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AUTHOR'S VIEW

Mechanistic insights into the role of truncating *PREX2* mutations in melanoma

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ABSTRACT

PREX2 is a PTEN binding protein that is significantly mutated in melanoma and pancreatic ductal adenocarcinoma. We recently reported the molecular mechanism of tumorigenesis associated with *PREX2* mutations: truncating *PREX2* mutations activate its RAC1 guanine nucleotide exchanger activity leading to increased PI3K/AKT signaling and enhanced cell proliferation.

Abbreviations: PREX2, phosphatidylinositol-3, 4, 5-triphosphate-dependent Rac exchange factor 2; PTEN, phosphatase and tensin homolog; RAC1, ras-related C3 botulinum toxin substrate 1; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase

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Cancer genomic studies have provided tremendous insight into the complexity of somatic mutations and delivered a long list of novel mutated cancer genes.[1] It is expected that a wave of functional studies will assign detailed molecular and functional roles for these mutations in tumor development. We identified *PREX2* (phosphatidylinositol-3, 4, 5-triphosphate-dependent Rac exchange factor 2) as a gene that is significantly mutated in human melanomas.[2] Interestingly, the International Cancer Genomics Consortium (ICGC) recently reported that *PREX2* is also significantly mutated in pancreatic ductal adenocarcinoma. [3]

PREX2 is a guanine nucleotide exchange factor (GEF) for ras-related C3 botulinm toxin substrate 1 (RAC1) and a known phosphatase and tensin homolog (PTEN) interacting protein.[4,5] Functionally, PREX2 has been shown to regulate Rac1-mediated cellular invasion in a manner that crosstalks with PTEN signaling and also regulates insulin signaling and glucose homeostasis through the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathway.[6,7] Our initial sequencing of the melanoma genome showed various patterns of *PREX2* mutations including missense and truncating mutations. Using xenograft models, we were able to show that truncating *PREX2* mutations have oncogenic activity;[2] however, how these *PREX2* mutations contribute to melanoma development was unclear.

To study *PREX2* mutations in a spatio-temporally restricted manner, we generated an inducible transgenic mouse model that expresses a truncating PREX2 mutant (*TetO-lox-STOP-lox-Prex2*$^{E824^*)}$ in melanocytes.[8] We crossed these transgenic mice with mice that have a melanoma sensitizing background (i.e., they lack the tumor suppressor *Ink/Arf* and inducibly express a constitutively active *Nras*^{Q61K}) to generate a genetically engineered mouse (GEM) model of melanoma. Tumor formation was induced by administration of tamoxifen (which induces Cre-mediated recombination and removal of the stopper cassette) and doxycycline (which allows expression of *Prex2*$^{E824^*}$ and *Nras*^{Q61K} transgenes from tet-responsive promoters). Interestingly, we observed an increased incidence of melanoma formation in mice harboring the inducible *Prex2*$^{E824^*}$ transgene. We also generated xenograft tumors by expressing control GFP, *PREX2* wild type, or various *PREX2* truncating mutations in primary immortalized human melanocytes. Again, truncating *PREX2* mutations induced increased tumor formation. To explore the molecular mechanisms behind the ability of *PREX2* mutations to induce increased tumor formation, we performed gene expression profiling of tumors from both xenograft and GEM models. Integrative cross-species analysis revealed regulation of cell cytoskeleton organization, cell cycle, and ribosome biogenesis as key biological pathways that were significantly enriched in tumors with *PREX2* truncating mutations. The connection of PREX2 to RAC1 can explain changes in the cell cytoskeleton signaling pathway, whereas the known role of PREX2 in PTEN biology is expected to explain the enrichment in ribosome biogenesis. However, it was not clear why cell cycle regulation is perturbed in *PREX2* mutant tumors and we therefore investigated this aspect further.

Histopathologically, we observed that *PREX2* mutant tumors are highly proliferative and show increased KI67 staining. Furthermore, we observed reduced expression of key negative cell cycle regulators such as CDKN1C (also known as p57) and CDKN1B (also known as p27) and increased expression of insulin like growth factor 2 (IGF2) in *PREX2* mutant tumors. How do truncating mutations in *PREX2* result in these biological changes? To answer this question, we next studied the biochemical and signaling consequences of truncating *PREX2* mutations. First, we purified recombinant full-length PREX2 or an N-terminal truncated PREX2 from Sf9 cells and performed guanine nucleotide exchange (GEF) activity assays using RAC1 as a substrate. This analysis revealed higher GEF activity of the truncated PREX2 compared to full-length PREX2. In support of these data, we also showed that cells with *PREX2* truncating

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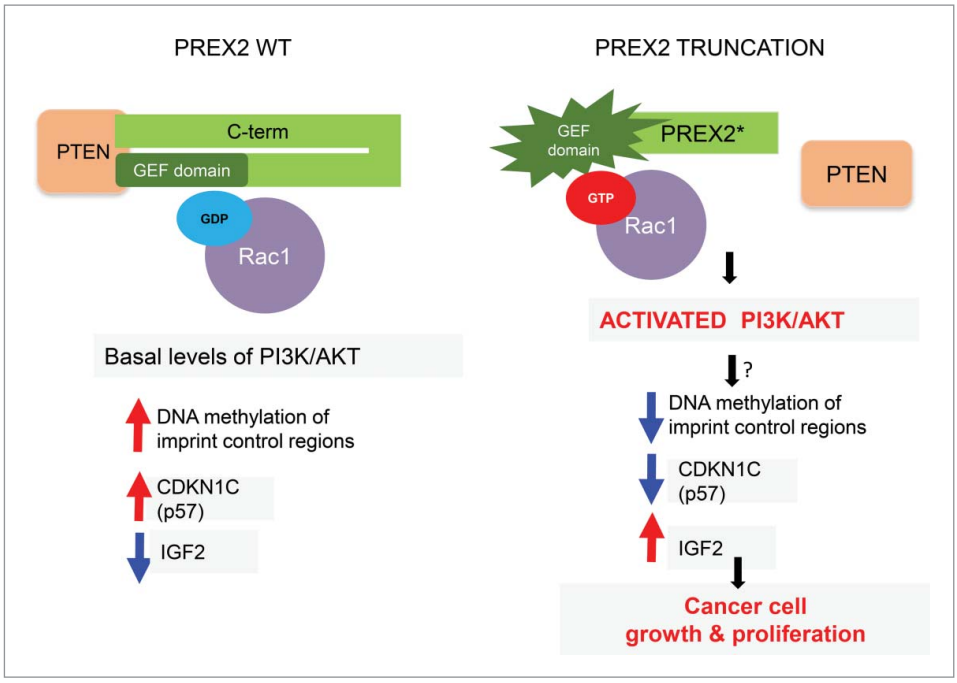

Figure 1. Mechanistic consequences of *PREX2* truncating mutations. In wild-type cells PREX2 is bound to PTEN and its GEF domain is kept in an inactive state through association with its C-terminus (C-term), with resultant basal levels of downstream signaling events such as GDP-loaded RAC1. In cells and tumors with truncating *PREX2* mutations, PREX2 is unable to bind to PTEN and its intramolecular inhibitory conformation is lost, resulting in activation of its GEF activity. This results in GTP loading of RAC1, which in turn leads to direct activation of the PI3K/AKT pathway. These alterations (through a still unknown link) perturb DNA methylation at a critical genomic imprint control region leading to downregulation of a key cell cycle regulator CDKN1C (also known as p57) and upregulation of IGF2, which favors accelerated cell growth and proliferation. GDP, guanosine diphosphate; GEF, guanine nucleotide exchange factor; GTP, guanosine triphosphate; IGF2, insulin like growth factor 2; PREX2, phosphatidylinositol-3, 4, 5-triphosphate-dependent Rac exchange factor 2; PTEN, phosphatase and tensin homolog; RAC1, ras-related C3 botulinum toxin substrate 1.

mutation have increased GEF activity as demonstrated by increased GTP-loaded Rac1.

How does truncation of PREX2 increase its GEF activity? Our data suggest the existence of 2 cooperating mechanisms that explain this effect. First, truncated PREX2 mutants lack binding to PTEN, which is known to have a suppressive effect on the GEF activity of PREX2. Hence, by avoiding the inhibitory influence of PTEN, PREX2 mutants have an intrinsically higher GEF activity. Second, using structural modeling of the PREX2:Rac1 interaction, we demonstrated that the GEF domain of PREX2 behaves similarly to the GEF domain of PREX1. It is known that the C-terminus of PREX1 has an auto-inhibitory effect on its N-terminal GEF activity.[9,10] Our structural model suggests that a similar mechanism exists in PREX2, and thus *PREX2* truncating mutations relieve the auto-inhibition of GEF activity by the C-terminus. Next, we examined the consequences of increased PREX2 GEF activity and RAC1 activation. Using reverse phase protein array (RPPA) and immunoblotting, we observed increased phosphorylation of AKT at Ser473 and Thr308 in *PREX2* mutant tumors. Gain-of-function and loss-of-function experiments revealed that the increased activation of Akt by *PREX2* mutations was dependent on activation of Rac1. Finally, we provided a mechanism to explain the gene expression changes induced by *PREX2* mutations: we showed that *PREX2* mutation or *PTEN* deletion induces DNA hypomethylation and downregulation of expression of p57, a critical cell cycle regulator.

In summary, our study demonstrated the oncogenic capacity of truncating *PREX2* mutations *in vivo*. We also identified a direct link to an established oncogenic signaling pathway—the PI3K/AKT pathway—and provided insights into the downstream regulation of a tumor suppressor—p57—in melanoma pathogenesis (Fig. 1). However, a number of important outstanding questions remain. High-resolution X-ray structures of full-length and truncated PREX2 protein in complex with its substrate will be of great help in elucidating the molecular basis of PREX2 GEF activation. Our understanding of the role of Rac1 in activation of the PI3K/AKT pathway is still rudimentary and must be explored in greater depth using biochemical, genetic, and pharmacologic tools. Finally, the link between changes in the PI3K/AKT pathway and downstream epigenetic and gene expression changes caused by *PREX2* mutations is not known and deserves further investigation.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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