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Post-transcriptional regulation of PTEN dosage by non-coding RNAs

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Abstract

The classic “two-hit” model of tumor suppressor inactivation, originally established by mathematical modeling of cancer incidence, implies that tumorigenesis requires complete loss of function of tumor suppressor genes. While this is true in some tumor types, the exact nature of tumor suppressor deregulation varies depending on tissue type, stage of cancer development, nature of co-existing molecular lesions, and environmental factors(1). Emerging evidence has indicated the functional importance of PTEN dosage during tumor development. Among the key regulators of PTEN dosage are a number of non-coding RNAs, including microRNAs (miRNAs) and pseudogenes, which regulate PTEN expression at the post-transcriptional level. Recent studies have revealed the functional importance of these PTEN-targeting non-coding RNAs during tumor development, and have provided a paradigm to explore the molecular mechanisms underlying the dosage-dependent effects of key oncogenes and tumor suppressors.

In addition to complete loss of function, alteration of tumor suppressor genes in cancer can also cause partial loss of function, gain of function, or dominant negative phenotypes, which are important for tumor initiation and/or progression in a context-dependent manner. In particular, there is growing interest in the variety of monoallelic inactivating tumor suppressor alterations occurring as an early event of tumorigenesis, which precede the ultimate biallelic inactivation of the same tumor suppressor at late stage of cancer. These findings implicate dosage-dependent effects on tumor suppression and suggest that the molecular basis for gene dosage regulation plays a key role in cancer development.

One of the best-studied dosage-dependent tumor suppressors is PTEN, the essential lipid phosphatase repressor of the highly oncogenic PI3K/AKT pathway(2, 3). In a variety of human cancers, such as prostate, breast, colon, and lung, monoallelic *PTEN* mutations or deletions occur frequently at an early stage of tumorigenesis, but the second *PTEN* allele remains active(4). It is in advanced and metastatic cancers that biallelic *PTEN* inactivation becomes prevalent(4). Furthermore, in mouse models where the endogenous level of *Pten* expression is genetically engineered, *Pten* inactivation has dosage-dependent effects on tumor progression, latency, and invasiveness (5-7). For example, heterozygous *Pten* loss in the mouse model for prostate cancer leads to prostate epithelial hyperplasia and low-grade lesions with incomplete penetrance(7). Additional reduction in *Pten* expression causes

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massive prostate hyperplasia with complete penetrance, accelerating tumor progression(7). Ultimately, complete inactivation of *Pten* gives rise to invasive and highly aggressive malignancies(7). Surprisingly, even subtly reduced *Pten* dosage, such as a hypomorphic allele with 80% of wildtype activity, increases tumor formation in mice, with cell type-dependent penetrance(5). It has been demonstrated that the complete loss of PTEN triggers cellular senescence, which protects against tumor initiation and/or progression(8). Therefore, it is tempting to speculate that partial loss of *PTEN* is more likely to promote tumorigenesis during the initial stage, while complete loss of PTEN promotes rapid tumorigenesis and metastasis after the senescence mechanisms are impaired in advanced tumors.

Although reduced *PTEN* activity in cancer is often associated with genetic mutations or chromosomal alterations, the level of *PTEN* expression and its activity are subject to other regulatory mechanisms. Transcriptional repression and epigenetic silencing, post-transcriptional gene regulation, posttranslational modification, and aberrant PTEN localization can all contribute to reduced PTEN activity, thus impacting the progression and the invasiveness of the resulted malignancies(4). If small changes in *PTEN* dosage cause significant phenotypical consequences in humans, as they do in mouse genetic models, it is likely that genetic or chromosomal alterations affecting these gene regulatory pathways could impact clinical outcome by modulating PTEN levels. This is consistent with the finding that in some sporadic tumors with monoallelic *PTEN* mutation, PTEN expression could be decreased or lost without detectable mutations or deletions of the second allele.

Emerging evidence highlights the importance of post-transcriptional silencing in gene regulation, including its essential roles in diverse developmental, physiological and pathological processes. A key player in this regulation is a family of small non-coding RNAs, microRNAs (miRNAs). First identified in *C. elegans* as essential regulators for the timing of larval development(9), miRNAs are now recognized as a large family of non-coding RNAs found in nearly all metazoans. Despite a high degree of functional divergence, most animal miRNAs share a common molecular structure, biogenesis machinery, and effector pathway. The mature miRNA is incorporated into the RNA-induced silencing complex (RISC), which subsequently mediates post-transcriptional gene silencing of specific mRNA targets by a combined mechanism of mRNA degradation and translational repression(10). The small size of miRNAs and imperfect base-pairing with their targets together give miRNAs the capacity to regulate many target mRNAs(11). Therefore, miRNAs often act as global regulators for gene expression, and a single mRNA can be subjected to regulation by multiple miRNAs. The collective action of several miRNAs on a particular mRNA may result in a continuum of gene dosage in a cell type and context-dependent manner. Such dosage-dependent modulation by miRNAs during tumor development could have a significant effect on the biological outcome.

The connection between miRNAs and cancer was first implicated by their frequent genomic alteration and dysregulated expression in various human tumors (12, 13). Interestingly, genetic and epigenetic alterations of global miRNA biogenesis machinery also exhibit oncogenic activity (14). Given the importance of *Pten* dosage during tumor development, it is not surprising that multiple miRNAs have been identified to modulate *PTEN* expression at

the post-transcriptional level in the context of malignant transformation. These PTEN-targeting miRNAs include those contain a single hairpin structure, such as *miR-21* (15, 16), *miR-22* (17) and *miR-214* (18) and *miR-205* (19), as well as those with a polycistronic structure, such as *mir-17-92* (20-22), *mir-106b-25* (17), *mir-367-302b* (17) and *mir-221-222* (15). Many polycistronic PTEN targeting miRNAs are particularly interesting. Unlike protein-coding genes where one transcript gives rise to one protein product, a single precursor transcript from a polycistronic miRNA gene yields multiple mature miRNAs. This gene structure gives miRNA clusters a unique regulatory ability, since specific components of the same miRNA polycistron often have synergistic effects on the same target mRNA (s). For example, *miR-19a* and *miR-19b*, two highly homologous components of *mir-17-92*, both target PTEN through the same binding sites in the 3'UTR (21). In *mir-106b-25*, two non-homologous components, *miR-25* and *miR-93*, repress PTEN through separate target sites (17). In both cases, although each individual miRNA component moderately dampens PTEN expression, cooperative effects among different components can achieve greater PTEN reduction. Complex modes of miRNA regulation of post-transcriptional PTEN levels present a redundant yet powerful mechanism to generate a range of PTEN dosages. Fine-tuned PTEN levels translate to precise PI3K-AKT signaling, which in turn determines the exact readout of PTEN physiological functions in a cell type and context-dependent manner.

Besides directly targeting PTEN, some PTEN-targeting miRNAs act to repress additional components of the same pathway, further increasing the PI3K-AKT readout. *miR-19* miRNAs, for example, repress both PTEN and PP2A, suggesting similar sequence motifs may exist in the 3'UTRs of functionally related genes to coordinate their post-transcriptional regulation (20, 21, 23). Given the polycistronic structure of a number of PTEN-targeting miRNA genes, it is conceivable that multiple mature miRNAs encoded by the same miRNA cluster (s) could target different components of the same signaling pathway, thus quantitatively modulating the dosage of the ultimate PI3K/AKT signaling readout.

The prominent biological effects of dosage-dependent tumor suppressor activity, as exemplified by PTEN, illustrate the functional importance of miRNAs in tumorigenesis. Both specific miRNAs and components of the miRNA biogenesis machinery undergo genetic and epigenetic alterations in human cancers. Deregulation of several PTEN targeting miRNAs, in particular, is prevalent in a variety of human cancers. Enforced expression of these miRNAs promotes malignant transformation by enhancing PI3K-AKT signaling in both tissue culture and animal models. Alteration of the PTEN 3'UTR is also likely to affect miRNA regulation and impact its dosage and activity through the altered post-transcriptional gene regulation. No PTEN 3'UTR mutations have been identified in human cancer so far. However, there are several examples where defected post-transcriptional gene regulation caused by 3'UTR alteration would lead to aberrant phenotypes. For example, mutations in the 3'UTR can change the polyadenylation site to either acquire or remove potential miRNA target sites. In addition, sequence polymorphism in the 3'UTR can alter the efficiency and specificity of miRNA targeting. Ongoing cancer genome sequencing projects are likely to reveal functionally important 3'UTR alterations in well-characterized oncogenes and tumor suppressors, which may result in defective miRNA regulation, thus promoting tumor initiation, progression, or metastasis.

A recent study revealed yet another unexpected mechanism for PTEN dosage regulation, where a PTEN pseudogene acts as a miRNA decoy to modulate PTEN levels during tumorigenesis(24). The protein-centric view of gene function considers pseudogenes non-functional variants of known genes because they have lost their protein-coding ability through genetic mutations. This viewpoint has been challenged in recent years, as examples of active transcription of pseudogenes emerged, and the gene regulatory role of pseudogenes was recognized (25). A *PTEN* pseudogene, *PTENP1*, shares extensive sequence homology with PTEN mRNA, particularly in the ORF region and within the first third of its 3'UTR, where known miRNA target sites are enriched(24). Since both PTEN and PTENP1 can be regulated by the same set of miRNAs, PTENP1 transcripts may sequester PTEN-targeting miRNAs, indirectly de-repressing PTEN expression and enhancing its tumor suppressor activity(24). Consistent with this hypothesis, chromosomal deletion of PTENP1 has been identified in colon and breast cancer samples with decreased PTEN levels(24). As exciting as this finding is, questions still remain. For example, how does PTENP1 act as a powerful and efficient decoy for PTEN-targeting miRNAs despite its lower expression level compared to endogenous PTEN mRNA? Do PTEN-targeting miRNAs regulate *PTEN* and *PTENP1* through the same mechanisms? Finally, are there additional non-coding RNAs that function as decoys for PTEN-targeting miRNAs? Answers to these questions will help us fully elucidate the molecular mechanisms underlying pseudogene-mediated post-transcriptional regulation of PTEN.

PTEN is not the only gene that exhibits dosage-dependent tumor suppressor activities at different stages of tumorigenesis. Similar scenarios have been described for a number of other tumor suppressors in many cancer types. For example, the role of p53 in mediating DNA repair and autophagic response makes partial p53 activity preferable for tumor cell survival at an early stage of cancer development. It is only at a later stage of tumorigenesis that additional mutations obviate the benefit of partial p53 activity (26). This situation may explain why complete p53 inactivation occurs relatively late in many tumor types. The importance of gene dosage for oncogene function is emerging as well. For example, high levels of aberrant c-Myc signaling can trigger widespread apoptosis to prevent tumorigenesis, while low-level c-Myc over-expression is advantageous in early cancer development, since it promotes proliferation without triggering apoptosis (27). As a result, robust c-myc signaling is only prevalent in late stage cancers that have impaired apoptotic responses (27). Given the pathological importance of gene dosage in tumor development, it is tempting to speculate that defective post-transcriptional gene regulation, combined with genetic mutations, chromosomal alterations and epigenetic modification, could generate a range of aberrant tumor suppressor and/or oncogene dosages that are advantageous for specific stages of malignant transformation. Identifying the crucial players that regulate gene dosage in the oncogene and tumor suppressor network will provide novel insights into the molecular basis underlying tumor development.

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Figure 1.

A diagram for the post-transcriptional PTEN regulation by miRNAs and pseudogenes.