PTEN, Stem Cells, and Cancer Stem Cells
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Like normal stem cells, “cancer stem cells” (CSCs) have the capacity for indefinite proliferation and generation of new cancerous tissues through self-renewal and differentiation. Among the major intracellular signaling pathways, Wnt, Shh, and Notch are known to be important in regulating normal stem cell activities and their alterations are associated with tumorigenesis. It has become clear recently that phosphatase and tensin homologue (PTEN) is also critical for stem cell maintenance and that PTEN loss can cause the development of CSCs and ultimately tumorigenesis.

Cells that fit the description of “cancer stem cells” (CSCs), a small population of cells which is capable of replenishing a tumor in its entirety, have been described in many human tumors including prostate (1), brain (2), colon (3,4), and pancreatic (5) cancers and it is widely believed that the majority, if not all tumors, develop from CSCs. The term CSC does not preclude that the cell of origin could be from non-stem cells, such as progenitor or more differentiated cell types. More specifically, the term indicates that these cells share many properties with normal stem cells and are likely to be both the initiating event in tumorigenesis and are also likely to be responsible for cancer relapse following what may have seemed at first to be successful therapeutic intervention. Much like a weed will not die until the root has been destroyed, CSCs may be the underlying roots of a tumor that are spared during treatment and allow cancer to survive.

In order to be classified as a CSC, a cell must possess the ability to self-renew, differentiate into the cell lineages present in the primary tumor, and sustain tumor growth. Recently much work has been done to prospectively isolate CSCs, experimentally test their function, and identify the genetics changes that lead to CSC transformation. Many signaling pathways known to control normal stem cell self-renewal and differentiation including NOTCH, WNT, and SHH have been reviewed intensively. In this review, we will focus on the role of PTEN and its controlled signaling pathway in the development of CSCs.

PTEN Controlled Signaling Pathways. The PTEN tumor suppressor gene encodes a lipid and protein phosphatase and is one of the most frequently mutated genes in human cancers (6). As a lipid phosphatase PTEN dephosphorylates phosphotidylinositol-3,4,5-triphosphate (PIP3), a product of phosphotidylinositol-3-kinase (PI3Kinase). PTEN loss results in an accumulation of phosphotidylinositol-3,4,5-triphosphate (PIP3) which activates a cascade of signaling molecules including the phosphotidylinositol-dependent kinases (PDKs), the serine/threonine kinases AKT/Protein kinase B, S6 kinase, and mTOR, as well as small GTPases Rac1 and Cdc42. PTEN exerts a wide range of effects on cell growth, cell migration, cell death, and cell differentiation (7). Activation of AKT, one of most studied PTEN downstream effectors, leads to inhibition of pro-apoptotic factors such as BAD and caspase 9, and stimulates cell cycle progression through down-regulation of G1 cell cycle inhibitor p27. In addition, recent work has demonstrated the role of PTEN in regulating p53 protein level and activity (8), the expression of NKX3.1 in prostate cancer development (9), in controlling genomic stability (10) and senescence (11). Taken together, it is clear that PTEN is responsible for regulating a variety of cellular processes (Figure 1). Emerging evidence shows that PTEN and its controlled pathway also control stem cell homeostasis and PTEN’s role in the development of CSCs in various malignancies is presented herein.

PTEN Controls the Activities of Somatic Stem Cells
Due to the early embryonic lethality of Pten conventional deletion (12), much of our current understanding of PTEN’s biological role in controlling somatic stem cells comes from analyzing various Pten conditional deletion models (13). In tissues or lineages known to correlate with major cancers in humans, Pten deletion results in either premalignant or fully developed cancer phenotypes, many of which also
show concomitantly altered stem/progenitor activities.

**PTEN and hematopoietic stem cells.** Hematopoietic stem cells (HSCs) are necessary for constant replenishment of the hematopoietic system and are among the most well characterized adult stem cell types. Alterations in the PTEN pathway are frequently associated with leukemogenesis (14) and recent works have shown that PTEN plays a critical role in controlling HSC proliferation and differentiation. Experiments in mice that bear a polyinosine–polycytidine (pIpC)-inducible Pten deletion in the HSC compartment show that following Pten depletion, the mice initially developed myeloproliferative disorder (MPD), followed by acute leukemia development (15,16). PTEN loss leads to enhanced G0-G1 cell cycle transition, similar to Pten null neural stem cells (see below), which yielded a short-term expansion of HSCs, suggesting that PTEN is a key regulator of HSC activity (16). However, Pten deletion eventually leads to HSC exhaustion with progressively decreased HSC presence in the bone marrow (15,16). One of the important downstream effectors activated by PTEN loss is the mammalian target of rapamycin (mTOR) whose kinase activity can be inhibited by the drug rapamycin. Interestingly, rapamycin treatment helped in maintaining the long-term multilineage reconstitution ability of Pten null HSCs as long as the mice were maintained on rapamycin, suggesting that Pten null HSCs exhaustion may be mediated by increased mTOR activation (16).

Although mice transplanted with Pten-deficient hematopoietic cells showed leukemia development, similar to the primary disease (16), the CSCs responsible for the transplantable nature of this disease were not identified in these initial studies. More significantly, the molecular mechanisms responsible for CSC formation had not been elucidated in these initial studies. Recently, various cell surface markers have been tested for enriching prostate stem cell activities, including CD133 (1) and Sca-1 (18). Through a systematic study, the Sca-1+ subpopulation was further enriched upon negative selection against the non-prostate cell lineage markers (CD45, Ter119, and CD31, collectively called “Lin”) and with additional basal cell surface marker CD49f (20). Recently, CD117 (c-kit, stem cell factor receptor) has been identified as a new marker for adult mouse prostate stem cells (21). Both the Lin−;Sca-1−;CD49fh high (termed LSC cells) and Lin Sca-1− CD133− CD44− CD117− subpopulations, are highly enriched in the proximal region of the prostate and are further

**PTEN and Prostate Stem Cells.** The growth of the prostate depends on the steroid hormone androgen, which undergoes involution following androgen withdrawal but can completely regenerate upon androgen restoration. The rodent prostate glands are capable of undergoing multiple rounds of such castration-replacement cycles, suggesting the existence of a long-lived prostate stem cell population in the prostatic gland. Many experimental systems, including in vivo kidney capsule reconstitution assays and in vitro 2D colony or Matrigel 3D sphere forming assays, have been developed for prospectively identifying prostate stem cells and for quantitatively measuring the capacity of stem cell self-renewal, proliferation and differentiation (18,19). Meanwhile, various cell surface markers have been tested for enriching prostate stem cell activities, including CD133 (1) and Sca-1 (18). Through a systematic study, the Sca-1+ subpopulation was further enriched upon negative selection against the non-prostate cell lineage markers (CD45, Ter119, and CD31, collectively called “Lin”) and with additional basal cell surface marker CD49f (20). Recently, CD117 (c-kit, stem cell factor receptor) has been identified as a new marker for adult mouse prostate stem cells (21). Both the Lin−;Sca-1−;CD49fh high (termed LSC cells) and Lin Sca-1− CD133− CD44− CD117− subpopulations, are highly enriched in the proximal region of the prostate and are further
enhanced following androgen withdrawal (18,20,21).

PTEN loss has been strongly linked to prostate cancer development and metastasis. Mice with prostate-specific \textit{Pten} deletion develop invasive prostate cancer with pathology mimicking the human disease by 9 weeks (22). During the course of prostate cancer initiation and progression, a significant increase in the number of p63\(^+\)CK5\(^+\) basal cells and CK5\(^+\)CK8\(^+\) transient amplifying cells are observed along the basement membrane in the proximal region of the dorsolateral lobe (23). PTEN negatively regulates basal cell proliferation, and \textit{Pten} deletion leads to expansion of Sca-1\(^+\) subpopulation (23) and LSC cells (our unpublished observation), in which the regenerative capacity of prostatic stem-progenitor cells has been shown to reside (20). Collectively, these results illustrate that perturbations of PTEN signaling in the stem/progenitor population of the prostate can serve as the tumor-initiating event in prostate cancer.

**PTEN and Neural Stem Cells.** Previous research has shown that only a small percentage of brain tumor cells possess the ability to self-renew and propagate tumor growth (24), suggesting that brain tumors contain CSCs. \textit{PTEN} is frequently mutated in glioblastomas (25). \textit{Pten} deletion in embryonic neural stem cells results in brain enlargement, increased cell proliferation, decreased cell death, and enlarged cell size (26). Using an \textit{in vitro} neurosphere culture system to study stem cell proliferation, it was observed that there were more stem/progenitor cells in the \textit{Pten} deficient brains (26,27). Microarray analysis revealed prominent dysregulation of cell cycle-related genes in \textit{Pten}-deficient neurospheres. In addition, flow cytometric analysis indicated that \textit{Pten} deficiency mediates enhanced neural stem/progenitor cell self-renewal by promoting G0-G1 cell cycle transition (27). Taken together, these data suggest that the loss of PTEN confers an increased self-renewal capacity to neural stem/progenitor cells, a potentially important mechanism for brain tumorigenesis.

Brain cells positive for nestin, a neuronal stem and progenitor cell marker (28), and the stem cell marker CD133 occupy a region adjacent to the blood vessels called the perivascular niche (PVN). In a mouse model of medulloblastoma with \textit{Pten} loss (29), cells in the PVN are highly proliferative and escape cell cycle arrest that would normally have occurred in the presence of functional PTEN. It was observed that while the majority of the cells in the tumor bulk underwent apoptosis upon irradiation treatment, the cells in the PVN survived and showed neither cell cycle arrest, nor elevated p53 levels that are normally associated with both radiation-induced cell cycle arrest and apoptosis (29), strongly suggesting that the radiation induced cell cycle arrest via p53 is PTEN-dependent. Moreover, it was shown that AKT inhibition could potentially be an effective adjunct treatment that may increase the efficacy of radiation treatment, highlighting the importance of the PI3K/AKT pathway in CSC radiation resistance.

**PTEN and Skin Stem Cells.** In the skin, papillomas that arise from the stem cell enriched bulge region of the hair follicle have a much higher chance of becoming malignant than papilomas originating in the more differentiated interfollicular region (30). It has been hypothesized that since the skin and the CNS are both derived from the ectodermal germ layer that PTEN, which plays a critical role in neural stem cell self-renewal and proliferation (26), may play a similar role in regulation of skin stem cell function. Deleting \textit{Pten} in CK5\(^+\) basal cells resulted in epithelial hyperplasia due to increased hair follicle density, a phenotype directly regulated by skin stem cells (31) and mutant mice later exhibited hyperkeratosis and spontaneous tumor formation (32). Recently, Dct-Cre mediated \textit{Pten} deletion in melanocytes and melanocyte stem cells (MSCs) displayed an increased number of dermal melanocytes during perinatal development (33). Interestingly, \textit{Pten} null melanocyte stem cells are resistant to exhaustion after repeated depletions, similar to phenotype observed in \textit{Pten} null neural stem cells (26,27) but opposite to hematopoietic stem cells with \textit{Pten} deletion (15-17), suggesting that the consequences of PTEN loss in stem cells may be cell lineage-dependent.

**PTEN and Intestinal Stem Cells.** Most intestinal cancers begin as benign polyps that undergo malignant transformation. The epithelium of the small intestine has been considered an ideal system to study stem cell biology because of its constant turnover. Intestinal stem cells (ISCs) are
located at the base of the crypt of the epithelia and differentiate to produce the three differentiated cell types that populate the villi. The pre-malignant condition, known as intestinal polyposis, results from an increase in the number of crypt cells, which begs the question whether polyp formation and ultimately intestinal tumorigenesis is caused by dysregulation of stem cells in the crypt cells. Ptene deletion leads to hamartomatous intestinal polyps (34) and this model has been used to investigate the role of PTEN in regulating intestinal stem cells and tumor formation. It was observed that loss of PTEN function resulted in an increase of ISC's in the crypt and altered cell differentiation (34). Interestingly, an increase in nuclear β-catenin staining was observed. In this respect Ptene deficient ISC's behave like Ptene deficient HSC's, which are also known to migrate away from the normal niche following Ptene abrogation and altered fate of cell differentiation (15).

PTEN and Breast Stem Cells. It was established early on that heterozygosity of Ptene in mice caused an increased risk for breast cancer development (35) and homozygous Ptene deletion in the mammary epithelium leads to precocious mammary gland development and breast cancer formation (36), similar to phenotypes associated with WNT activation. Crossing Wnt transgenic mice with Ptene +/- mice also led to accelerated tumor development (37), suggesting that cross-talk exists between the WNT and PTEN/PI3K pathways. Ptene null breast cancers showed an increase in CK5+ and CK6+ cells, a phenotype similar to human breast cancer of the basal subtype. Interestingly, Wnt/Ptene +/- mice exhibited great heterogeneity with an expansion of cells positive for CK6 and Sca-1. The fact that tumors arising in these mice contain both luminal epithelial and myoepithelial tumor cells suggest that they arose from a common progenitor cell. Loss of the wild-type Ptene allele in both of these cell lineages suggests that Ptene was lost in the common progenitor cell that gives rise to tumorigenesis and its loss is necessary for tumor progression.

PTEN and Primordial Germ Cells and Oocytes. Primordial germ cells (PGCs) are precursors to germ cells that normally differentiate into eggs and sperm but can also give rise to teratomas. Ptene deletion in the male PGCs leads to increased PGC self-renewal, survival, proliferation, differentiation, and ultimately teratoma formation in the gonads (38). Similar findings were made when Ptene was deleted in the oocytes. Normally, a small number of primordial follicles are recruited from the resting follicle reserve to replenish the follicle pool. The ovaries in Ptene deleted females appeared larger and possessed more activated follicles and less primordial follicles (39). Eventually this unchecked activation of primordial follicles leads to follicle depletion and premature ovarian failure, a condition seen in humans, much like Ptene loss compromises the ability of the HSC compartment to maintain an adequate number of stem cells (15,16). This data shows that PTEN functions as a suppressor of follicular activation and is critical for maintenance of oocyte homeostasis.

Molecular Mechanisms Responsible for PTEN Controlled Stem Cell Activities

While the evidence described above (Table 1) makes a convincing case that PTEN plays an important role in controlling the homeostasis of stem/progenitor cells in multiple tissues, the development of successful treatments for CSC-initiated cancers will require the deeper understanding and efficient targeting of the downstream pathways perturbed by dysregulation of the PTEN-PI3K signaling axis. The general signaling pathways controlled by PTEN and the cross-talk between the PTEN controlled pathway and other major intracellular signaling pathways have been intensively reviewed recently (40). However, less is clear about the key components within this general signaling pathway essential for PTEN controlled stem cell activities, especially in CSC formation. Summarized below are the results derived from some of the most recent studies.

PI3K Isoform Mutations. Perturbation of the activities of various PI3K isoforms have been linked to human cancer. Mutation of the PI3KCA, which encodes for the catalytic domain of Class IA PI3Ks, have been implicated in many human cancers including glioblastoma, gastric cancers, hepatocellular carcinomas, breast cancers, ovarian cancers, and lung cancers (41). Recent work has
shown that targeting the class IA PI3K subgroup strongly suppresses cell growth, metabolism and tumorigenesis (42). Importantly, ablation of the PI3K p110β (Pik3cb) catalytic subunit, but not p110α (pik3ca), significantly impeded prostate cancer development and AKT hyperphosphorylation in the Pten null prostate cancer model (22,42), suggesting that p110β may be a promising target in cancers caused by PTEN loss.

**FOXO Pathway Activation.** Another downstream target of AKT is the FOXO transcription factors that are negatively regulated by AKT. FOXO transcription factors control cell proliferation, cell survival, and help eliminate reactive oxygen species (ROS). FOXO also plays an important role in stem cell maintenance as demonstrated by loss of LT-HSCs in Foxo-null bone marrow (43). Mirroring the effects of Pten deleted bone marrow cells, FoxO3a-deficient HSCs also exhibited an increased proliferation rate, decreased quiescence and a reduced ability to repopulate bone marrow after transplantation into recipient mice (44). Although these results could seem to indicate that the FOXO pathway is exclusively the target of Pten deletions in HSCs, direct disruption of the FoxO gene family by genetic deletions shows that perturbation of the FOXO pathway alone is not capable of mirroring all of the tumorigenic qualities of Pten deletion (45), suggesting that while the FoxO genes are a critical downstream target of Pten deletion, the effects from other PTEN downstream pathways are also required for the full development of cancer phenotypes.

**Wnt/β-catenin Pathway Activation.** The Wnt/β-catenin signaling pathway is known to be important for HSC self-renewal and its activation is required for the in vitro reprogramming activity of the LSCs from myeloid blast crisis of human chronic myeloid leukemia (46). In the VE-cadherin-Cre leukemia model, Guo and colleagues (17) showed that Pten inactivation in HSCs serves as the first hit, by activating the PI3K–AKT pathway, conferring survival and proliferative advantages, and promoting genomic instability. Interestingly, although PTEN loss and AKT activation in HSCs leads to a moderate increase in the level of unphosphorylated β-catenin, presumably due to inactivation of GSK3α/β, such an increase is not sufficient to maintain Pten null HSC self-renewal (15-17). Via currently unknown molecular and genetic alterations, either parallel or synergistic with PTEN-PI3K-AKT pathway, β-catenin gets further activated in the self-renewable LSCs and enriched in the c-Kit<sup>mid</sup>CD3<sup>−</sup>Lin<sup>−</sup> compartment and blast cells (17). Importantly, simultaneously deleting Pten and one allele of β-catenin (Pten and Ctnnb1, respectively) in HSCs impaired LSC formation or self-renewal and T-ALL development caused by Pten loss (17), suggesting that specific inhibitors for β-catenin may have therapeutic effect on Pten null CSCs. Similarly, increased β-catenin activation has been shown in Pten intestinal epithelial deletion models (34).

**NOTCH1 and c-myc Activation.** NOTCH signaling is known to play a role in regulating neural stem cell expansion in vivo and in vitro (47) and is found to be dysregulated in about 56% of human T-ALL (48). NOTCH pathway activation leads to reduced PTEN expression in human T-ALL via the transcriptional repressor HES-1 (49) and enhanced c-MYC oncogene expression (50), suggesting the NOTCH pathway plays a critical role in regulating PTEN controlled stem cell activity. Results from the Pten null leukemia model further support this notion. Although no alterations in NOTCH1 signaling are detected in the Pten leukemia model (17), a recurring chromosomal translocation, T(14;15), was found to result in aberrant overexpression of the c-myc oncogene in c-Kit<sup>mid</sup>CD3<sup>−</sup>Lin<sup>−</sup> LSCs and CD3+ leukemic blasts. Therefore, Pten inactivation and c-myc overexpression may substitute functionally for NOTCH1 pathway activation, recapitulating a subset of human T-ALL (49). Consequently, one could predict that T-ALL with PTEN loss may be resistant to treatments targeted against the NOTCH pathway.

**SUMMARY**

Although our understanding of the biology of stem cells and CSCs is increasing, many questions still remain unanswered. We know that the PTEN pathway controls normal stem cell maintenance, self renewal and migration, but we don't know why PTEN loss in some stem cell population leads
to enhanced self-renewal and proliferation and in others causes exhaustion. The cell-of-origin of CSCs in various cancers caused by PTEN loss is largely unknown. More specifically, we need to determine what are the key mutations or pathway alterations that are linked to the formation of CSCs and understand why *Pten* null CSCs are resistant to current therapeutic agents. Finding ways to eliminate these cells will require a much better understanding of the nature of the molecular mechanisms that lead to the formation of CSCs and the development of drugs that target these dysregulated pathways.

**Acknowledgments**

The authors thank Sherly Mosessian and Wei Guo for helpful comments and suggestions. This work is partly supported by the Prostate Cancer Foundation, DOD PC031130, National Cancer Institute UO1 CA84128-06, P50 CA092131, and RO1 CA107166 (H. Wu). R.H. is supported by the Damon Runyon Cancer Foundation Fellowship Award.

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| Tissue                  | Phenotype                                                                                                                                                                                                 | References |
|------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Hematopoietic Stem Cells | *Pten* deletion leads to short-term expansion of hematopoietic stem cells (HSCs) which eventually leads to HSC exhaustion. Two additional molecular genetic events, namely, β-catenin activation and a recurring T(14;15) chromosome translocation, were identified and showed to be associated with leukemia stem cells (LSC) transformation and leukemogenesis. | 15-17      |
| Prostate Stem Cells    | PTEN negatively regulates basal cell proliferation, and *Pten* deletion leads to expansion of the Sca-1 subpopulation in which the regenerative capacity of prostatic stem-progenitor cells has been shown to reside. | 20,23      |
| Neural Stem Cells      | *Pten* deletion leads to an increase in stem/progenitor cells and it has been demonstrated that *Pten* deficiency mediates enhanced neural stem/progenitor cell self-renewal by promoting G0-G1 cell cycle transition. | 26-27      |
| Skin Stem Cells        | Deleting *Pten* in CK5⁺ basal cells resulted in epithelial hyperplasia due to increased hair follicle density, a phenotype directly regulated by skin stem cells. | 31-33      |
| Intestinal Stem Cells  | *Pten* deletion in intestinal stem cells (ISCs) resulted in an increase of ISCs in the crypt and altered cell differentiation. ISCs also migrated away from the normal niche following *Pten* abrogation and altered fate of cell differentiation. | 34         |
| Breast Stem Cells      | Homozygous *Pten* deletion in the mammary epithelium leads to precocious mammary gland development and breast cancer formation. *Pten* null breast cancers showed an increase in CK5⁺ and CK5⁺ cells, with an expansion of cells positive for Sca-1. | 35-37      |
| Primordial Germ Cells  | *Pten* deletion in the male Primordial germ cells (PGCs) leads to increased PSC self-renewal, survival, proliferation, differentiation, and ultimately teratoma formation in the gonads with similar results being found when *Pten* was deleted in the oocytes. | 38-39      |
Figure 1. Loss of PTEN function results in an accumulation of PIP3 which activates a cascade of signaling molecules. Activation of AKT leads to the inhibition of pro-apoptotic factors such as BAD and stimulates cell cycle progression through down-regulation of G1 cell cycle inhibitor p27. PTEN and its effectors also interact with other signaling pathways known to be essential for stem cell maintenance, including the Wnt/β-catenin pathway. Emerging evidence shows that the loss of PTEN function leads to activation of the active form of β-catenin which leads to the acquisition of self-renewal ability. PTEN loss also activates the mammalian target of rapamycin (mTOR) whose kinase activity is inhibited by the drug rapamycin. Increased mTOR activity can lead to increased cell survival and proliferation. Other downstream targets of AKT include the FOXO transcription factors that are negatively regulated by phosphorylation by AKT. FOXO transcription factors control cell proliferation, cell survival, and help eliminate reactive oxygen species (ROS). Cells without FOXO activity have an increased proliferation rate and decreased quiescence.
