methylation and the

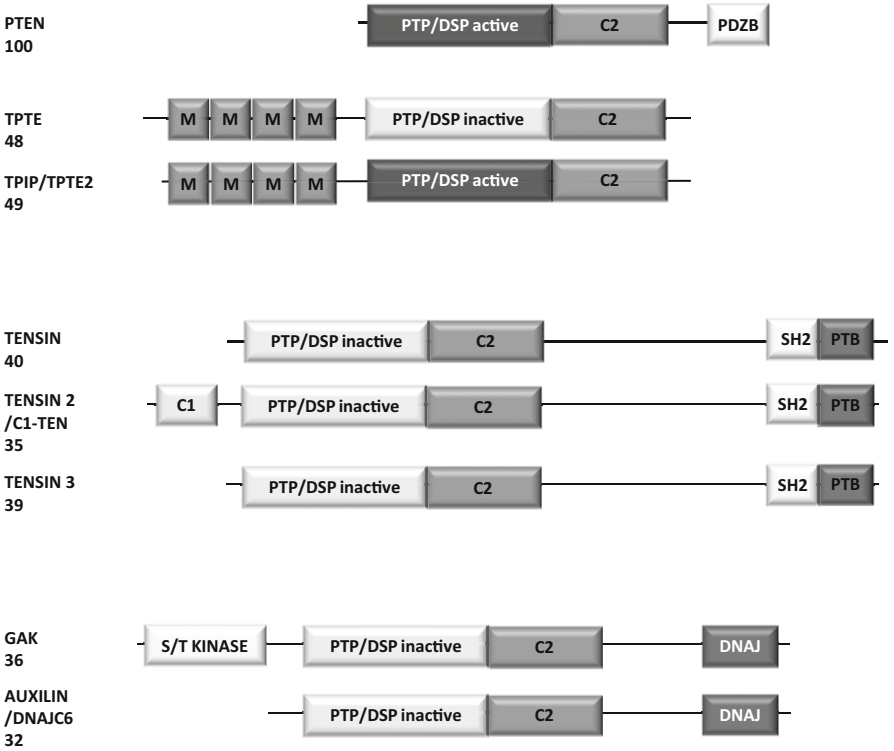

Fig. 8.1 Schematic representation of the major domains of the human PTEN family. The domain structure of human PTEN is shown, along with its closest 7 relatives within the human genome. More distantly related phosphatases display both lower sequence identity through the phosphatase domain and lack an adjacent recognizable C2 domain. Numbers below each protein name show the sequence identity with PTEN through the phosphatase domain. Domains are those identified in the NCBI/CDD database. Abbreviations: *GAK* Cyclin G-associated Kinase, *PTP/DSP* Protein tyrosine phosphatase/dual specific phosphatase, M Transmembrane domain, *SH2* Src homology 2, *PTB* Phosphotyrosine binding, S/T Kinase Serine/Threonine Protein Kinase, PDZB binding motif for PDZ domains (Post synaptic density protein, Drosophila disc large tumor suppressor, zonula occludens-1 protein)

actions of several oncogenic microRNAs also decrease PTEN protein expression in sporadic cancers (Khan et al. 2004; Whang et al. 1998; Mirmohammadsadegh et al. 2006; Salvesen et al. 2004; Poliseno et al. 2010a).

Post-translational modifications of PTEN may regulate protein stability, expression, catalytic activity or sub-cellular localization. PTEN interacts with PCAF (p300/CBP-associated factor), a histone acetyltransferase that regulates gene transcription (Okumura et al. 2006; Yao and Nyomba 2008). Interaction of PTEN and PCAF results in increased acetylation of Lys^{125} and Lys^{128}, within the catalytic cleft of PTEN, inhibiting PTEN's phosphatase activity. Several studies have shown that phosphorylation of specific residues within the carboxyl-terminal of PTEN by a number of kinases also regulates protein stability and turnover. Phosphorylation

of PTEN at a cluster of phosphorylation sites (Ser^{380}, Thr^{382}, Thr^{383} and Ser^{385}) in the carboxyl-terminal tail region by casein kinase 2 promotes a conformational change in PTEN, indirectly increasing PTEN's resistance to proteosome-mediated degradation, but decreasing its membrane association and cellular activity (Vazquez et al. 2000, 2001, 2006; Torres and Pulido 2001; Maccario et al. 2010). Conversely, glycogen synthase kinase 3β (GSK3β)-mediated phosphorylation of PTEN at Thr^{366} in its carboxyl-terminal tail results in protein destabilization (Maccario et al. 2007). Phosphorylation of PTEN by the Src family of protein-tyrosine kinases, probably within the C2 domain, may also regulate protein stability or its sub-cellular localization (Lu et al. 2003). Additionally, the candidate tumor suppressor, PICT-1 (also known as GLTSCR2) promotes PTEN phosphorylation and stability, although the precise mechanism is unclear (Okahara et al. 2004). PEST sequences are commonly found in proteins that are targeted for degradation within the ubiquitin pathway. Phosphorylation-dependent polyubiquitination has been proposed as a potential molecular mechanism targeting PTEN for proteosomal degradation (Tolkacheva et al. 2001), however, the identification of the physiological ubiquitin ligase remains to be confirmed. Studies in which NEDD4-1 levels were manipulated through ectopic expression or RNA interference, identified this protein as the E3 ubiquitin ligase that polyubiquinates PTEN (Wang et al. 2007, 2008). However, studies in Nedd4-1 knockout mice, showed that Nedd4-1 was dispensable for the regulation of PTEN stability, activity and/or localization (Fouladkou et al. 2008). The reasons for these apparent contradictory findings are yet to be resolved.

Recently PTEN was demonstrated to interact with P-Rex2, a multi-domain protein that contains a Rac GEF domain and a domain with homology to the inositol polyphosphate 4-phosphatases. Within this latter domain P-Rex2 contains a CX_5R motif, but there is no evidence that P-Rex2 or the related P-Rex1 are catalytically active phosphoinositide phosphatases (see Chap. 7). The interaction between P-Rex2 and PTEN inhibits PTEN catalytic activity, and as a consequence cell proliferation and survival is enhanced (Fine et al. 2009).

8.2.3 Functional Roles of the Protein Versus Lipid Phosphatase Activity of PTEN

Lipid phosphatase-independent roles for PTEN are currently emerging; however, the identification of the G129E missense mutation in Cowden disease kindred, which selectively eliminates the lipid phosphatase activity of PTEN, while retaining its protein phosphatase activity, demonstrates that the lipid phosphatase activity of PTEN is essential for tumor suppression (Furnari et al. 1998; Myers et al. 1998). However, while PTEN is the central regulator of the PI3K signaling pathway, reports from many studies suggest a role for the protein phosphatase activity of PTEN, particularly in adhesion and cell migration, and the functional role of PTEN may indeed require both its lipid and protein phosphatase activities. Potential PTEN protein substrates include FAK, Shc and platelet-derived growth factor receptor (PDGFR) (Gu et al. 1999; Tamura et al. 1998; Mahimainathan and Choudhury 2004); however, whether

these are *bona fide* physiological targets of PTEN remains unresolved (Davidson et al. 2010).

In *Dictyostelium discoideum* (*D. discoideum*), PTEN sub-cellular localization is restricted to the rear and lateral aspects of the cell, ensuring PtdIns(3,4,5)P_3 is localized to the leading edge of chemotaxing cells (Funamoto et al. 2002; Iijima and Devreotes 2002). The reconstitution of wild-type PTEN into PTEN-null mouse fibroblasts inhibits cell migration, and decreases the activation of the small GTPases Rac1 and Cdc42, dependent on the lipid phosphatase activity of PTEN (Liliental et al. 2000). However, studies using the G129E mutant (Furnari et al. 1998; Myers et al. 1998) have shown that PTEN can inhibit mammalian cell migration through a mechanism that is dependent on PTEN's protein phosphatase activity (Tamura et al. 1998, 1999a; Dey et al. 2008; Leslie et al. 2007; Gildea et al. 2004; Gu et al. 1999). The most compelling function of the protein phosphatase activity of PTEN in the regulation of mammalian cell migration is its proposed role in the auto-dephosphorylation of its carboxyl-terminal tail to reveal its lipid-binding C2 domain. PTEN inhibits cell migration in glioblastoma cells, independent of its lipid phosphatase activity, but reliant on its protein phosphatase activity (Raftopoulou et al. 2004). The C2 domain alone can also inhibit cellular migration in microinjection experiments, suggesting that this activity of the C2 domain may be regulated by the full-length protein. The dephosphorylation of PTEN is essential to C2 domain activation and is dependent solely on the protein phosphatase activity of PTEN. Raftopoulou et al. identified the specific dephosphorylation of residue Thr^{383} as important in this process; however analysis in many cell types by multiple groups using phospho-specific antibodies has failed to delineate the significance of this site as compared to the other phosphorylation cluster sites Ser^{380}, Thr^{382} and Ser^{385} in the regulation of PTEN's activity and function (Odriozola et al. 2007; Leslie et al. 2007; Rahdar et al. 2009). PTEN has previously been shown to exhibit preferential protein substrate specificity toward highly acidic proteins and peptides (Myers et al. 1997). The PTEN carboxyl-terminal tail is predominantly acidic, which may implicate the protein phosphatase activity of PTEN in its autodephosphorylation, leading to the activation of the C2 domain and inhibition of cellular migration. In support of this, a 71 amino acid region within the carboxyl-terminal tail of PTEN has been identified as an auto-inhibitory domain that regulates membrane localization and catalytic activity through an intramolecular association with the CBRIII motif of the C2 domain (Odriozola et al. 2007). One report suggests that PTEN is involved in the regulation of two distinct processes that require the co-operation of both its lipid and protein phosphatase activities to mediate cell migration during embryonic development (Leslie et al. 2007). The protein phosphatase activity of PTEN is required for the control of epithelial-to-mesenchymal transition (EMT) via the autodephosphorylation of its carboxyl-terminal domain, while the lipid phosphatase activity of PTEN regulates PtdIns(3,4,5)P_3-dependent cell polarization and directionality of mesodermal cell migration. A recent study has also contributed to the contention that both the lipid and phosphatase activities of PTEN are required to act in co-operation to regulate physiological processes. Davidson et al. generated a novel PTEN mutant, Y138L, which retains lipid phosphatase activity, but lacks phosphatase activity toward protein substrates (Davidson et al.

2010). Using this mutant alongside the well-described G129E mutant, the role of the lipid and protein phosphatase activities of PTEN in physiological processes was further delineated. The lipid phosphatase activity of PTEN regulated cell proliferation in soft agar and cell spreading. Adherent cell migration was regulated by either the protein or lipid phosphatase activities of PTEN; whereas cellular invasion required the coordinated actions of both activities. Therefore, the lipid and protein phosphatase activities of PTEN may be required for the regulation of cellular processes important in development and disease prevention.

8.2.4 A Nuclear Function for PTEN

Apart from its function as a negative regulator of PI3K-mediated signaling pathways at the plasma membrane, a role for PTEN within the cell nucleus is currently emerging. The localization of PTEN within the nucleus has been described in a range of both normal and tumor cells, with nuclear exclusion of PTEN associated with increased cancer progression (Zhou et al. 2002; Perren et al. 2000; Fridberg et al. 2007). At the functional level, targeted expression of PTEN within the nucleus does not affect catalytic activity *in vitro*, but leads to loss of PTEN function in cellular assays of proliferation, promotes cell cycle arrest and inhibits anchorage-independent cell growth (Ginn-Pease and Eng 2003; Liu et al. 2005b; Chung and Eng 2005; Denning et al. 2007). Therefore, control of PTEN localization may become a future therapeutic tool.

While a traditional NLS (nuclear localization signal) has not been identified in PTEN to date, a number of mechanisms regulating PTEN nuclear localization have been proposed. PTEN has been shown to enter the nucleus through passive diffusion in a RAN (Ras-related nuclear protein)-independent manner (Liu et al. 2005a). Putative NLS-like sequences have additionally been identified in PTEN that are required for nuclear import, mediated through interaction with MVP (major vault protein) (Chung et al. 2005). A further mechanism has identified both putative NLS and nuclear exclusion motifs as necessary for nuclear localization of PTEN, mediated through currently unidentified importin proteins and RAN (Gil et al. 2006). Finally, mono-ubiquitination is emerging as a critical means of regulating PTEN localization. NEDD4-1-mediated mono-ubiquitination of Lys^{289} or Lys^{13} residues within PTEN has been identified as a molecular mechanism that regulates nuclear import of the protein (Trotman et al. 2007). Although the nuclear function of PTEN remains to be fully characterized, the K289E mutation in the carboxyl-terminal tail of PTEN is associated with Cowden disease. This point mutation does not affect catalytic activity or plasma membrane localization (Georgescu et al. 2000), but prevents mono-ubiquitination at this site (Trotman et al. 2007). Alternative regulation of mono-ubiquitination of PTEN occurs through the opposing actions of HAUSP (herpesvirus-associated ubiquitin-specific protease) and PML (promyelocytic leukemia protein) via the adapter protein DAAX (death domain-associated protein) (Song et al. 2008). PTEN localization is abnormal in acute promyelocytic leukemia where PML function is impaired; and HAUSP is over-expressed in human prostate cancer and is associated with nuclear exclusion of PTEN.

While a nuclear pool of PtdIns(3,4,5)P_3 has been identified, this may be insensitive to PTEN expression (Lindsay et al. 2006), suggesting a phosphoinositide phosphatase-independent role for nuclear PTEN. In support of this, several groups have described phosphatase-independent functions of PTEN within in the nucleus, which may promote chromosome stability. These functions are predominantly associated with the regulation of protein interactions within the nucleus. Phosphatase-independent protein interactions between PTEN and p300 in the nucleus induce hyper-acetylation of p53, inducing cell cycle arrest in response to DNA damage (Liu et al. 2006). An association between loss of PTEN and chromosomal fragmentation has recently been described, suggesting a possible role for PTEN in DNA repair mechanisms. Endogenous PTEN was identified at the centromere, where it associated with the core centromeric protein Cenp-C, a protein required for kinetochore assembly and also during mitosis for metaphase to anaphase transition (Shen et al. 2007). This association was mediated via the carboxyl-terminus of PTEN, independently of catalytic activity.

8.2.5 PTEN Function as Revealed by Mouse Knock-out Studies

To dissect PTEN function, both global and tissue-specific deletion of PTEN in mice have been undertaken over the last 10 years. These studies have revealed roles for PTEN in autoimmune disease, non-alcoholic steatohepatitis, insulin hypersensitivity, heart failure, angiogenesis via regulation of endothelial cell function, macroencephaly, bone density, respiratory distress syndrome, immunoglobulin class switching, and resistance to hair graying to name a few (Knobbe et al. 2008). Homozygosity for a null mutation of *Pten* in mice results in early embryonic lethality (Di Cristofano et al. 1998, 1999; Podsypanina et al. 1999; Stambolic et al. 1998; Suzuki et al. 1998). Many different tissue-specific mouse *Pten* knockouts have been generated and their phenotypes are summarized in Table 8.1. For more detailed descriptions of conditional Pten mutant mice the reader is referred to recent reviews (Suzuki et al. 2008; Knobbe et al. 2008).

8.2.6 Disruption of PTEN Correlates with Tumorigenesis and Cancer Progression

After p53, *PTEN* is the second most frequently mutated tumor suppressor gene in human cancer. It was identified as the tumor suppressor gene at the 10q23 human chromosomal locus, a region frequently mutated in a vast range of sporadic cancers (Li and Sun 1997; Steck et al. 1997). The classification of PTEN as a tumor suppressor is further sustained through the identification of germline mutations of *PTEN* in the autosomal dominant cancer predisposition syndromes, Cowden disease, Lhermitte-Duclos disease and Bannayan-Zonana syndrome. While targeted disruption of *Pten* in mice results in early embryonic death between embryonic day 6.5 and 9.5, the phenotype exhibited in heterozygotes varies, possibly as a result of variations in targeting constructs; however, increased susceptibility to tumor formation is

Table 8.1 Genetically modified PTEN murine models

PTEN knockout mouse model	Phenotype	Reference
Constitutive Pten knockout mice		
Global	Homozygotes: Embryonic death at E6.5-E9.5 Heterozygotes: Increased susceptibility to tumor development in multiple tissues. Increased autoimmune responses	(Di Cristofano et al. 1998, 1999; Podsypanina et al. 1999; Stambolic et al. 1998; Suzuki et al. 1998)
Conditional Pten knockout mice (*single mutants*)		
Adipocyte-specific (*aP2Cre*)	Improved systemic glucose tolerance and insulin sensitivity. Increased resistance to diabetes	(Kurlawalla-Martinez et al. 2005)
B-cell-specific (*CD19Cre*)	Impaired immunoglobulin class switching and defective B-cell homeostasis. Hyper-proliferation, resistance to apoptosis and enhanced migration of splenic B-cells. Abrogation of BCR-mediated apoptosis and restoration of BCR-induced cell cycle progression via PtdIns(3,4,5)P_3-dependent signaling pathways in immature B cells	(Suzuki et al. 2003b; Anzelon et al. 2003; Cheng et al. 2009)
Cardiomyocyte-specific (*MckCre*)	Cardiac hypertrophy from 10 weeks of age and decreased cardiac contractility	(Crackower et al. 2002)
Cerebellum-specific (*En2Cre-neuronal and glial cells of the vermis of the cerebellum*) (*L7Cre-Purkinje cells*)	Reduced proliferation and progressive loss of Purkinje cells, beginning in early postnatal development, characterized by increasing vacuolation of the cells and the accumulation of fibrillary inclusions. Increased cerebellar size, neurons with larger soma size and thickened dendrites, dysplastic astrocytes and abnormally localized oligodendrocytes	(Marino et al. 2002)
Chrondrocyte-specific (*Col2a1Cre*)	Contrasting phenotype reported in two independent studies. Ford-Hutchinson et al. showed increased skeletal size, increased vertebrae size, and primary spongiosa development. They described disorganization of long bone growth plates, matrix overproduction and accelerated hypertrophic differentiation. No evidence of hamartoma, benign bone lesions, or chondrosarcoma was reported, however, 2/12 mice followed for a period of 12 months, developed metastatic osteosarcoma. However, Yang et al. described a phenotype with chondrocyte-specific deletion of *Pten* resulting in dyschondroplasia, as a result of delayed chondrocyte differentiation and decreased proliferation. Pathological cartilaginous neoplasms were evident from birth resembling human enchondroma	(Ford-Hutchinson et al. 2007; Yang et al. 2008)

Table 8.1 (continued)

PTEN knockout mouse model	Phenotype	Reference
Endothelial cell-specific (*Tie2Cre*)	*Homozygotes:* Embryonic death prior to E11.5, associated with bleeding and cardiac failure as a result of impaired recruitment of pericytes and vascular smooth muscle cells to blood vessels, and cardiomyocytes to the endocardium *Heterozygotes:* Enhanced tumorigenesis due to increased angiogenesis, associated with altered expression of endothelial cell receptor proteins, vascular adhesion molecules and vascular growth factors	(Hamada et al. 2005)
Hematopoietic stem cells (*pIpc-inducible Mx1Cre*)	Rapid development of myeloproliferative disorders within 4–6 weeks of age, which progressed to acute myeloid leukemia or acute lymphoblastic leukemia	(Yilmaz et al. 2006; Zhang et al. 2006)
Hepatocyte-specific (*AlbCre*)	Increased hepatomegaly, steatohepatitis and an accumulation of triglycerides similar to that in human non-alcoholic steatohepatitis (NASH). Increased levels of C16:1 and C18:1 acids within the liver. Increased induction of adipocyte-specific genes (adipsin, adiponectin, and aP2) and lipogenic genes. Increased development of liver adenoma and hepatocellular carcinoma within 78 weeks of age. Decreased serum glucose levels due to insulin hypersensitivity, and reduced serum insulin. Hepatic steatosis, inflammation, and carcinogenesis in Pten-deficient mice were attenuated in females compared to males. Decreased hepatic protein levels of apoB100 and microsomal triglyceride transfer protein	(Horie et al. 2004; Anezaki et al. 2009; Stiles et al. 2004; Qiu et al. 2008)
Hypothalamic POMC-specific (*PomcCre*)	Development of hyperphagia and sexually-differential diet-induced obesity. In male mice, increased body weight was associated with increased consumption of a normal diet. Females maintained normal body weight on a normal chow diet, but became obese on a high-fat diet. Pomc-expressing neurons were larger in Pten-null cells and had more efferent fibers than wild-type neurons	(Plum et al. 2006)
Intestinal epithelial cell-specific (*Tg(Cyp1a1-cre)1Dwi or Tg(Vil-cre/ESR1)23Syr*)	No effect on the normal architecture or homeostasis of the epithelium within adult or embryonic epithelial cells	(Marsh et al. 2008)
Intestinal stem cell-specific (*Mx1Cre*)	Deletion of PTEN in the epithelial and stromal cells of the small intestine results in increased proliferative intestinal stem cells that initiate intestinal polyp formation, resembling intestinal polyposis, within 1 month	(He et al. 2007)

Table 8.1 (continued)

PTEN knockout mouse model	Phenotype	Reference
Keratinocyte-specific (*k5Cre, MMTVCre*)	Epidermal hyperplasia, hyperkeratosis, and shaggy hair. Decreased body weight, with approximately 90% of mutants dying from malnutrition within 3 weeks of birth, as a result of esophageal hyperkeratosis. Mice that survive beyond 2 months of age show increased susceptibility to the development of squamous papilloma squamous cell carcinoma, sebaceous carcinoma and adenocarcinoma of the sweat gland within 9 months	(Suzuki et al. 2003a; Yang et al. 2005; Backman et al. 2004)
Lung epithelial cell-specific (*Doxycycline-inducible SP-C-rtTA/(tetO)$_7$-Cre*)	Ninety percent of mutant mice that receive doxycycline in utero (E10-16) die of hypoxia within 2 h of birth. Hyperplasia of bronchioalveolar epithelial cells and myofibroblast precursors. Enlarged undifferentiated alveolar epithelial cells, and impaired production of surfactant proteins. Increased numbers of bronchioalveolar stem cells, which are putative initiators of lung adenocarcinoma. Increased susceptibility of the surviving mutants, and mice receiving doxycycline postnatally (P21-27), to spontaneous or induced lung adenocarcinoma	(Yanagi et al. 2007)
Macrophage-specific (*LysMCre*)	Increased susceptibility to infection and reduced clearance of infection, resulting from decreased secretion of tumor necrosis factor, correlating with reduced expression of inducible nitric oxide synthase and reduction in nitric oxide production	(Kuroda et al. 2008)
Mammary-specific (*MMTVCre*)	Enhanced lobulo-alveolar development, excessive ductal branching, delayed involution and severely reduced apoptosis in mutant mammary tissue. Increased development of mammary tumors in mutant females within 2 months of age	(Li et al. 2002a)
Melanocyte-specific (*DctCre*)	Increased melanocytes in the dermis of perinatal mice. Protection against hair graying. No change in spontaneous tumor development, but increased susceptibility to the development of large nevi and melanoma after carcinogen exposure	(Inoue-Narita et al. 2008)

Table 8.1 (continued)

PTEN knockout mouse model	Phenotype	Reference
Neuronal cell-specific (*GfapCre*)	Increased neurological defects including seizures and ataxia within 6–9 weeks of birth, death within 29–48 weeks of age. Macroencephaly, hydrocephaly and dysplasia of several neural cell populations, including enlarged soma in Pten-null neurons, accompanied by enlarged caliber of neuronal projections and increased dendritic spine density. Abnormal synaptic structures and severe myelination defects, with weakened synaptic transmission and plasticity at excitatory synapses. Disorganized architecture as a result of neuronal migratory defects that lead to abnormal accumulation of granule cells in the external granule cell layer	(Backman et al. 2001; Kwon et al. 2001; Yue et al. 2005; Fraser et al. 2008)
Neuronal (differentiated cells of cortical layer III-IV *NseCre*)	Increased soma hypertrophy, macroencephaly and premature death in the forebrain and hippocampus. Increased axonal outgrowth and altered spine morphology. Abnormal social interaction, increased anxiety, hyperactivity and increased sensory sensitivity, reminiscent of autistic spectrum disorders in humans. Increased sporadic spontaneous seizures	(Kwon et al. 2006; Ogawa et al. 2007)
Neuronal progenitor cell-specific (*NestinCre*)	Early perinatal death. Enlarged, histoarchitecturally abnormal brains, as a result of increased cell proliferation, decreased cell death and enlarged cell size	(Groszer et al. 2001)
Neutrophil-specific (*LysMCre*)	Increased superoxide production. Enhanced actin polymerization, membrane ruffling, and pseudopod formation, which resulted in increased chemotaxis and migratory speed, but loss of directionality	(Zhu et al. 2006a)
NKT cell-specific (*LckCre*)	Reduced levels of serum γ-interferon, in response to NKT activation. Impaired NKT cell development, with increased numbers of immature NKT cells and decreased numbers of mature NKT cells, with impaired functionality. Increased tumorigenesis, resulting from decreased immune surveillance	(Kishimoto et al. 2007)
Oocyte-specific (*GDF9Cre*)	Premature activation of the complete primordial follicle pool, resulting in ovarian failure	(Reddy et al. 2008)
Osteoblast-specific (*OcCre*)	Progressively increasing bone mineral density throughout life, as a result of increased differentiation and reduced apoptosis	(Liu et al. 2007)

Table 8.1 (continued)

PTEN knockout mouse model	Phenotype	Reference
Ovarian granulosa cell-specific (*Cyp19Cre*)	Increased ovary volume, increased production of oocytes during ovulation and increased number of pups	(Fan et al. 2008)
Pancreatic-specific (*Pdx1Cre, RipCre*)	Increased islet cell numbers and total islet mass evident at P15 and persisting through adulthood in RipCrePten$^{flox/flox}$ mice, in which Pten is deleted in pancreatic β-cells only. Hypoglycemic and diabetic resistance in adult mice, with smaller body mass and reduced lifespan, but no evidence of pancreatic tumor development. However, Pdx1CrePten$^{flox/flox}$ mice, which lack Pten expression in all pancreatic cells, show progressive replacement of the acinar pancreas with highly proliferative ductal structures, containing mucins and expressing markers of pancreatic progenitor cells, with increased development of ductal malignancy. Mice exhibited delayed onset of streptozotocin-induced diabetes and sex-biased resistance to high-fat-diet -induced diabetes	(Stanger et al. 2005; Stiles et al. 2006; Nguyen et al. 2006; Tong et al. 2009)
Primordial germ cell-specific (*TNAPCre*)	Bilateral testicular teratoma development in all new-born males, as a result of impaired mitotic arrest and outgrowth of cells with immature characters. Increased pluripotent embryonic germ cell production in both sexes	(Kimura et al. 2003)
Prostate-specific (*PbCre, PbCre4, MMTVCre PSACre, PSACreER*(*T2*))	Development of prostatic hypoplasia within the early postnatal period, rapidly progressing to high-grade prostatic intraepithelial neoplasia, then to invasive adenocarcinoma, and metastatic carcinoma	(Ma et al. 2005; Backman et al. 2004; Trotman et al. 2003; Wang et al. 2003; Ratnacaram et al. 2008)
Retinal ganglionic cell-specific (*AAVCre surgical delivery*)	Increased RGC survival, protein synthesis and axon regeneration following optic nerve injury	(Park et al. 2008)
Retinal pigment epithelium-specific (*TRP1Cre*)	Progressive degeneration of both RPE cells and their photoreceptors due to an inability of RPE cells to maintain basolateral adhesions, the development of an epithelial-to-mesenchymal transition (EMT), and subsequent cellular migration out of the retina	(Kim et al. 2008)

Table 8.1 (continued)

PTEN knockout mouse model	Phenotype	Reference
Smooth muscle cell-specific (*TaglnCre, Sm22*αCre)	Elevated incidence of smooth muscle cell hyperplasia and abdominal leiomyosarcomas, within 2 months of age Early perinatal lethality, increased development of medial and intimal smooth muscle cell hyperplasia, and vascular recruitment of progenitor/proinflammatory cells	(Hernando et al. 2007; Nemenoff et al. 2008)
Skeletal muscle-specific (*MckCre*)	Enhanced protection from insulin resistance and diabetes on a high-fat diet Reversal of high-fat diet-induced impairment of muscle regeneration	(Wijesekara et al. 2005)
T-cell-specific (*LckCre, CD4Cre*)	Defective thymic negative selection, increased autoimmune responses. Lymphadenopathy, splenomegaly, and enlarged thymus within 6–8 weeks. Post-natal death prior to 20 weeks of age as a result of malignant T-cell lymphoma	(Suzuki et al. 2001; Hagenbeek and Spits 2008; Hagenbeek et al. 2004; Xue et al. 2008)
Thyroid follicular cell-specific (*TpoCre*)	Increased induction of thyroid hyperplasia and diffuse colloid goiter, caused by an increased thyroid mitotic index. Increased neoplastic transformation within 10 months of age	(Yeager et al. 2007)
Ureteric bud epithelial cell-specific (*HoxB7Cre*)	Defective branching morphogenesis in developing mouse kidneys, mislocalization of glomeruli and post-natal lethality before P26	(Kim and Dressler 2007)
Urothelial cell-specific (*FabpCre*)	Increased urothelial hyperplasia with complete penetrance at 6–8 weeks after birth. Increased spontaneous pedicellate papillary transitional cell carcinoma and increased susceptibility to chemically-induced carcinogenesis	(Tsuruta et al. 2006; Yoo et al. 2006)
Conditional Pten knockout mice (*double mutants*)		
B-cell-specific (*CD19Cre*) *Pten & Ship1*	Development of spontaneous and lethal B cell neoplasms consistent with marginal zone lymphoma or follicular or centroblastic lymphoma	(Miletic et al. 2010)
Central nervous system-specific (*hGFAPCre*) *Pten & p53*	Complete CNS deletion of *Pten* resulted in lethal hydrocephalus in early postnatal life. *hGFAP-Cre*$^{+}$*;p53*$^{lox/lox}$*;Pten*$^{lox/+}$ mice presented with acute-onset neurological symptoms, including seizure, ataxia and/or paralysis. Mice developed penetrant acute-onset high-grade malignant glioma with clinical, pathological and molecular resemblance to primary glioblastoma multiforme in humans	(Zheng et al. 2008)

Table 8.1 (continued)

PTEN knockout mouse model	Phenotype	Reference
Endothelial cell-specific (*Tie2Cre*) *Pten & Pdk1*	Slight delay in embryonic lethality, resulting from defective vascular remodeling and cardiac development	(Feng et al. 2010)
Intestinal epithelial cell-specific (*Tg(Cyp1a1-cre)1Dwi or Tg(Vil-cre/ESR1)23Syr*) *Pten & Apc*	Accelerated tumorigenesis through increased activation of Akt, resulting in the rapid development of adenocarcinoma	(Marsh et al. 2008)
Keratinocyte-specific (*k5Cre*) *Pten & Smad4*	Development of early onset hyperplasia and dysplasia in the esophageal and forestomach epithelia and accelerated tumor formation in the forestomach. Squamous cell carcinomas developed at 1 month of age, which progressed to invasive SCC with 100% penetrance by 2 months. Mice exhibited progressive growth retardation and post-natal death between P10 and P100	(Teng et al. 2006)
Prostate-specific (*PBCre4*) *Pten & Trp53*	Development of invasive prostate cancer by 2 weeks post-puberty, with disease progression and death prior to 7 months of age	(Chen et al. 2005)
Renal tubular cell-specific (*NseCre*) *Pten & TSC1*	Development of severe polycystic kidney disease and increased post-natal lethality	(Zhou et al. 2009)
T-cell-specific (*LckCre*) *Pten & Mnk1/Mnk2*	Suppression of malignant T-cell lymphoma associated with T-cell-specific deletion of Pten in mice	(Ueda et al. 2010)
Urothelial cell-specific (*AdenoCre—surgical delivery*)*Pten & p53*	Development of bladder tumors with 100% penetrance by 6 months of age, displaying the histological features of carcinoma in situ, as well as high-grade invasive carcinoma	(Puzio-Kuter et al. 2009)

observed in multiple tissues, signifying haploinsufficiency of *Pten* in mouse models (Di Cristofano et al. 1998; Podsypanina et al. 1999; Suzuki et al. 1998).

Indeed, PTEN is frequently mutated or its expression lost in many human cancers including glioblastomas, breast, kidney and uterine endometrioid carcinomas, lung cancer, colon cancer, and melanoma (Jiang and Liu 2008; Salmena et al. 2008; Steck et al. 1997). The incidence of somatic mutation or deletion of *PTEN* is high in high-grade glioblastoma (estimated prevalence 30–40%), breast (10%), melanoma (7–20%), prostate cancer (15%), and endometrial cancer (50%). *PTEN* mutations have been reported at lower rates in bladder, lung, ovary, colon cancers, and in lymphoma (Cairns et al. 1997; Gronbaek et al. 1998; Kim et al. 1998; Kohno et al. 1998;

Sansal and Sellers 2004). *PTEN* inactivation results in multiple abnormal processes leading to abnormal cell polarity and invasion, cell proliferation and survival, cell architecture, chromosomal integrity, cell cycle progression, and stem cell self-renewal. In the early stages of some cancers, including prostate, breast, colon, or lung cancers, monoallelic *PTEN* mutation or deletion is detected, however, the second *PTEN* allele remains active (Salmena et al. 2008), and it is only in late stage or metastatic cancers that biallelic loss of *PTEN* is observed. Most *PTEN* mutations lead to loss of phosphatase activity both to phosphoinositide and protein substrates.

Examination of tissue-specific *Pten* deletion in mice further supports a role for Pten in the prevention of tumorigenesis, with hyper-proliferation and neoplastic change observed in tissues where *Pten* is selectively inactivated (Backman et al. 2004; Horie et al. 2004; Yanagi et al. 2007; Yeager et al. 2007). Furthermore, injection of *Pten*-deficient ES cells, PTEN-null tumor cells or catalytically inactive PTEN mutants in nude or syngeneic mice results in enhanced tumor generation, due to increased anchorage-independent cell growth and abnormal differentiation (Di Cristofano et al. 1998; Li and Sun 1998). Studies in cell culture models have revealed loss of PTEN function results in increased proliferation, growth and survival, correlating with increased basal PtdIns(3,4,5)P_3 levels and enhanced Akt activation (Subramanian et al. 2007; Li and Sun 1998; Furnari et al. 1998; Myers et al. 1998; Stambolic et al. 1998; Lee et al. 1999; Davies et al. 1998; Haas-Kogan et al. 1998). Conversely, expression of PTEN induces apoptosis and promotes cell cycle arrest (Furnari et al. 1998; Li and Sun 1998; Maehama and Dixon 1998; Davies et al. 1998; Koul et al. 2001; Tamura et al. 1999a; Stambolic et al. 1998).

PTEN's haploinsufficiency is sufficient to promote tumor formation. Analysis of a series of hypomorphic mouse mutants, developed in order to assess a correlation between PTEN levels and cancer progression, reveals that heterozygous *Pten* loss in a mouse model of prostate cancer leads to prostate epithelial hyperplasia and low-grade lesions with incomplete penetrance (Trotman et al. 2003). Further reduction in *Pten* is associated with massive prostate hyperplasia in all mice, with accelerated tumor progression, and complete loss of *Pten* leads to highly invasive and aggressive cancer. These studies suggest the reduction in PTEN expression below the heterozygous loss of function level may lead to more aggressive cancer. Recently it was reported even a subtle reduction (20%) in *Pten* expression may promote cancer susceptibility in mice. *Pten* hypermorphic mice ($Pten^{hy/+}$), which express 80% of normal levels of Pten, develop a variety of tumors, with the most common being breast cancer (Alimonti et al. 2010). These mice show reduced survival, develop autoimmune disease, with lymphadenopathy and splenomegaly, but significantly, females show an increased susceptibility to epithelial cancers including breast cancer (~75%) and uterine cancer (67%). Even this small reduction in PTEN by 20% is sufficient to promote the activation of a pro-proliferative gene expression signature.

Interestingly, in some tissues complete loss of PTEN from untransformed cells may trigger cellular senescence, a process which protects against tumor initiation (Alimonti et al. 2010). Cell senescence is a very stable form of cell cycle arrest, which is activated in response to stress, including oncogenic signaling and telomere shortening (Collado and Serrano 2010). Recent studies have revealed constitutive

activation of Akt promotes senescence via inhibition of the transcription factors FOX01/03 (Nogueira et al. 2008).

Altered PTEN expression in cancer may be due to inherited germ line mutations, sporadic mutations or chromosomal alterations, transcriptional repression, epigenetic silencing, post-transcriptional gene regulation, post-translational modification, and aberrant PTEN localization, which can in turn regulate the initiation, progression and long term survival from cancer (Sasaki et al. 2009). Recent studies have revealed a significant role for post-transcriptional silencing of PTEN by multiple microRNAs (miRNAs) from precursors with a single hairpin structure (*miR-21*, *miR-22*, *miR-214* and *miR-205*) or from a polycistronic structure (*mir-17-92*, *mir-106b*, *mir-367-302b* and *mir-221-22*) that recognizes target sequences in PTEN and thereby regulates its expression, reviewed in (He 2010). Interestingly, the related *PTENP1* may act as a decoy for the same miRNA species to rescue *PTEN* loss of expression (He 2010). Consistent with this contention, *PTENP1* chromosome deletion has been reported in colon and breast cancer associated with decreased PTEN expression (Poliseno et al. 2010b).

PTEN localizes to the cytosol, plasma membrane and nucleus, and its function as a tumor suppressor appears to be dependent on its appropriate localization within the cell. Numerous inactivating mutations of *PTEN* have been identified in both hereditary and spontaneous human cancers that map to the phosphatase domain of *PTEN*, however, many mutations map to areas outside of this region (Marsh et al. 1998). These residues may be crucial for the regulation of protein stability, or localization patterns. For example, mutations that map to the C2 domain, or that disrupt the phosphatase/C2 domain interface, have been identified, which prevent the correct positioning of PTEN at the plasma membrane and thereby access to PtdIns(3,4,5)P_3 (Lee et al. 1999). Loss-of-function hereditary mutations have also been described within the promoter region of *PTEN*, leading to increased Akt signaling (Zhou et al. 2003). As described earlier, the nuclear import-defective PTEN mutants, K289E and K13E, are associated with hereditary and spontaneous cancer respectively (Trotman et al. 2007; Duerr et al. 1998). PTEN mutations within the conserved polybasic amino-terminal motif required for PtdIns(4,5)P_2 binding have also been identified in sporadic cancer. While retaining catalytic activity, mutation of PTEN within this region disrupts PtdIns(4,5)P_2 binding, thus preventing the correct orientation of PTEN at the plasma membrane and access to PtdIns(3,4,5)P_3 (Han et al. 2000; Walker et al. 2004).

Loss of PTEN is associated with highly invasive cancer, implicating this enzyme in cell motility and invasion. Indeed, disruption of *Pten* in *D. discoideum* impairs cell polarization, actin polymerization and both the speed and directionality of cell migration (Iijima and Devreotes 2002; Funamoto et al. 2002). In contrast, disruption of PTEN in numerous mammalian cells increases cell migration (Gao et al. 2005; Gu et al. 1999; Liliental et al. 2000; Suzuki et al. 2003b; Tamura et al. 1998, 1999b). Chemoattractant-induced Transwell migration of Pten-deficient murine neutrophils is increased, however, defects in directionality are evident in single-cell chemotaxis assays (Subramanian et al. 2007) and in the prioritization of chemoattractant cues (Heit et al. 2008). On the other hand, these results contrast with studies in human

cell lines that show that while loss of PTEN increases the rate of chemokine-induced migration, it does not affect directionality (Lacalle et al. 2004). Also in primary neutrophils a more dominant role for the PtdIns(3,4,5)P_3 5-phosphatase, SHIP1, than PTEN has been shown (Nishio et al. 2007). Interestingly, subventricular zone precursor cells (Li et al. 2002a) and isolated primary B cells (Fox et al. 2002) from *Pten*$^{+/-}$ mice also exhibit greater motility than wild-type cells, providing further evidence that in mice, *Pten* is haploinsufficient.

PTEN may exert a tumor suppressive effect via regulation of surrounding fibroblasts. Recently PTEN loss of function has been linked to the regulation of stroma and tumor cell signaling (Trimboli et al. 2009). Genetic inactivation of *Pten* in stromal fibroblasts of mouse mammary glands accelerates the initiation and malignant transformation of mammary epithelial tumors. Notably there was significant remodeling of the extracellular matrix with increased angiogenesis. Global gene expression profiling of PTEN-depleted mammary stromal cells reveals activation of the Ets2-specific transcription program (Trimboli et al. 2009). *Ets2* inactivation in *Pten* stroma-deleted tumors decreases tumor growth and progression, revealing the Pten–Ets2 axis as a stroma-specific signaling pathway that suppresses mammary epithelial tumors.

8.2.7 The Tumor Context of PTEN Loss of Function

There are several points which will be considered here regarding the occurrence of changes in other oncogenes and tumor suppressors in tumors, in addition to PTEN. Firstly, does additional activation of other components of the broad PI3K/growth factor receptor signaling network occur in PTEN null tumors? Secondly, are there other independent pathways that are favorably mutated in parallel with PTEN in certain tumor types? And thirdly, are there oncogenes and tumor suppressors that affect malignant transformation indirectly through their influence on PTEN function, in the way that murine double minute 2 (MDM2) amplification drives tumorigenesis through suppression of p53 function?

In other major functional pathways implicated in cancer, such as the Rb and MDM2/p53 pathways, mutation of more than one component of the same definable functional pathway is generally very rare (Cancer Genome Atlas Research Network 2008). However, in the PI3K pathway, mutation of multiple components, such as both PTEN and p110α PI3K is not uncommon (Yuan and Cantley 2008; Cancer Genome Atlas Research Network 2008). Because many factors independently influence cellular PtdIns(3,4,5)P_3 levels and localization, and because there are many PtdIns(3,4,5)P_3-binding proteins and Akt substrates, that regulate diverse biological processes with different dose responses to changes in PtdIns(3,4,5)P_3 levels, it seems unsurprising that multiple mutations within the pathway are able to drive further selective advantages to a tumor.

Extensive genomic and expression analyses of large numbers of tumors has confirmed their classification into identifiable groups that share patterns of genomic and

expression changes. For example, 94% of the "classical" sub-type of glioblastomas share high level amplification of EGFR and homozygous deletion of CDKN2A (Verhaak et al. 2010). However, in many tumor types, PTEN loss is common in many independently identifiable sub-groups of tumors. For example, in a categorization of glioblastomas, loss of one allele of PTEN occurs at very high levels in all four categories of glioblastoma, with lowest frequency being in "proneural" tumors, but still as high as 67% (Verhaak et al. 2010). However, there are cases with notable association of PTEN mutation with other events. For example, several studies have identified a strong association between TMPRSS2-ERG translocations and loss of PTEN in a large fraction of human prostate cancers (Taylor et al. 2010) and have demonstrated the functional significance of this genetic interaction in transgenic mice (Carver et al. 2009). Similarly, co-operating pathways have been identified in experiments in mice, with for example, experimental deletion of Pten from T-cells leading to lymphomas that are also found to display myc translocations (Liu et al. 2010).

Finally, data has emerged over the last few years showing that several regulators of PTEN function are mutated or over-expressed in tumors and that this may represent a mechanism by which they affect tumor development indirectly through the regulation of PTEN. These include P-REX2 and SIPL1 that appear to directly inhibit PTEN activity, RAK and PICT1/GLTSCR2 that stabilize the PTEN protein and the E3 ubiquitin ligase, NEDD4 (Fine et al. 2009; He et al. 2010; Okahara et al. 2006; Wang et al. 2007; Yim et al. 2009). These findings have been recently reviewed in depth (Leslie et al. 2010).

8.2.8 PTEN and Cellular Senescence

Loss of PTEN function appears to be a common early event in endometrial cancer (Mutter et al. 2000). However, this appears not to be the case in most other tumor types, and PTEN loss has been described at highest frequencies in late stage tumors in many tissues (Salmena et al. 2008). In particular, complete *PTEN* loss is infrequent in early stages of prostate cancer (Taylor et al. 2010). Interestingly, acute complete loss of PTEN may induce cell senescence, a cellular program in mitotic cells that induces irreversible growth arrest as an anti-tumor mechanism. This program may be initiated by tumor suppressor genes in response to DNA damage and/or oncogene activation (Campisi and d'Adda di Fagagna 2007). Complete acute loss of *Pten* from untransformed cells, rather than promoting enhanced proliferation, induces a significant senescence response that opposes tumor progression associated with enhanced p19Arf–p53 signaling (Chen et al. 2005). Therefore, complete loss of PTEN can oppose tumorigenesis by triggering a p53-dependent cellular senescence response. Inactivation of PTEN concomitantly with *p53* allows escape from senescence, promoting invasive cancer. PTEN-induced cell senescence has also been shown in primary human epithelial cells (Kim et al. 2007). PTEN-loss—induced cellular senescence occurs rapidly after Pten inactivation, in the absence of

cellular proliferation and DNA damage checkpoint responses, and is associated with enhanced p53 translation. Pharmacological inhibition of PTEN or p53-stabilizing drugs potentiates senescence and its tumor suppressive potential (Alimonti et al. 2010).

Pten-loss—induced cellular senescence exerts its effects using molecular pathways distinct from oncogenic-induced cell senescence. Skp2 protein is an E3-ubiquitin ligase that mediates degradation of a number of proteins, including p21 and p27. Mice lacking Skp2 are viable, but *Skp2* inactivation restricts tumorigenesis by promoting cellular senescence during oncogenic conditions, such as following expression of the Ras oncoprotein. In the absence of Skp2, cells lacking Pten become more sensitized to senescence (Lin et al. 2010). Mice with loss of one copy of *Pten* and deficient in Skp2 ($Pten^{+/-}$; $Skp2^{-/-}$) are protected from cancer. Recent studies have revealed PTEN also acts as a critical determinant of cell fate between senescence and apoptosis in several glioma cell lines in response to ionizing radiation. Depletion of Akt or scavenging of reactive oxygen species (ROS) prevents radiation-induced senescence in PTEN-deficient glioma (Lee et al. 2011).

8.2.9 PTEN and the Brain

As previously described, germline mutations in *PTEN* are associated with Cowden disease, Lhermitte-Duclos disease and Bannayan-Zonana syndrome, which are collectively described as the PTEN hamartoma tumor syndromes (PHTS) (Salmena et al. 2008). Whether these are indeed separate syndromes, or only one syndrome with a broad clinical spectrum remains to be determined, however, neurological abnormalities are a prominent feature of these disorders. PTEN is widely expressed in the mammalian brain and is enriched in large pyramidal, Purkinje, olfactory and mitral neurons. Conditional deletion of *Pten* specifically in the mouse brain results in macrocephaly and increased neurological defects including seizures and ataxia, commencing within 6–9 weeks of birth (Groszer et al. 2001; Backman et al. 2001; Kwon et al. 2001). The macrocephaly is associated with abnormal brain patterning, as a consequence of increased neuronal cell number, enhanced cell survival, and enlarged cell size, as a result of enhanced PI3K/Akt signaling, subsequent activation of the mTOR complex and the promotion of protein translation. $Pten^{-/-}$ mice additionally exhibit dysplasia of neuronal cell populations, abnormal synaptic structures and severe myelination defects. The number of neuronal stem cells in the fetal brain is also regulated by PTEN, with loss of PTEN in neuronal stem cells shown to stimulate stem cell proliferation and enhance self-renewal capacity (Groszer et al. 2006). The observed phenotypes in $Pten^{-/-}$ mice recapitulate many of those described in PHTS, in particular Lhermitte-Duclos disease (Waite and Eng 2002). In addition, PTEN function is implicated in depression and anxiety (Bandaru et al. 2009), and also in response to drug addiction (Ji et al. 2006). Pharmacological inhibition of PI3K by wortmannin treatment leads to increased depression and anxiety (Bandaru et al. 2009) and impaired fear memory (Lin et al. 2001). Conditional knockout of

Pten in specific differentiated neurons results in increased anxiety and decreased social interaction suggesting that either up- or down-regulation of PI3K signaling, and thus too much or too little PtdIns(3,4,5)P_3, is critical to these processes (Kwon et al. 2006).

PTEN not only controls neuronal cell number and size but is also implicated in regulating responses to neuronal injury, including brain ischemia (Chang et al. 2007). Increased expression of PTEN in hippocampal neurons promotes neuronal cell death following exposure to the excitatory amino acid glutamate via regulation of the Akt signaling pathway, while decreased expression of endogenous Pten increases neuron resistance to seizure-induced cell death (Gary and Mattson 2002). In a rat model of transient cerebral ischemia, PTEN phosphorylation is enhanced in the ischemic core (Omori et al. 2002), which is proposed to decrease PTEN phosphatase activity (Torres and Pulido 2001) and thereby increase Akt signaling. In a rat model of chronic exposure to ethanol, increased PTEN expression is associated with cerebellar hypoplasia and increased neuronal cell death (reviewed in (Chang et al. 2007)). Therefore, downregulating the activity and/or expression of PTEN may be a novel therapeutic treatment for brain injury.

The precise molecular mechanisms surrounding the role of PTEN in ischemic neurodegeneration remain to be determined, and PTEN may co-operatively regulate numerous down-stream effectors. A direct role for PTEN has been described in the regulation of neurodegeneration during oxidative stress via mitochondria-dependent apoptosis. Neuronal precursor cells, that lack one copy of *PTEN*, exhibit increased resistance to oxidative stress-induced apoptosis (Li et al. 2002b). In many cells in response to growth factor stimulation, PTEN translocates to the plasma membrane to degrade PtdIns(3,4,5)P_3 and inhibit Akt signaling; however in staurosporine-treated hippocampal neurons, mitochondrial accumulation of PTEN is observed, consistent with a role for PTEN in the regulation of mitochondria-dependent apoptotic pathways. Knockdown of PTEN protects hippocampal cells from apoptotic damage by inhibiting staurosporine-induced release of cytochrome c and caspase-3 activity (Zhu et al. 2006b). Down-regulation of PTEN decreases the activity of apoptosis signal-regulating kinase 1 (ASK1), an upstream component of the mitogen-activated protein kinases (Wu et al. 2006). The activity of the pro-apoptotic c Jun N-terminal kinases 1/2 is reduced as a result of decreased PTEN activity and increased Akt activation following ischemic injury (Zhang et al. 2007). PTEN regulates the generation of intracellular ROS in response to oxygen–glucose deprivation (OGD) and neurotoxin 1-methyl-4-phenylpyridinium iodide (Zhu et al. 2007). Suppression of PTEN activity also complexes with the *N*-methyl-d-aspartate (NMDA) receptor (NMDAR), a subtype of excitatory glutamate receptor that promotes excitotoxicity-induced neuronal death, to inhibit extrasynaptic NMDAR activity, leading to protection against ischemic neuronal death *in vitro* and *in vivo* (Ning et al. 2004).

PTEN may also play a role in regulating neurodegeneration associated with Alzheimer disease (AD) and Parkinson disease. Decreased PTEN expression has been reported in the hippocampus of AD brains (Griffin et al. 2005). Three mitochondrial associated genes *parkin*, *DJ-1* and *PINK1* (PTEN-induced putative kinase 1),

have recently been shown to be associated with early-onset recessive parkinsonism (reviewed in (van der Vegt et al. 2009)). PINK1 is a serine/threonine kinase, which is transcriptionally activated by PTEN (Valente et al. 2004), and phosphorylates TRAP1, preventing oxidative stress-induced mitochondrial cytochrome *c* release (Pridgeon et al. 2007). Parkin is an E3 ubiquitin ligase and DJ-1 functions in parallel to the PINK1/parkin pathway to maintain mitochondrial function in the presence of oxidative stress (Inzelberg and Jankovic 2007). Mutations in *PINK1* occur in familial Parkinson disease and loss of PINK1 function increases sensitivity to oxidative stress, followed by neuronal death (Gispert et al. 2009). DJ-1 inhibits PTEN's negative regulation of the PI3K/Akt/mTOR pathway, which exerts a pro-survival effect during oxidative stress (Delgado-Esteban et al. 2007). siRNA knock-down of DJ-1 enhances cellular sensitivity to oxidative stress in Drosophila and mice brains (Kim et al. 2005; Shendelman et al. 2004). DJ-1 and PINK1, with PTEN, may contribute to the regulation of neuroprotection and neurodegeneration. Thus when PTEN levels are high, DJ-1 signaling will decrease and the PI3K/Akt/mTOR pathway will be suppressed, enhancing sensitivity of neuronal cells to oxidative stress. When PTEN levels are low, PINK1 levels will also be low, and oxidative stress-induced neuronal mitochondrial death pathways may be enhanced (Bonifati et al. 2003).

8.2.10 The PTEN and p53 Association

Over several years, many studies have implied a specific functional connection between PTEN and p53, the stress-induced transcription factor that is itself the most frequently mutated tumor suppressor in human cancers (Liu et al. 2008). p53 may influence PTEN expression through the presence of potential p53-binding sites in the *PTEN* promoter. This mediates induction of *PTEN* transcription, as part of a p53 transcriptional program in response to stresses, such as gamma irradiation (Stambolic et al. 2001). Conversely, PTEN has been reported to affect p53 in several ways. p53 expression is normally maintained at low levels by the action of a proto-oncogenic E3 ubiquitin ligase, MDM2. Two reports simultaneously described the phosphorylation of MDM2 by the PTEN-regulated kinase, AKT, and showed that MDM2 ligase activity is increased by phosphorylation on these sites (Mayo and Donner 2001; Zhou et al. 2001). PTEN activity, or inhibition of PI3K, suppresses MDM2 transcription by negatively regulating its P1 promoter (Chang et al. 2004), thereby enhancing p53 levels. A direct physical interaction between PTEN and p53 has also been reported, and PTEN-null ES cells and immortalized mouse embryonic fibroblasts (MEFs) exhibit reduced p53 levels (Freeman et al. 2003). Together these studies provide good evidence that loss of PTEN can lead to reduced p53 function in several settings. However, more recent work has added a complication to this picture, showing that genetic deletion of both *Pten* copies from primary MEFs, or in the murine prostate, leads to an induction of p53 expression and cellular senescence as described here earlier (Chen et al. 2005). A resolution to these somewhat contradictory findings and a better understanding of the complex relationship(s) between PTEN and p53

will require further study, and may be critical given the frequency with which both tumor suppressors are deregulated in cancer.

8.2.11 PTEN Function and Stem Cell Fate

Much of our knowledge of PTEN function comes from tissue-specific genetic deletion of *Pten* in mouse models (see Table 8.1). Several such mouse models have revealed the specific effects of Pten in the regulation of stem cell fate in different lineages. Deletion of Pten early in development from the mouse brain leads to increased numbers of neural stem cells and increases in differentiated cell numbers and size (Groszer et al. 2001). These increases in neural stem cell number are caused by promotion of the G_0-G_1 cell cycle progression and a gene expression signature associated with rapid proliferation and a rapid cell cycle (Groszer et al. 2006). In contrast, studies of mice lacking Pten in hematopoietic stem cells (HSCs) have indicated that Pten loss promotes a short term proliferation of these cells, yet leads to a long term depletion of the self-renewing stem cell compartment (Yilmaz et al. 2006). Crucially, Pten loss in HSCs leads to the rapid development of leukemia in these mice, yet this disease is characterized by the presence of Pten-null self-renewing leukemic stem cells (LSCs). These apparently distinct characteristics of normal HSCs versus LSCs suggests that their self-renewal is regulated by different mechanisms and provides hope for therapies that target LSCs, without killing normal HSCs. The short term proliferation of HSCs driven by Pten loss is associated with enhanced G_0-G_1 cell cycle transition, as observed in neuronal stem cells. However, why in HSCs, this leads to a transient, rather than long term expansion in stem cell numbers, as seen in the brain, is unclear. Studies of other lineages imply that other stem cell populations can be similarly classified, with Pten loss in melanocyte stem cells also leading to long term stem cell expansion (Inoue-Narita et al. 2008). This proposed role for PTEN in the maintenance of the normal stem cell compartment is supported by the identification of PTEN as the most significantly reduced transcript in microarray analyses of gene expression differences between normal fetal neuronal stem cells and glioblastoma stem cells isolated from brain tumor patients (Pollard et al. 2009). By regulating PTEN expression this may allow in future therapeutic approaches that target cancer stem cells (or cancer-initiating cells) without affecting normal stem cell compartments (Rossi and Weissman 2006).

8.2.12 PTEN and the Immune System

The role of the PI3K signaling network in the immune system has been studied extensively. This research activity has been driven by the recognition that PI3K signaling plays key roles in the survival and proliferation of several normal and transformed immune cell populations and also in other immune cell functions such as migration,

phagocytosis and reactive oxygen species generation (Fruman and Bismuth 2009). Of particular significance was the initial discovery that mice selectively lacking the gamma catalytic isoform of PI3K (p110γ) were viable and fertile, yet their T-cells and neutrophils displayed reduced chemotaxis *in vitro* and recruitment in *vivo* (Hirsch et al. 2000; Sasaki et al. 2000; Li et al. 2000). This has lead to intense drug discovery activity attempting to target p110γ, and also p110δ, for inflammatory diseases, that have been sustained by subsequent discoveries (Ruckle et al. 2006).

The tumor suppressor activity of PTEN is important in the avoidance of both T cell and B cell malignancies, due to its roles in promoting non-proliferative states and apoptosis. Studies of mice, in which Pten has been specifically deleted in immune cell populations have also identified roles for the phosphatase in the suppression of B-cell and neutrophil migration *in vivo* (Suzuki et al. 2003b; Subramanian et al. 2007; Heit et al. 2008; Li et al. 2009). Furthermore, Pten-null macrophages exhibit elevated phagocytosis and Fcγ-mediated signaling (Cao et al. 2004). Given these specific actions of PTEN to limit the selection and proliferation of immune cell populations and also in suppressing immune cell activities, it is not surprising that autoimmunity is a striking phenotype of Pten loss in mice. As discussed, tight regulation of PI3K and thus PtdIns(3,4,5)P_3 levels is important for normal development and responsiveness of the immune system and also to prevent immunopathology. Some studies have shown that diminished PI3K activity can also lead to similar autoimmune conditions (Oak et al. 2006), while hyperactivation of this pathway in T-cells also leads to lymphoproliferation and systemic immunity (Suzuki et al. 2001). Consistent with this, Pten heterozygous ($Pten^{+/-}$) mice develop lymphoproliferative syndrome with autoimmune features (Di Cristofano et al. 1999; Suzuki et al. 2001). Several mechanisms have been proposed for PTEN's role in suppressing autoimmunity. PTEN can regulate Fas-mediated apoptosis (an autoimmunity repression mechanism) and also IL-4 production and thus regulate CD4(+) T-cell function (Di Cristofano et al. 1999; Liu et al. 2010). Mice with higher expression of microRNA miR-17-92 have been shown to have similar autoimmune disorders and this is believed to be due to miR-17-92-mediated suppression of PTEN expression (Xiao et al. 2008).

8.2.13 PTEN Function in Epithelial Biology

The frequent loss of PTEN function observed in epithelial-derived solid tumors (carcinomas) has stimulated investigations into the functions of PTEN in normal and transformed epithelial biology. Significantly, several pieces of evidence point towards distinct roles for PTEN and PI3K signaling in epithelia, regulating both cell growth, proliferation and also cell polarity/architecture (Liu et al. 2004; Leslie et al. 2008). In this context, it is notable that PtdIns(3,4,5)P_3 is found to be enriched in the basolateral membrane of several epithelial cell types (Liu et al. 2004; Watton and Downward 1999) and PTEN appears to be strongly enriched in the apical domains of some but not all, epithelial cell types, such as the *Drosophila melanogaster* (*D. melanogaster*) photoreceptors and embryonic epithelia (von Stein et al. 2005; Pinal

et al. 2006), chick epiblast (Leslie et al. 2007) and murine retinal pigment epithelium (Kim et al. 2008). These localization data and the identified interactions of PTEN with proteins such as PAR3 and MAGI1 indicate that PTEN is enriched in apical cell-cell junctional complexes, almost certainly including adherens junctions (Pinal et al. 2006; von Stein et al. 2005; Kotelevets et al. 2005). Functional studies in cultured epithelial cells suggest that PTEN acts to suppress PI3K-dependent epithelial cell-cell junctional destabilization (Kotelevets et al. 2001; Martin-Belmonte et al. 2007; Liu et al. 2004). In particular, in epithelial cell lines cultured in 3D matrices, the knockdown of PTEN expression leads to a dramatic loss of cell polarity and tissue organization (Martin-Belmonte et al. 2007). Knockdown of PTEN in normal human mammary epithelial cells causes the formation of disorganized hyperplastic lesions when introduced into humanized murine mammary fat pads (Korkaya et al. 2009).

The phenotypes of mice with different epithelial tissue-specific deletion of Pten, reveals a range of related effects but dramatic differences in the severity of the phenotype. Inducible deletion of Pten from the intestinal epithelium has no overt effects on the morphology of the tissue in the absence of further alterations (Marsh et al. 2008). Deletion of PTEN from the kidney leads to defects in branching morphogenesis within the organ, which although not affecting the integrity of the epithelium still appear responsible for the death of these animals within a month of birth (Kim and Dressler 2007). Similarly, MMTV-Cre driven mammary-specific deletion of PTEN in mice leads to hyperproliferative development of the gland with dysregulated ductal branching and mammary tumors (Li et al. 2002a). In addition, deletion of Pten in the lung leads to increased epithelial cell size, abnormal lung morphogenesis and adenocarcinoma in mice that escape the hypoxic postnatal lethality (Yanagi et al. 2007). Perhaps most remarkably, deletion of Pten from the retinal pigment epithelium, that, significantly, displays a strongly polarized localization of the Pten protein, causes a full EMT and complete disruption of the tissue (Kim et al. 2008). This supports a model in which PTEN function plays a role in establishing, although perhaps less significantly maintaining, epithelial cell-cell junctions, with loss of PTEN driving a shift to a more motile, mesenchymal phenotype (Leslie et al. 2007; Song et al. 2009; Kim et al. 2008).

8.3 Myotubularins

The myotubularins are a large family of conserved proteins in eukaryotes. They dephosphorylate PtdIns(3)P and PtdIns(3,5)P_2, to form PtdIns and PtdIns(5)P respectively. Despite the large number of myotubularins (MTMs) and shared specificity for their substrates, mutations in individual myotubularins can cause different human diseases. Myotubularins exhibit varied sub-cellular localization, and integrate into protein/signaling complexes. These lipid phosphatases are required for the cellular control of diverse processes, including ion channel-stimulated excitation-contraction coupling and signaling, endocytic trafficking, autophagy and cell proliferation.

The myotubularins are defined by conserved myotubularin-related and dual specific protein tyrosine phosphatase CX_5R-containing domains. 16 myotubularins have been described in mammalian cells, the first was designated myotubularin (MTM1), and the other family members "myotubularin-related phosphatases" (MTMR1-15) (Table 8.2). Seven of the myotubularins possess inactivating mutations within the catalytic motif, and are phosphatase dead, however, a number of these proteins still play an active role in cellular functions and when mutated can cause disease (Begley et al. 2006). Myotubularins possess a number of additional domains, conserved across most family members; including PH-GRAM (Pleckstrin-Homology-Glucosyltransferase, Rab-like GTPase Activator), and coiled-coil domains (Laporte et al. 2003) (Fig. 8.2). However, the two most recently described myotubularins, MTMR14 and MTMR15, exhibit very little homology at the PH-GRAM or coiled-coil regions. Myotubularins also contain conserved SID (Set Interacting Domain), and RID (Rac Induced Recruitment Domain) domains (Begley et al. 2003, 2006; Laporte et al. 2003). Individual myotubularins also contain a number of additional protein or lipid interacting modules, described in more detail below.

A phylogenetic survey of 31 different species across broad taxonomic groups counted varied complements of different myotubularins, ranging from one in fungi such as *Saccharomyces cerevisiae* and *Aspergillus niger*, to 19 in *Entamoeba histolytica* (Kerk and Moorhead 2010). Additionally, the inactive myotubularin homologs appear to have evolved on three separate occasions in different eukaryotic lineages, highlighting their presence as more than simply an interesting observation. Phylogenetic analysis also permits the grouping of the myotubularins into similarity clusters—three for active myotubularins, and three for inactive (See Fig. 8.2) (Laporte et al. 2003; Kerk and Moorhead 2010). Some organisms with lower complements of myotubularins have representatives of these groups (Robinson and Dixon 2006; Laporte et al. 2003). For example, for *Caenorhabditis elegans* (*C. elegans*) these are: M1-mtm-1 (CeY110A7A.5); R3-mtm-3 (CeT24A11.1); R6-mtm-6 (CeF53A2.8); R5-mtm-5 (CeH28G03.6) and mtmr-9 (CeY39H10A) (Xue et al. 2003). Recently, MTMR14 (also known as hJumpy or MIP) has been characterized, and it contains an active CX_5R phosphatase motif and homology to the myotubularin-related domain, but no PH-GRAM domain (Alonso et al. 2004; Tosch et al. 2006; Vergne et al. 2009). A sixteenth myotubularin has also been identified, MTMR15, which shares little homology with other myotubularins, and has an inactive phosphatase site (Alonso et al. 2004). This protein functions as a DNA repair enzyme and as such, its characterization as a myotubularin awaits further clarification. Many myotubularins (MTM1, MTMR1-6 and MTMR12) are expressed in a broad range of tissues and cell types (Laporte et al. 1998; Nandurkar et al. 2001; Zhao et al. 2001). In contrast, MTMR5 is concentrated in the testis, and MTMR7 shows a brain-specific expression (Laporte et al. 1998; Firestein et al. 2002). Non-redundancy may be a result of differences in the sub-cellular distribution of various myotubularins, and also by different protein and lipid interactions. This in turn may regulate myotubularin access to specific pools of PtdIns(3)P and/or PtdIns(3,5)P_2 in a directed manner, with different functional consequences (Kim et al. 2002; Lorenzo et al. 2006), as described below.

Table 8.2 Genetically modified myotubularin animal models

Protein name	Alias(es)	Activity	Knockout animal models	References
MTM1 (myotubularin)	CNM, MTMX, XLMTM	Active	Mouse constitutive KO—some embryonic lethality, growth retardation, muscle weakness, premature death, ultrastructural changes in muscle triads, decreased calcium release from SR Labrador retriever model—muscle weakness, ultrastructural changes in triads	(Buj-Bello et al. 2002b; Beggs et al. 2010)
MTMR1		Active	Not reported	
MTMR2	CMT4B, CMT4B1, KIAA1073	Active	Mouse constitutive KO report (1)—some embryonic lethality, growth retardation, minimal motor change, abnormal neurophysiology and histopathology, azospermia Mouse constitutive KO report (2)—minimal motor change, abnormal neurophysiology and histopathology	(Bonneick et al. 2005; Bolino et al. 2004)
MTMR3	FYVE-DSP1, KIAA0371	Active	Not reported	
MTMR4	FYVE-DSP2, KIAA0647	Active	Not reported	
MTMR5	sbf1	Inactive	Mouse constitutive KO—smaller testes and infertile, azospermia	(Firestein et al. 2002)
MTMR6		Active	Not reported	
MTMR7		Active	Not reported	
MTMR8	FLJ20126, FLJ60798	Active	Not reported	
MTMR9	LIP-STYX	Inactive	Not reported	
MTMR10	FLJ20313	Inactive	Not reported	
MTMR11	CRA	Inactive	Not reported	
MTMR12	3-PAP	Inactive	Not reported	
MTMR13	sbf2, CMT4B2, FLJ22918, FLJ41627, KIAA1766	Inactive	Mouse constitutive KO (two reports)—mild motor phenotype, abnormal neurophysiology and nerve histopathology	(Tersar et al. 2007; Robinson et al. 2008)
MTMR14	hJumpy, MIP, FLJ22405	Active	Mouse constitutive KO—decreased performance on strenuous exercise, abnormalities of excitation contraction coupling, calcium leak from SR, decreased stimulated calcium release from SR	(Shen et al. 2009)
MTMR15	KIA1018	Inactive	Not reported	

XLMTM X-linked myotubular myopathy, *CMT* Charcot-Marie-Tooth, *SR* sarcoplasmic reticulum

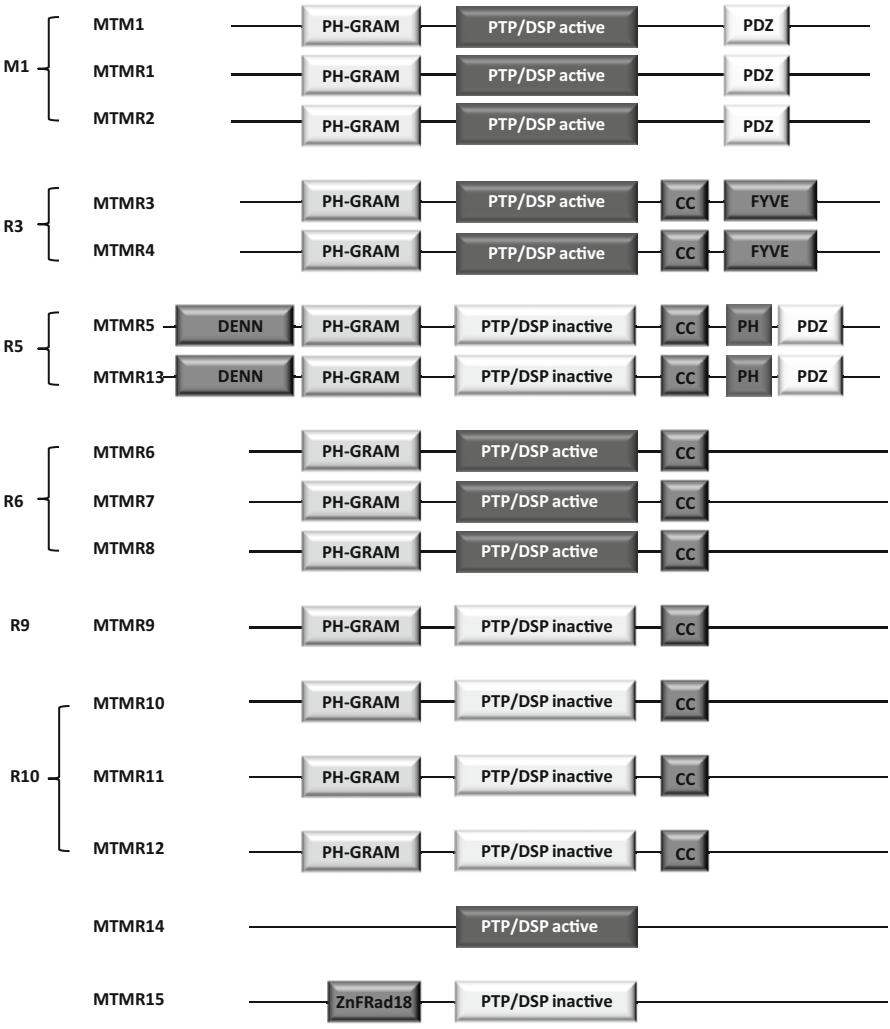

Fig. 8.2 Schematic representation of the major domains of the human myotubularins. Groups are named as reported by (Laporte et al. 2003; Kerk and Moorhead 2010). Domain associations were obtained from searches on conserved domains using NCBI/CDD and SMART databases, as well as those reported by (Laporte et al. 2003). Abbreviations: *PH-GRAM* Pleckstrin-Homology-Glucosyltransferase, Rab-like GTPase Activator; *PTP/DSP* Protein tyrosine phosphatase/dual specific phosphatase; *CC* Coiled-coil; *PDZ* Post synaptic density protein, Drosophila disc large tumor suppressor, zonula occludens-1 protein; FYVE, Fab1, Yotb, Vac1p and EEA1; *DENN* Differentially Expressed in Neoplastic versus Normal cells; ZnFRad18, Rad18-like CCHC Zinc finger

8.3.1 Myotubularin Structure and Substrate Specificity

The crystal structure of MTMR2 has been solved, and shows a larger active site pocket than most other protein tyrosine phosphatases, which is postulated to be required to accommodate the large inositol head group of phosphoinositides (Begley et al. 2003). The "WPD" loop also differs from most protein tyrosine phosphatases, lacking an aspartic acid, prompting Begley et al. to speculate a unique phosphatase action for the myotubularins (Begley et al. 2003). The tertiary structure also reveals that the then-named "GRAM domain" (Doerks et al. 2000) is similar to the PH domain, a phosphoinositide, phosphotyrosine and protein-protein interacting region, and that the SID domain is part of the protein phosphatase domain (Begley et al. 2003). The PH-GRAM domain may interact with PtdIns(5)P (Lorenzo et al. 2005), PtdIns(3,5)P_2, PtdIns(4)P, and PtdIns(3,4,5)P_3 (Berger et al. 2003), although this predicted role in lipid binding is not fully established. MTMR2 binds PtdIns(3)P and PtdIns(3,5)P_2 in its active site, however, no lipid binding was detected at the PH-GRAM domain (Begley et al. 2006).

Despite showing the properties consistent with the tyrosine phosphatase superfamily, the myotubularins exhibit predominant activity against 3-phosphorylated phosphoinositides (Taylor et al. 2000a; Zhao et al. 2001). PtdIns(3)P and PtdIns(3,5)P_2 are hydrolyzed at their 3-position phosphate by the MTMs, *in vitro* forming PtdIns and PtdIns(5)P respectively (Velichkova et al. 2010; Naughtin et al. 2010; Tosch et al. 2006; Berger et al. 2002, 2006; Tronchere et al. 2004; Schaletzky et al. 2003; Begley et al. 2003: Zhao et al. 2001; Walker et al. 2001; Taylor et al. 2000a; Blondeau et al. 2000). Myotubularins exhibit significantly less catalytic activity against PtdIns(3,4,5)P_3, PtdIns(3,4)P_2, PtdIns(4,5)P_2, PtdIns(4)P or PtdIns(5)P (Blondeau et al. 2000; Walker et al. 2001; Zhao et al. 2001; Berger et al. 2002; Schaletzky et al. 2003; Tronchere et al. 2004; Tosch et al. 2006). One exception is MTMR14, which exhibits phosphatase activity against a larger number of phosphoinositides (PtdIns(3,5)P_2 > PtdIns(3,4)P_2 > PtdIns(4,5)$_2$ > PtdIns(3)P) (Shen et al. 2009). However, in a separate study using immunoprecipitates from HEK293 cells, MTMR14 selectively degraded only PtdIns(3)P and PtdIns(3,5)P_2 (Tosch et al. 2006).

The cellular localization of PtdIns(3)P and PtdIns(3,5)P_2 is predominantly endosomal, and some of the myotubularins are predicted to control endosomal PtdIns(3)P levels, although there are conflicting reports. The different effects of MTM overexpression or decreased expression on endosomal PtdIns(3)P may relate to different experimental approaches. Many microscopy-based studies have detected endosomal PtdIns(3)P, by expression of GFP-tagged constructs containing two FYVE domains in tandem, with co-localization with endosomal markers. Another approach has utilized purified GST-2xFYVE as a probe, detected by GST antibodies after cellular fixation and permeabilization (Gillooly et al. 2000). Over-expression of MTM1, MTMR2-4, and MTMR14 decreases endosomal PtdIns(3)P in both cell lines as well as in primary cells (Kim et al. 2002; Kelley and Schorey 2004; Lorenzo et al. 2006; Tosch et al. 2006; Cao et al. 2007; Naughtin et al. 2010; Taguchi-Atarashi et al. 2010). Expression of the *D. melanogaster* mtm decreases endosomal PtdIns(3)P in

hemocytes (Velichkova et al. 2010). However, other studies have shown that expression of MTM1 (Laporte et al. 2002), or MTMR2 (Kim et al. 2002) does not influence endosomal PtdIns(3)P levels, and in fibroblasts from human patients with different MTM1 mutations, no alteration in endosomal PtdIns(3)P probe fluorescence was detected (Tronchere et al. 2004). Fili and colleagues used a chimeric MTM1 protein designed to be recruited to Rab5 positive membranes only upon addition of rapalogue (Fili et al. 2006). Expression of the MTM1-FKB chimera in HeLa cells did not decrease GST-2xFYVE staining until rapalogue was added, which induced MTM1 targeting to early endosomes (Fili et al. 2006). In contrast, siRNA knockdown of different myotubularins increases endosomal PtdIns(3)P in some studies (Cao et al. 2008; Naughtin et al. 2010; Taguchi-Atarashi et al. 2010; Velichkova et al. 2010). Some of the differences in the effects of myotubularin expression on PtdIns(3)P might be accounted for by the frequent reliance of over-expression of myotubularins and/or GST-2xFYVE probe over-expression and the associated difficulties in comparing cells with different expression levels. Expression of a GFP-2xFYVE probe *per se*, as opposed to application of a recombinant GST-2xFYVE probe after cellular fixation, itself interferes with PtdIns(3)P-dependent events (Vieira et al. 2004; Gillooly et al. 2000). In addition, most studies have presented qualitative representative images of cells with little quantification. Of special note, Cao et al. used siRNA to MTM1 or MTMR2, and with quantitation demonstrated increased PtdIns(3)P in cells by analysis of GFP-2xFYVE fluorescence (Cao et al. 2008). Interestingly, MTM1 knockdown did not alter total GFP-2xFYVE fluorescence, but increased the fraction of GFP-2xFYVE which co-localized with the early endosomal marker, EEA1. In contrast MTMR2 siRNA increased late endosomal GFP-2xFYVE (Cao et al. 2008). Additionally, GST-2xFYVE staining was quantified in HeLa cells treated with MTMR4 siRNA (Naughtin et al. 2010). This study reported that in knockdown cells, the increased GST-2xFYVE signal did not reflect an increase in the fluorescence associated with individual endosomes, but rather was a result of a large increase in the number of GST-2xFYVE-positive endosomes per cell. This suggests a function of MTMR4 is to restrict PtdIns(3)P from certain endosomal populations.

Despite being characterized as a dual specific phosphatase, the ability of a myotubularin to utilize phosphoprotein(s) as substrates has only been identified in one study. Recently, negative regulation of TGFβ signaling by MTMR4 was demonstrated, via binding to and dephosphorylation of the R-SMADS, SMAD2 and SMAD3 (Yu et al. 2010). Expression of MTMR4 rendered cells resistant to the anti-proliferative effects induced by TGFβ treatment (Yu et al. 2010). This raises the possibility that MTMR4 and other myotubularins may exhibit biological activity against additional phosphoprotein substrates.

To determine the sub-cellular localization of specific MTMs, many studies have relied on ectopic over-expression of myotubularin in cells (Blondeau et al. 2000; Taylor et al. 2000a; Firestein et al. 2002; Walker et al. 2001; Kim et al. 2002; Berger et al. 2003, 2006; Chaussade et al. 2003; Kim et al. 2003; Tsujita et al. 2004; Robinson and Dixon 2005; Lorenzo et al. 2006; Tosch et al. 2006; Vergne et al. 2009; Taguchi-Atarashi et al. 2010; Plant et al. 2009). These various studies have reported

discordant results for the sub-cellular localization of some of the myotubularins, perhaps as a result of different experimental techniques or differences in cell types and/or the level of over-expression achieved. Multiple reports have localized recombinant MTM1 to the cytosol, at the plasma membrane and on membrane ruffles (Blondeau et al. 2000; Fili et al. 2006; Kim et al. 2002; Laporte et al. 2002; Lorenzo et al. 2006; Nandurkar et al. 2003; Taylor et al. 2000a; Tsujita et al. 2004). In contrast, endogenous myotubularin is reported to localize to early endosomes, where it regulates a pool of PtdIns(3)P (Cao et al. 2007, 2008). The only myotubularin which has been consistently reported to localize to endosomes is MTMR4, which contains a FYVE domain. When expressed in cells recombinant MTMR4 exhibits an endosomal distribution, co-localizing with early, late and recycling endosomal markers (Lorenzo et al. 2006; Naughtin et al. 2010; Plant et al. 2009; Yu et al. 2010).

Several reports have localized recombinant MTMR2 to the cytosol, with some perinuclear enrichment (Kim et al. 2002, 2007; Robinson and Dixon 2005) as well as the nucleus (Lorenzo et al. 2006), whilst endogenous MTMR2 is detected on late endosomes (Cao et al. 2008). Some of the inconsistencies in these reports on the different sub-cellular localizations of myotubularin and the MTMs may be a consequence of different protein:protein interactions between myotubularins, and/or the localization may change with agonist specific-cell stimulation, or may be cell type-specific. For example stimulation of COS7 or FlpIn293 cells with EGF results in a MTM1 or MTMR2 cytosolic redistribution to punctate and vesicular structures (Tsujita et al. 2004; Berger et al. 2011).

Several other myotubularins exhibit a cytosolic localization, with non-specific patterns of punctate or reticular localization (Lorenzo et al. 2006). Over-expressed wild-type MTMR3 localizes to a cytosolic and reticular distribution, with some overlap with the endoplasmic reticulum (Walker et al. 2001), and despite the presence of a FYVE domain it shows little endosomal localization. In addition, mutations in MTMR3 have been associated with significant alterations in its sub-cellular distribution (Walker et al. 2001; Lorenzo et al. 2005). A single mutation at the cysteine in the MTMR3 catalytic site (C413S) results in its punctate cellular distribution, co-localizing with structures which by ultrastructural analysis have the appearance of autophagosomes, and by light microscopy co-localize with autophagosome markers such as WIPI-1α and DFCP1 (Walker et al. 2001; Taguchi-Atarashi et al. 2010). Double mutation of MTMR3 C413S, together with deletion of the PH domain, surprisingly induces Golgi localization of MTMR3 (Lorenzo et al. 2005).

8.3.2 Myotubularin Association with Human Disease

Mutations in either active or inactive myotubularins lead to human disease. MTM1 is mutated in X-linked centronuclear (myotubular) myopathy (Laporte et al. 1996). This congenital disease is characterized by severe muscle weakness in affected males, which often results in death in infancy from respiratory failure (Laporte et al. 1996).

Pathological analysis of muscles from affected patients reveals small rounded muscle cells with centrally placed nuclei and a surrounding halo devoid of contractile elements (Laporte et al. 1996). *Mtm1*$^{-/-}$ mice exhibit growth retardation with a shortened life span, associated with a progressive muscle phenotype that resembles the human disease, attributed to a defect in muscle maintenance, but not myogenesis (Biancalana et al. 2003). MTMR2 mutation is associated with the peripheral neuropathy Charcot-Marie-Tooth disease type 4B1, and mutation in its inactive binding partner MTMR13 leads to a similar clinical syndrome, Charcot-Marie-Tooth disease type 4B2 (Bolino et al. 2000; Berger et al. 2002; Azzedine et al. 2003; Senderek et al. 2003). Pathological analysis of affected patients reveals a demyelinating neuropathy with focally folded myelin sheaths. Mice have also been generated with a deletion of *Mtmr2,* or *Mtmr13* genes (Bolino et al. 2004; Tersar et al. 2007; Robinson and Dixon 2005). *Mtmr2*$^{-/-}$ mice with a complete deletion are viable, but underrepresented according to Mendelian predictions, and exhibit growth retardation, but no apparent weakness (Bolino et al. 2004). These mice show a peripheral neuropathy on nerve conduction and histopathological analyses, and also exhibit azospermia (Bolino et al. 2004). Conditional loss of *Mtmr2* in Schwann cells, but not neurons, reproduces the neuropathy phenotype (Bolis et al. 2005). In a separate study, mice expressing a truncated transcript lacking the phosphatase domain of *Mtmr2* were born within the expected Mendelian frequency, and showed normal growth and physical appearance (Bonneick et al. 2005). Histopathological analysis, however, revealed the characteristic features observed in humans with Charcot-Marie-Tooth 4B1 (Bonneick et al. 2005). *Mtmr13*$^{-/-}$ mice are viable and are born at the predicted Mendelian frequency (Tersar et al. 2007; Robinson et al. 2008). These mice do not exhibit an obvious disease phenotype, however, defects were observed on detailed motor testing at 12 months age, with electrophysiological and histopathological evidence of neuropathy (Tersar et al. 2007). A mouse knock-out of *Mtmr5* exhibits azospermia, perhaps related to abnormalities in Sertoli cell function (Firestein et al. 2002). Mutations of *MTMR14* are implicated in some cases of human autosomal recessive centronuclear myopathy (Tosch et al. 2006). *Mtmr14*$^{-/-}$ mice have a normal appearance, but exhibit abnormal motor testing during strenuous exercise, and abnormalities of excitation-contraction coupling (Shen et al. 2009).

8.3.3 Inactive Myotubularins and Protein Complex Formation

Inactive myotubularins have been speculated to regulate the catalytic activity of the active myotubularins, or alternatively to interact with active myotubularins and direct their sub-cellular localization (Berger et al. 2003, 2006; Kim et al. 2003; Nandurkar et al. 2003). For example, the inactive MTMR13 increases the activity of MTMR2 toward PtdIns(3)P and PtdIns(3,5)P_2, by 10- and 25-fold amounts respectively (Berger et al. 2006). This might provide a molecular basis for the observation that mutations in the inactive MTMR13 result in Charcot Marie Tooth type 4B2 neuropathy in humans and in mouse models. Both endogenous and recombinant MTMR2

and MTMR13 form homodimers in cells via interaction of their coiled-coil domains (Berger et al. 2006). These myotubularins also interact with each other in a tetrameric protein complex (Berger et al. 2006). The sub-cellular distribution of MTMR13 overlaps with MTMR2 under resting conditions, but diverges under conditions of hypo-osmotic stress, suggesting that in addition to regulating enzyme activity of an active phosphatase, the inactive phosphatase regulates the sub-cellular localization of the active phosphatase (Berger et al. 2006). Similarly, MTMR12 forms a complex with MTM1, which requires the SID domain. This interaction directs the localization of MTM1 away from the plasma membrane with reversal of MTM1-induced changes in filopodial formation in COS7 cells (Nandurkar et al. 2003). Interactions have also been observed for many other recombinant myotubularins, but whether these interactions occur *in vivo* is unclear due to a general difficulty in this field in making specific high affinity antibodies to any of the myotubularins: MTM1 interacts with MTMR10 (Lorenzo et al. 2006); MTMR2 interacts with MTMR5, MTMR10, MTMR12 and MTMR13, and the interaction between MTMR5 and MTMR13 requires the coiled-coil domain and increases phosphatase activity (Nandurkar et al. 2003; Kim et al. 2003; Robinson and Dixon 2005; Lorenzo et al. 2006; Berger et al. 2006). MTMR3 interacts with MTMR4 and co-expression of MTMR3 and MTMR4 alters the sub-cellular localization of each phosphatase (Lorenzo et al. 2006); MTMR6, MTMR7 and MTMR8 interact with MTMR9; the MTMR7/MTMR9 interaction requires the coiled-coil domain and results in increased MTMR7 phosphatase activity (Mochizuki and Majerus 2003; Lorenzo et al. 2006). Protein complex formation is not restricted only to other myotubularins. MTM1 and MTMR2 interact in a multi-protein complex with the Class III PI3K (hVps34) and hVps15 (Cao et al. 2007, 2008). MTMR2 also interacts with PSD95, an excitatory postsynaptic scaffolding protein, as well as with disc-large1 (Dlg1) (Bolis et al. 2009; Lee et al. 2010). MTRM4 interacts with R-SMADs, and the ubiquitin ligase, NEDD4 (Plant et al. 2009; Yu et al. 2010).

8.3.4 Myotubularins and Cellular Function

The functional role that individual myotubularins play in the cellular control of phosphoinositide signaling is currently a target of much work. PtdIns(3)P localizes to early and late endosomes, the plasma membrane and the forming autophagosome (Gillooly et al. 2000, 2003; Tooze and Yoshimori 2010). The localization of PtdIns(3,5)P_2 is within endosomes/lysosomes (Dove et al. 2009). A number of the myotubularins may regulate endosomal PtdIns(3)P. This suggests potential roles for the myotubularins in regulating endocytic trafficking, including endocytosis, degradative and recycling pathways and autophagy. In *C. elegans*, mutations in mtm-6 or mtmr-9 impair endocytosis by coelomocytes (Fares and Greenwald 2001; Xue et al. 2003). siRNA depletion of MTMR4 decreases endocytosis of transferrin by HeLa cells (Naughtin et al. 2010). Conversely, siRNA depletion of MTMR2 in neuronal cells enhances endocytosis and impairs synaptic maintenance (Lee et al. 2010). Sorting of EGFR for degradation is an important PI3K-dependent pathway. Expression of MTM1 or

MTMR2 inhibits EGFR degradation (Tsujita et al. 2004; Berger et al. 2011). In contrast, Lorenzo and colleagues found that over-expression of MTMR4, but not MTM1, MTMR2 or MTMR3, impaired EGFR degradation in some cells (Lorenzo et al. 2006). Expression of a catalytically inactive MTMR4, or treatment with MTM1 or MTMR2 siRNA also results in impairment of EGFR degradation, possibly suggesting that tight control of phosphoinositide levels is necessary for normal degradative receptor sorting (Lorenzo et al. 2006; Cao et al. 2008). Recycling of transferrin can occur by both PI3K-dependent and independent pathways, and over-expression of MTMR4 impairs the sorting of transferrin from early endosomes (Naughtin et al. 2010). In contrast, expression of a phosphatase inactive MTMR4 mutant increases transferrin recycling (Naughtin et al. 2010). Recycling of MIG-14/Wls is necessary for Wnt secretion, a signaling process important in tissue development and disease, and it has recently been reported that this is disrupted in *C. elegans* mtm-6 and mtmr-9 mutants (Silhankova et al. 2010). The authors also reported that mtm-6 and mtmr-9 regulate the localization of sorting nexin 3 to PtdIns(3)P-positive endosomes and maintain normal trafficking of MIG-14/Wls to control Wnt secretion (Silhankova et al. 2010).

Autophagy is a cellular process whereby portions of the cellular contents are internalized in double membrane autophagosomes and degraded. PtdIns(3)P plays a significant role in autophagosome initiation (Tooze and Yoshimori 2010). MTMR3 and MTMR14 localize to the site of autophagosome formation and negatively regulate autophagy (Vergne et al. 2009; Dowling et al. 2010; Taguchi-Atarashi et al. 2010). Additionally MTMR14 *Danio rerio* (*D. rerio*) morphants show elevated autophagic markers, and a double morphant embryo of MTM1 and MTMR14 exhibits significantly greater autophagic markers and autophagic structures by ultrastructural analysis (Dowling et al. 2010). Taken together, this data suggests that at least three of the myotubularins may inhibit autophagy.

A number of reports have recently linked the muscle defects observed in myotubularin mutations with alterations in skeletal muscle triad structure and function. Ultrastructural defects in T-tubules and the sarcoplasmic reticulum have been described in animal models of myotubular myopathy, including *Mtm1*$^{-/-}$ mice, knockdown of mtm1 in *D. melanogaster* and *D. rerio* and a spontaneous *Mtm1* mutation in Labrador retrievers, as well as in the tissues of patients with myotubular myopathy (Al-Qusairi et al. 2009; Dowling et al. 2009, 2010; Beggs et al. 2010; Toussaint et al. 2010). In skeletal muscle, MTM1 localizes to the region of the T-tubule (Buj-Bello et al. 2008; Dowling et al. 2009). *Mtm1*$^{-/-}$ mice show depressed calcium release from the sarcoplasmic reticulum with lower protein levels of the type 1 ryanodine receptor (RyR1). MTM1 *D. rerio* morphants show abnormal excitation-contraction coupling (Al-Qusairi et al. 2009; Dowling et al. 2009). MTMR14 *D. rerio* morphants show normal muscle triad ultrastructure, but defective excitation-contraction coupling and a motor phenotype (Dowling et al. 2010). A mouse *Mtmr14*$^{-/-}$ model exhibits prolonged muscle relaxation and fatigability, a result of spontaneous calcium leakage from the sarcoplasmic reticulum (Shen et al. 2009). Intriguingly, the *Mtmr14*$^{-/-}$ mouse shows increased PtdIns(3,5)P_2 (Shen et al. 2009). PtdIns(3,5)P_2 and PtdIns(3,4)P_2 directly activate the RyR1 calcium channel,

linking the observed muscle weakness with an accumulation of these phosphoinositides (Shen et al. 2009). In addition to the effect of MTM1 and MTMR14 on muscle calcium channel signaling, a third myotubularin has also been linked to ion channel signaling. MTMR6 negatively regulates the calcium-dependent activated potassium channel, KCa3.1 (Srivastava et al. 2005; Choudhury et al. 2006). MTMR6 functions as a negative regulator of calcium influx and proliferation of reactivated CD4 + T cells (Srivastava et al. 2006). Very recently myotubularin has been shown to bind desmin, and regulate desmin function (Hnia et al. 2010). Desmin is a major intermediate filament protein in skeletal muscle, and mutations in desmin are associated with cardiomyopathy and myopathy (Omary et al. 2004). Loss of MTM1 results in abnormal desmin intermediate filament assembly and mitochondrial positioning, and this is independent of the phosphatase activity of MTM1 (Hnia et al. 2010).

Several MTMs have also been associated with abnormal muscle maturation and atrophy. Abnormal regulation of the MTMR1 gene has been described in myotonic dystrophies types 1 and 2 (Buj-Bello et al. 2002a; Santoro et al. 2010). MTMR4 interacts with the ubiquitin ligase NEDD4, and in atrophying muscle increased levels of NEDD4 correlate with decreased levels of MTMR4 (Plant et al. 2009). Additionally, aged mice show progressive loss of Mtmr14, together with altered motor function and calcium homeostasis, suggesting a role for the loss of MTMR14 in sarcopenia (Romero-Suarez et al. 2010).

The molecular basis of myotubularin mutations and resultant neurological manifestations are also slowly emerging. MTMR2 forms a protein complex, interacting with the scaffolding protein Dlg1, the plus-end kinesin motor protein, Kif13b, and the exocyst component Sec8 (Bolis et al. 2009). Dlg1 and Kif13b transport MTMR2 to sites of membrane remodeling, where MTMR2 restricts and Sec8 promotes membrane addition to regulate Schwann cell myelination (Bolis et al. 2009). Additionally, expression of recombinant MTM1 or MTMR2 mutants, but not wild type MTM1, results in aggregation of neurofilaments in an adrenal carcinoma cell line (Goryunov et al. 2008). MTMR2 interacts with the scaffolding protein, PSD95, and knockdown of MTMR2 results in reduced excitatory synapse number and synapse transmission (Lee et al. 2010).

Two of the myotubularins have also been linked in gene association studies to metabolic defects. Single nucleotide polymorphisms (SNPs) of *MTMR4* are associated with elevated plasma total cholesterol values, and SNPs in *MTMR9* associate with obesity and hypertension (Dolley et al. 2009; Yanagiya et al. 2007). However the molecular basis of these interesting observations remains currently unknown.

A number of the myotubularins may also positively regulate cell proliferation and/or inhibit apoptosis. MTMR6 expression promotes resistance to etoposide-induced apoptosis, an effect enhanced by MTMR9 (Zou et al. 2009a). Silencing of MTMR2 in cultured Schwann cells results in decreased proliferation and enhanced caspase-dependent cell death (Chojnowski et al. 2007). MTMR2 and MTMR13 positively regulate Akt signaling and prevent the degradation of EGFR (Berger et al. 2011). MTMR4 expression suppresses growth inhibition induced by TGFβ, through its action in dephosphorylating R-SMADS (Yu et al. 2010). Taken together this data suggests that at least five of the myotubularins are pro-proliferative, by yet to be

Table 8.3 PTEN and Myotubularin family members

Locus	Common name	Swiss prot protein	Accession	Reference MIM	Gene map Hs/Mm
PTEN	PTEN	PTEN_HUMAN	NM_000314	MIM: 601728	10q23.3
MTM1	Myotubularin	MTM1_HUMAN	NM_000252	MIM: 300415	Xq28
MTMR1	Myotubularin related protein 1	MTMR1_HUMAN	NM_003828	MIM: 300171	Xq28
MTMR2	Myotubularin related protein 2	MTMR2_HUMAN	NM_016156	MIM: 603557	11q22
MTMR3	Myotubularin related protein 3	MTMR3_HUMAN	NM_153051	MIM: 603558	22q12.2
MTMR4	Myotubularin related protein 4	MTMR4_HUMAN	NM_004687	MIM: 603559	17q22-q23
SBF1	SET binding factor 1 Myotubularin related protein 5	MTMR5_HUMAN	NM_002972	MIM: 603560	22q13.33
MTMR6	Myotubularin related protein 6	MTMR6_HUMAN	NM_004685	MIM: 603561	13q12
MTMR7	Myotubularin related protein 7	MTMR7_HUMAN	NM_004686	MIM: 603562	8p22
MTMR8	Myotubularin related protein 8	MTMR8_HUMAN	NM_017677		Xq11.2
MTMR9	Myotubularin related protein 9	MTMR9_HUMAN	NM_015458	MIM: 606260	8p23-p22
MTMR10	Myotubularin related protein 10	MTMRA_HUMAN	NM_017762		15q13.3
MTMR11	Myotubularin related protein 11	MTMRB_HUMAN	NM_181873		1q12-q21
MTMR12	Myotubularin related protein 12	MTMRC_HUMAN	NM_001040446	MIM: 606501	5p13.3
SBF2	SET binding factor 2 Myotubularin related protein 13	MTMRD_HUMAN	NM_030962	MIM: 607697	11p15.4
MTMR14	Myotubularin related protein 14 Jumpy	MTMRE_HUMAN	NM_001077526	MIM: 611089	3p26
FAN1	Myotubularin related protein 15 Fanconi associated nuclease 1	FAN1_HUMAN	NM_014967	MIM: 613534	15q13.2-q13.3

defined molecular mechanisms. RNAi-mediated knockdown of the *D. melanogaster* homologs of human MTM1, MTMR5/13 or MTMR 6/7/8 result in mitotic defects, suggesting a role for myotubularins in the regulation of cell division (Chen et al. 2007). These findings are of interest given recent evidence that shows PtdIns(3)P localizes to the midbody during division (Sagona et al. 2010).

Additional diverse functions of myotubularins have been reported. MTMR8 may be involved in angiogenesis as Mtmr8 knockdown in *D. rerio* results in abnormal vascular development (Mei et al. 2010). Mtm1 knockdown in *D. melanogaster* produces defects in cell cytoskeletal responses to hormone stimulation, and alters the wound recruitment of hemocytes (Velichkova et al. 2010). Mtm1 also functions in *C. elegans* as a negative regulator of corpse engulfment during development (Zou et al. 2009b).

8.4 Concluding Remarks

We have described a wealth of research conducted over 15 years or so, that has shown us how these two contrasting groups of PI 3-phosphatases, which play roles in the fundamental cellular processes of signal transduction and of membrane traffic, have important influences on human health and disease. The ability to use biochemistry and genetics to link strongly the related catalytic activities of these phosphatases to human diseases provides an excellent framework for future studies, which are both biological and translational. Ongoing work should provide a deeper understanding of the complex biology by which aberrant PI 3-kinase functions cause these pathologies and it is hoped that drug discovery efforts in these areas, many targeting the lipid kinases, may lead to successful treatments Table 8.3.

Additional information regarding sequences and online database entries is shown on Table 8.3.

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