The role of PTEN in myeloid malignancies

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Abstract

PTEN deletion in the mouse and in the zebrafish highlights the essential role of this tumor suppressor in the development of myeloid malignancies, in particular acute myeloid leukemia and myeloproliferative disorders. In humans, extensive genetic sequences of myeloid malignancies did not reveal recurrent PTEN mutations and deletions. However, PTEN was shown to be functionally inactivated in several acute myeloid leukemia and chronic myeloid leukemia samples, through both post-trasductional modifications, changes in protein levels and cellular compartmentalization. Notably, non genomic inactivation of PTEN in myeloid malignancies could represent a challenging therapeutic opportunity for these diseases. Targeting those mechanisms that affect PTEN function could indeed promote PTEN reactivation with consequent cancer selective apoptosis induction. In this review we will describe the role of PTEN in the development of myeloid malignancies.

Introduction

PTEN is an essential tumor suppressor with similar mutation/deletion rates as those of TP53.[1] PTEN acts mostly as a phosphatase that de-phosphorylates the Phosphatidylinositol (3,4,5)-trisphosphates (PIP3), therefore counteracting the activation of PI3-kinase (Figure 1A). Beside this major mechanism of action, PTEN tumor suppressive functions can be independent of its phosphatase activity. In particular, PTEN was shown to act in the nucleus, where it mediates cellular proliferation through the interaction with the APC/CDH1 complex (Figure 1B).[2] Furthermore in the nucleus, PTEN promotes the stabilization of the genome, through the interaction with CENP-C, an essential component of the kinetochore.[3] Disruption of PTEN causes centromeric instability and spontaneous DNA double-strand breaks.[3] Similarly, nuclear PTEN was also shown to regulate DNA repair and sensitivity to genotoxic stress, through the involvement of ataxia telangiectasia mutated protein, ATM.[4] PTEN is regulated in an highly complex network.[1] The function of the protein is indeed affected by proper levels of expression, which in turn are mediated by genetic and non-genetic mechanisms.[1,5] In particular, PTEN level of expression are regulated by several miRNA, with important implications in cancer pathogenesis.[6] PTEN phophatase activity is also controlled by post-trasductional modifications.[1] In particular, PTEN phosphorylation by Casein Kinase II promotes PTEN inactivation through the stabilization of PTEN in a *closed* conformation.[7] Notably, even proper PTEN cellular compartmentalization is essential for the regulation of its tumor suppressive functions, as observed for p53 and FOXOs proteins.[8] In particular, loss of the nuclear pool of a genetically wild-type PTEN was associated with development of cancer.[9] PTEN shuttling from the nucleus to the cytoplasm is regulated by mono-ubiquitination[9] and sumoylation.[4] Both PTEN phosphorylation, changes in protein levels and cellular compartmentalization are associated with tumorgenesis leading to the definition of PTEN as the paradigm for the non genomic loss of function of tumor suppressors.[5,10] The identification of those cancer characterized by functional inactivation of PTEN could have important implications from the therapeutic standpoint. Targeting those mechanisms that inactivate genetically wild-type PTEN could indeed be associated with strong and cancer selective apoptosis induction.

Myeloid malignancies in PTEN murine models

The PI3K-AKT pathway was shown to play a role in the development of myeloid malignancies. Expression of an active form of AKT in the mouse was associated with the development of myeloproliferative disorders (MPD) and acute myeloid leukemia (AML).[11] Due to the ability of PTEN to regulate PI3K-AKT signaling, several models of PTEN deletion in the murine hemopoietic compartment have been described. In particular, deletion of PTEN was associated with the development of a myeloproliferative disorder which eventually evolves into an acute leukemia.[12-15] Recently, PTEN was also shown to play an essential role in the regulation of protein synthesis of the hemopoietic stem cell compartment, with consequent involvement in myeloid leukemogenesis.[16] PTEN tumor suppressive functions were also associated with the pathogenesis of CML in a murine model,[17] and with the development of myelodysplasia, through the interaction with C/EBP-alpha.[18] Furthermore, in some models characterized by the association of different genetic lesions, such as the PTEN+/-SHIP-/- compound mice, were also associated with the development of MDS.[19] All together, these phenotypes highly suggest that PTEN could play a tumor suppressive role in myeloid malignancies development.

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Hemopoietic role of PTEN in the zebrafish

Similarly, PTEN was also shown to regulate both normal and pathological hemopoiesis in the zebrafish.[20,21] In particular, the aberrant myelopoiesis observed in PTEN null embryos was explained due to the ability of PTEN to regulate CEBPA expression, which is as essential protein involved in myeloid malignancies.[22]

PTEN in human myeloid malignancies

PTEN deletion in murine and zebrafish models clearly attribute to PTEN a putative role in the development of myeloid malignancies. In particular, AML and CML appeared to be associated with PTEN deletion. In the following part of the review, we will focus on the role of PTEN in human myeloid malignancies.

PTEN in acute myeloid leukemia

Original reports by Aggerholm described PTEN mutations in just 1 out of 59 AML patients, suggesting that PTEN is rarely genetically involved in AML pathogenesis.[23]

Similarly, Liu and colleagues did not detect PTEN mutations in 62 AML patients.[24] Also, no PTEN LOH was observed. However, PTEN transcripts were aberrantly expressed in 24% of AML patients,[24] suggesting that PTEN may be targeted by aberrant splicing machinery. In more recent AML massive genome surveys, no PTEN mutations was never been reported in AML.[25] Only occasionally, PTEN was found deleted.[26] All together, these analyses did not support the genetic involvement of PTEN in AML pathogenesis. However, PTEN is not only acting as a tumor suppressor through its genetic inactivation (mutations and deletions), but it can be functionally inactivated with important therapeutic implications.[1] Early investigations showed that PTEN is highly phosphorylated in AML samples. In particular, almost 70% AML samples display Casein Kinase II (CKII)-mediated serine phosphorylation of PTEN tail, which is associated with PTEN inactivation.[27] Notably, PTEN inactivation by tail-phosphorylation is potentially reversible through CKII inhibitors treatment. Furthermore, it was also shown that in acute promyelocytic leukemia PTEN is aberrantly expressed with complete nuclear exclusion of the protein.[9] Interestingly, treatment with arsenic trioxide and all-trans retinoic acid, reversed the aberrant expression of PTEN. In this work, authors demonstrated that the aberrant PML-RAR-alpha protein is able to regulate the localization of PTEN through the de-ubiquitinase HAUSP. This work settles a new interpretation of the role of PTEN as a tumor suppressor. Following these observations, Noguera and colleagues discovered that even NPMc+ AML is characterized by aberrant PTEN cellular compartmentalization.[28] This work further highlights the complexity of the regulation of PTEN shuttling but also offers challenging therapeutic opportunities. The identification of those AML samples that are associated with inactive PTEN, through PTEN tail phosphorylation or PTEN delocalization, could be indeed targeted to promote PTEN reactivation with cancer selective apoptosis induction.

Another layer of complexity for PTEN regulation in AML is represented by the aberrant PTEN expression regulation by miRNAs. Several miRNA have been shown to regulate PTEN expression and to be aberrantly expressed in AML.[29] In this respect, the AML/ETO translocation/miR-193a/PTEN network is a well described mechanism of PTEN involvement in cancer through miRNA.[30] All together, these data indicate that AML is characterized by a aberrant inactivation of PTEN functions.

PTEN in myelodisplasia

Sequencing analyses of human MDS patients did not revealed genetic involvement

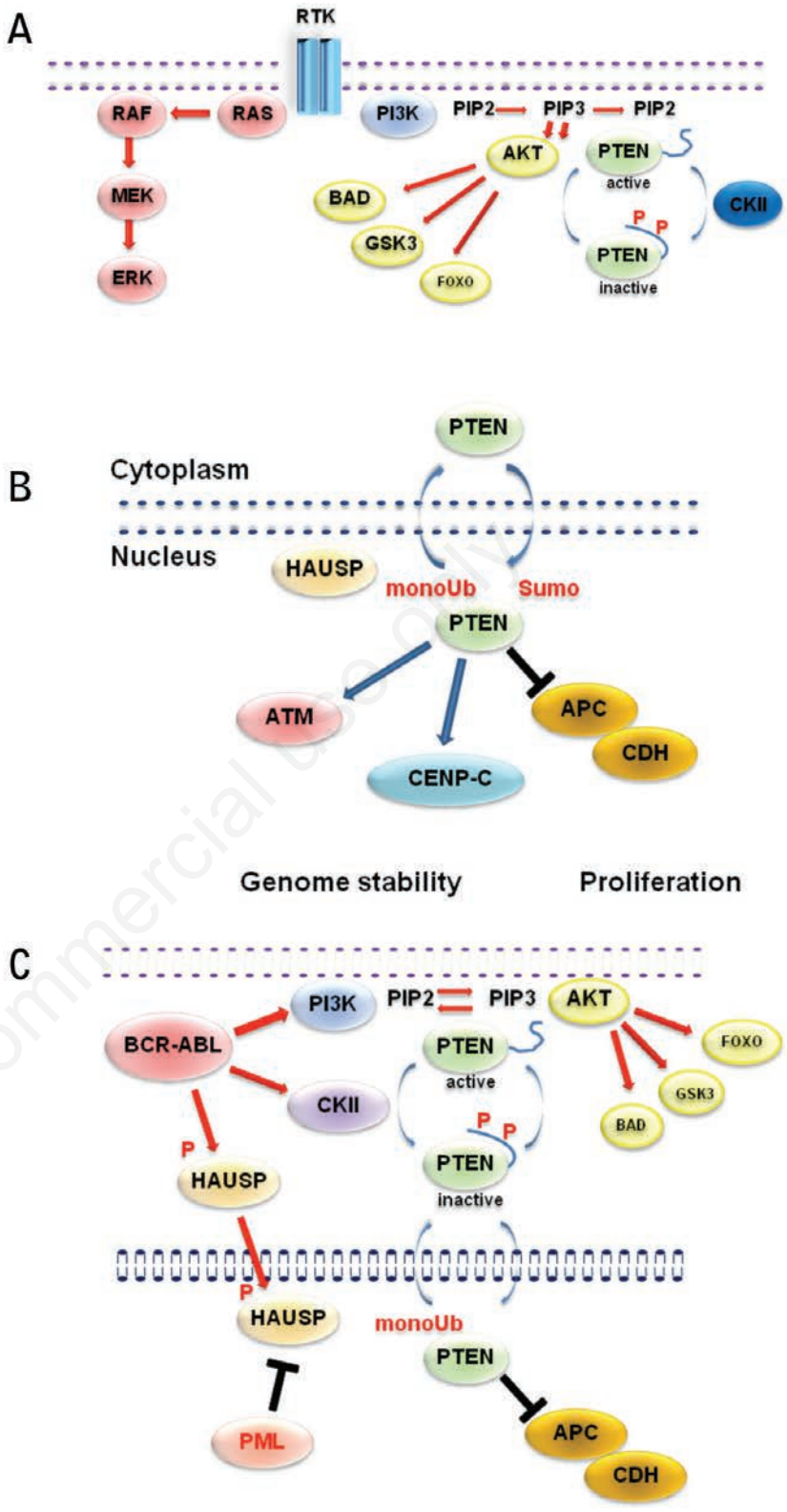

Figure 1. A) PTEN regulation of the PI3K-AKT signaling. PTEN promotes PIP3 dephosphorylation with consequent inactivation of the PI3K-AKT signaling. Casein Kinase II (CKII) induces PTEN-tail phosphorylation with consequent switch to a tail-closed inactive conformation. RTK: receptor tyrosine kinase; PIP3: Phosphatidylinositol (3,4,5)-trisphosphates; PIP2: Phosphatidylinositol (3,4)-bisphosphates; CKII: casein kinase II. B) Representation of the nuclear-cytoplasmic shuttling of PTEN with the identification of PTEN nuclear targets. C) Representation of PTEN signaling in chronic myeloid leukemia.

of PTEN in these diseases.[23] Similarly to what observed in AML, non genomic mechanisms of PTEN regulation were observed in MDS patients. In particular, PTEN was shown to be under-expressed in high-risk MDS,[31] with a concomitant increased phosphorylation of AKT. Importantly, in the 5q+ MDS the deletion of the *EGR1* gene was associated with an aberrant regulation of the expression of PTEN itself.[32]

PTEN in chronic myeloid leukemia

Chronic myeloid leukemia is a myeloproliferative disorder that is caused by the translocation t(9;22), coding for the chimeric protein BCR-ABL.[33-35] PTEN was clearly shown to play a tumor suppressive role in a murine model of CML.[17] In primary CML, PTEN was shown to be aberrantly excluded from the nucleus.[36] In particular, PTEN is nuclear excluded in the progenitors and mature cells, while it maintains the proper diffuse cellular compartmentalization in CML stem cells. As observed in AML, PTEN cellular compartmentalization is a regulated process. In particular, we demonstrated that BCR-ABL promotes HAUSP activation through tyrosine phosphorylation, with consequent PTEN nuclear exclusion.[36] In the stem cell compartment, PTEN maintains its nuclear pool due to high levels of PML, which are able to counter act the BCR-ABL/HAUSP activation. PTEN expression was also shown to be regulated by BCR-ABL.[37,38] In particular, we observed that BCR-ABL regulates PTEN expression through the RAS-MEK pathway.[37] Finally, BCR-ABL was also shown to functionally inactivate the phosphatase activity of PTEN.[39] The BCR-ABL substrate Casein Kinase II is indeed able to promote PTEN phosphorylation of the tail, with consequent inactivation. This mechanism appears to be a common mechanism of inactivation of PTEN in hematological cancers.[40]

In CML, both PTEN nuclear exclusion, inactivation and down-regulation were shown to be tightly regulated and targetable. In particular, arsenic trioxide was shown to promote PML degradation with consequent PTEN delocalization in the CML stem cell compartment and cell exhaustion.[36] Casein kinase II inhibitors promotes PTEN reactivation with consequent PI3K-AKT inactivation,[39] and Ras-MEK inhibitors can restore proper PTEN levels (Figure 1C).[37]

Essential thrombocythemia, polycythemia vera, and myelofibrosis

To our knowledge, this group of classical Philadephia negative myeloproliferative disorder has never been investigated for the expression and mutational status of PTEN. Although massive parallel sequencing of ET and PV focused results on the most relevant *JAK2* and *Calreticulin* genes, the absence of PTEN in the list of mutated genes sounds as the absence of any genetic involvements of PTEN in such disorders.[41] Recently, we showed that PTEN expression can be regulated in atypical CML, through the involvement of a Morgana/chp1-ROCK network.[42] However, the real consequence of PTEN regulation by this novel network in aCML is still under investigation.

Therapeutic implications

Overall, these observations highly suggest that PTEN is an essential tumor suppressor in human myeloid malignancies. Notably, PTEN involvement in AML and CML does not depend on its genetic inactivation but mostly on non-genomic mechanisms of inactivation. In particular, in AML and CML PTEN-tail phosphorylation was associated with PTEN inactivation and in APL, NPC+ AML and CML, PTEN nuclear exclusion promotes loss of the essential nuclear tumor suppressive functions of PTEN. Importantly, PTEN inactivation by tail phosphorylation and nuclear exclusion has tremendous implications from the therapeutic standpoint. In particular, while genetic inactivation of PTEN can be counteracted only with PI3-K inhibitors, which do not affect PTEN phosphatase-independent tumor suppressive functions, PTEN phosphorylation and PTEN compartmentalization can be targeted by casein kinase II inhibitors and de-ubiquitination targeting drugs with restoration of its tumor suppressive functions. Inhibitors of CKII were indeed shown to promote AML and CML apoptosis and PTEN-shuttling inhibitors, such as arsenic trioxide and HAUSP inhibitors, could relocate PTEN into the nucleus with astonishing pro-apoptotic effects.

Conclusions

PTEN deletion in the mouse and in the zebrafish highlighted a putative role for PTEN in myeloid malignancies. Several phenotypes have indeed been associated with the development of acute myeloid leukemias and myeloproliferative disorders. However, extensive genetic analyses did not revealed recurrent genetic aberrations of PTEN in human myeloid malignancies. Over the last years, the role of PTEN in tumorigenesis has been completely revised. In particular, it was demonstrated that even genetically wild-type PTEN could be involved in cancer pathogenesis when aberrantly down-regulated or aberrantly delocalized in the cells. In this review, we have highlighted that PTEN is mostly functionally inactivated in several myeloid malignancies. In particular, PTEN was shown to be tail-phosphorylated in AML and CML, with consequent inactivation. Both AML, in particular NPM-c+ and APL, and CML was also associated with PTEN nuclear exclusion, due to aberrant activity of the de-ubiquitinase HAUSP. The major consequences of these observations rely on the fact that inactivated-PTEN could be targeted to promote its re-activation. In particular, casein kinase II inhibitors and HAUSP inhibitor strategies have been shown to promote PTEN reactivation and proper re-localization in the cellular compartments with strong apoptosis induction. All together, these observations attribute to PTEN the role of a challenging targetable tumor suppressors in myeloid malignancies.

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