Power of PTEN/AKT: Molecular switch between tumor suppressors and oncogenes (Review)

YINGQIU XIE[1*], SANZHAR NAIZABEKOV[1*], ZHANLIN CHEN[2] and TURSONJAN TOKAY[3]

[1]Department of Biology, Nazarbayev University, School of Science and Technology, Astana 010000, Republic of Kazakhstan; [2]Lipscomb University, Lipscomb Academy, Nashville, TN 37204, USA; [3]Center for Life Sciences, National Laboratory Astana, Nazarbayev University, Astana 010000, Republic of Kazakhstan

Abstract. An increasing amount of evidence has shown that tumor suppressors can become oncogenes, or vice versa, but the mechanism behind this is unclear. Recent findings have suggested that phosphatase and tensin homolog (PTEN) is one of the powerful switches for the conversion between tumor suppressors and oncogenes. PTEN regulates a number of cellular processes, including cell death and proliferation, through the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway. Furthermore, a number of studies have suggested that PTEN deletions may alter various functions of certain tumor suppressor and oncogenic proteins. The aim of the present review was to analyze specific cases driven by PTEN loss/AKT activation, including aberrant signaling pathways and novel drug targets for clinical application in personalized medicine. The findings illustrate how PTEN loss and/or AKT activation switches MDM2-dependent p53 downregulation, and induces conversion between oncogene and tumor suppressor in enhancer of zeste homolog 2, BTB domain-containing 7A, alternative reading frame 2, p27 and breast cancer 1, early onset, through multiple mechanisms. This review highlights the genetic basis of complex drug targets and provides insights into the rationale of precision cancer therapy.

Contents

Correspondence to: Dr Yingqiu Xie, Department of Biology, Nazarbayev University, School of Science and Technology, 53 Kabanbay Batyr Avenue, Astana 010000, Republic of Kazakhstan
E-mail: xieautumnus@gmail.com

*Contributed equally

1. Introduction

Phosphatase and tensin homolog (PTEN), a lipid phosphatase, is one of the frequently mutated or deleted tumor suppressor genes during cancer progression (1). In particular, it is deleted in >50% of prostate cancer cases. PTEN is a well-known tumor suppressor protein that regulates a number of cellular processes, including cell death and proliferation, through the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway (2,3). However, PTEN function is phosphatase-independent, as well as being dependent upon events in the nucleus (4,5). It has been found that inactivated PTEN can switch oncogenes to oncosuppressors in certain mouse models, which suggests a dependence on the genetic context. In other words, PTEN inactivation may change the progression of disease from an aggressive state to safe and vice versa. In the present review, examples of altered function in the p53, enhancer of zeste homolog 2 (EZH2), alternative reading frame 2 (ARF), zinc finger and BTB domain-containing 7A (ZBTB7A), p27 and breast cancer 1, early onset (BRCA1) proteins by PTEN loss/AKT activation signaling will be discussed.

2. PTEN loss switches MDM2-dependent p53 downregulation through ARF

The first and classical example of PTEN inactivation as a switch is the modulation of tumor suppressor p53 (6). p53 and PTEN control cell death and proliferation, and they are often expressed simultaneously in various types of tumor (7,8). Due

to its short half-life, the suppressor function of p53 heavily relies on this stabilization (9). In normal cells, p53 levels are kept at insignificant levels by ubiquitin-mediated proteolysis. PTEN and p53 may form a positive feedback loop. In detail, p53 can upregulate PTEN by binding to the PTEN promoter, thereby activating PTEN transcription (8). Mechanistically, PTEN inhibits AKT-mediated MDM2 phosphorylation to prevent MDM2 from translocation into the nucleus to degrade p53 (10). Thus, wild-type PTEN can stabilize p53. It can be stated that AKT activation with PTEN deletion may result in the rapid degradation of p53, leading to further PTEN-dependent tumorigenesis (11).

However, in mouse embryonic fibroblasts (MEFs) and a mouse model of prostate cancer, it has been found that PTEN loss does not induce p53 degradation or instability (12). On the contrary, Pten loss can also upregulate p53, which leads to Pten loss-induced cellular senescence through p53, and to a certain degree, restricted tumor growth (13). Mechanistically, PTEN loss induces ARF elevation and elevated ARF may degrade MDM2, thereby stabilizing p53 (14,15). Indeed, in MEFs, acute loss of Pten did not decrease p53 stability, but actually increased stability, with co-upregulation of p19^{Arf} (ARF in mouse) (13). Thus, PTEN loss can also induce elevation of p53 as a molecular switch by regulating p53 stability through the ARF-MDM2 pathway. However, the mechanism via which PTEN can induce ARF upregulation remains unclear. Moreover, the method by which ARF and AKT compete for the regulation of MDM2 for the stability of p53 also requires further investigation.

3. PTEN loss/AKT activation switches *EZH2* from a tumor suppressor to an oncogene

Epigenetic regulators are a relatively new class of therapeutic target for cancer treatment. The enhancer of zeste homolog (EZH2), a catalyst of polycomb repressive complex 2 (PRC2), is a well-known epigenetic regulator that plays oncosuppressive roles by silencing gene expression via its histone methyltransferase activity (16). Moreover, it silences transcription by trimethylating H3 histone on lysine 27 (16). Nevertheless, EZH2 expression correlates to the progression of prostate cancer, particularly to that of castration-resistant prostate cancer (CRPC) (17).

A recent study has reported that EZH2 can act as an oncogenic protein. The oncogenic function of EZH2 is independent of its role as a transcriptional repressor in cases of CRPC (17). This functional change requires phosphorylation of EZH2 by AKT, as well as an intact methyltransferase domain. Study results have shown that EZH2 phosphorylation at serine 21 can easily change its function from a PRC2 catalyst to an androgen receptor transcriptional co-activator, as it is mediated by the PI3K/AKT pathway directly and indirectly (17). These findings indicate that the oncogenic activity of EZH2 in CRPC is not dependent on its polycomb-related oncosuppressive function. Furthermore, the findings indicate that it is possible to develop inhibitors that may specifically target the activation of EZH2 without altering its PRC2 repressive function. Recent studies have suggested that PTEN loss correlates with EZH2 elevation in the invasion and metastasis of gallbladder adenocarcinoma (18). Moreover,

PTEN loss downregulates p16 expression to decrease cellular senescence, thereby increasing cell proliferation, an oncogenic process. This process is induced through the EZH2 elevation-mediated methylation change of the p16 promoter. In other words, PTEN loss may upregulate EZH2 for the inhibition of cell cycle arrest (19). Thus, PTEN loss/AKT activation may switch EZH2 function.

4. Pten loss switches *ZbTb7a* from an oncogene to a tumor suppressor

ZBTB7A (also known as Pokemon, LRF, OCZF and FBI-1) is a part of the POK (Kruppel and POZ/BTB) transcription factor family, and plays a major role during oncogenesis and cell differentiation (20). ZBTB7A was previously believed to be a proto-oncogene in various cancer types, including prostate cancer (21,22). ZBTB7A is highly expressed in Hodgkin's lymphoma, as it represses the expression of AFR, a tumor suppressor (23). Nevertheless, a recent study conducted by Wang *et al* showed that it may have oncosuppressive functions in the case of prostate cancer upon PTEN loss (24). The prostate-specific transgene of *Zbtb7a* has no association with tumorigenesis, which is contrary to the previous hypothesis that it is an oncogene. Unexpectedly, the inactivation of Zbtb7a accelerates Pten loss-induced prostate cancer progression. Mechanistically, the downregulation of retinoblastoma-associated protein by hyper-elevated Sox9 was found to occur in Pten/Zbtb7a double-null prostate tumors (24); this resulted in Pten loss-induced cellular senescence being overcome (24). Therefore, *Zbtb7a* can also be identified as a PTEN context-dependent cancer gene that may have oncosuppressive and oncogenic functions in PTEN-null tumors.

5. PTEN loss switches ARF function through sumoylation

ARF is another transcript of the *ARF-INK4a* locus (CDKN2a) on human chromosome 9p21 (25). The *CDKN2a* locus encodes p14^{ARF} and p16INK4a, which are two cyclin-dependent kinase inhibitors (26). *CDKN2a* deficiency may lead to susceptibility to carcinogen-induced tumors via antagonism of Mdm2-mediated p53 degradation (27). The canonical pathway of ARF induces senescence through degradation of MDM2, thereby stabilizing p53 (27).

Multiple studies have suggested that ARF has oncosuppressive and oncogenic functions. For instance, it has been shown that p19^{Arf} deficient mice develop various types of cancers, suggesting that ARF has oncosuppressive roles (26). On the other hand, a study by Chen *et al* (28) showed that p19^{Arf} inactivation decreases tumorigenesis in PTEN-deficient prostate cancers, indicating the oncogenic role of ARF. Moreover, a number of previous studies have shown the oncogenic role of ARF in a genetic context-dependent manner (29,30). However, the manner by which ARF function is switched from tumor suppressor to oncogene is unclear. In a previous study, we found that PTEN loss may be the switch during Zinc finger protein SNAI2 (SLUG)-mediated epithelial-mesenchymal transition (EMT). SLUG as a transcription factor can represses E-cadherin transcription to promote EMT in various cancers, including
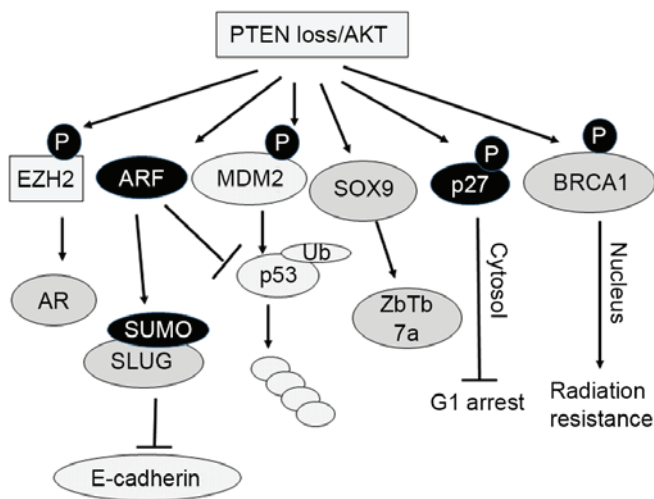
Figure 1. PTEN loss/AKT activation converts a subset of tumor suppressor proteins to oncogenic proteins or vice versa through multiple pathways. PTEN, phosphatase and tensin homolog; Akt, protein kinase B; EZH2, enhancer of zeste homolog 2; AR, androgen receptor; ARF, alternative reading frame 2; SUMO, small ubiquitin-like modifier; SLUG, ces-1-related zinc finger transcription factor; E-cadherin, epithelial cadherin; MDM2, mouse double minute 2; Ub, ubiquitin; SOX9, SRY-related HMG-box 9; ZbTb7a, zinc finger and BTB domain-containing 7A; BRCA1, breast cancer 1, early onset; P, phosphorylation.

prostate cancer (31). The mechanisms underlying these pathways are not described well, particularly when it comes to *in vivo* tumorigenesis during PTEN loss. In another previous study, we showed that p14^{ARF} (ARF in humans) stabilizes SLUG through sumoylation at lysine residue 192 (32). This stabilization results in the inhibition of E-cadherin in prostate cancer mouse models. On the other hand, p19^{Arf} inactivation leads to the reduction of Slug levels, resulting in high E-cadherin expression (32). This inactivation delays the onset and progression of prostate cancer in Pten/Trp53 double null mice (32). This study suggested that PTEN loss may be the switch for ARF function from a p53-dependent tumor suppressor to an SLUG-EMT-dependent oncogenic protein. These novel findings may have implications for clinical research. Chemotherapeutic compounds that target ubiquitination to control cancer progression are currently in clinical trial. A similar approach can be used to develop inhibitors that will prevent cancer metastasis by blocking SLUG sumoylation. Moreover, it is possible to target ARF and make the treatment of prostate cancer more efficient.

6. PTEN loss/AKT activation switches *p27* from a tumor suppressor to an oncogene

p27 is a tumor suppressor that represses cell cycle progression as an inhibitor of cyclin-dependent kinase (CDK) in the nucleus. However, the cytoplasmic localization of p27 has been found in a number of cancers and correlates with PTEN loss in prostate cancer (33). The cytoplasmic localization of p27 has been also found to be correlated with the activation of AKT in primary breast cancer samples (34). Mechanically, the AKT-mediated phosphorylation of p27 at threonine 157 determines cellular p27 translocation from the nucleus to the cytoplasm (34-36). As a consequence, cytoplasmic localized

p27 inhibits G1 cell cycle arrest (34,35). Thus, the PTEN loss/AKT activation pathway may switch p27 from a tumor suppressor to an oncogenic protein through phosphorylation mediated nuclear-cytoplasmic translocation.

7. PTEN loss/AKT activation switches *BRCA1* from a tumor suppressor to an oncogenic resistant gene in radiation therapy

BRCA1 is believed to act in the nucleus as a tumor suppressor that can repair DNA (37). The cytoplasmic localization of BRCA1 has been found in numerous cancers, where it results in the sensitization of DNA damage and increased apoptosis (38). Wild-type p53 promotes BRCA1 nuclear export upon irradiation (38). Consistently, dysregulated p53 induces the nuclear retention of BRCA1, which results in the resistance to radiation therapy in breast cancer cells (39). While p53 is regulated indirectly or directly by PTEN, PTEN loss may affect BRCA1 cellular localization and thereby switch the *BRCA1* from a tumor suppressor to an oncogenic resistant gene in radiation therapy. On the other hand, AKT has been found to phosphorylate BRCA1 to enhance its nuclear localization and stability, which results in the inhibition of radiation sensitivity (40). Thus, the PTEN loss/AKT activation pathway is most likely able to convert BRCA1 function.

In summary, with PTEN loss/AKT activation, functional switches of targets from oncogenes to suppressors and vice versa have been observed. These switches are associated with a subset of previously believed oncogenes or tumor suppressors (Fig. 1). It can be concluded that PTEN may be a powerful switcher, playing an oncosuppressive/oncogenic role in a context-dependent manner through multiple pathways.

8. Conclusion and prospective outlook

The results of this review suggest that PTEN inactivation may impact prostate cancer development significantly. This issue becomes particularly important in case of cancer treatment. For example, drugs targeting ZBTB7A may facilitate tumor growth if PTEN is inactivated. Therefore, a personalized medicine treatment approach should be taken into consideration, as gene deletions or mutations induce switches of oncogenic/tumor suppressive signaling, such as PTEN loss or mutations among individual patients.

Finally, with the development of methods for the transcriptional and genomic analysis of cancer cells, PTEN inactivation can potentially be used as a prognostic and diagnostic marker for various types of cancer, including, but not limited to, prostate cancer. As the understanding of the role of PTEN in the progression and initiation of cancer increases, this accumulating knowledge may be used for the design of novel therapeutics methods that will target PTEN-dependent pathways to treat different stages of prostate cancer.

Acknowledgements

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