RNA polymerase II and are processed in the nucleus by the Drosha RNase into pre-miRNAs (comprising 60 to 70 nucleotides). After transport into the cytoplasm, the pre-miRNAs are further processed by an RNase enzyme termed Dicer. miRNAs (and experimentally designed siRNAs) are generated consisting of 22 nucleotide RNA duplexes with two-nucleotide 3′ overhangs. Dicer is part of the RNA-induced silencing complex that functions downstream of miRNA processing.

Early studies demonstrated that constitutive loss of *Dicer1* results in embryonic lethality at E8.5 because of loss of brachyury-expressing stem cells.[4] Conditional alleles of *Dicer1* have shown the requirement of the enzyme in B cell[5] and T cell development.[6,7] Interestingly, conditional deletion of *Dicer1* in mouse osteoprogenitors resulted in a myelodysplasia phenotype, which progressed into a secondary leukemia with splenomegaly, anemia, an increase in leukemic blasts in the blood and bone marrow, and myeloid sarcomas in a small subset of animals.[8] Inducible deletion of *Dicer1* using *Mx1-Cre* resulted in defective competitive repopulation and reduced reconstitution in secondary transplant recipients. miRNA profiling revealed a locus on chromosome 19 expressing 3 miRNAs. Remarkably, expression of miR-125a increased long-term multilineage reconstitution and one of miR-125a targets was shown to be the proapoptotic protein Bak1.[9]

These previous studies help to frame the current analysis. A conditional *Dicer1* allele was crossed with *Cebpa-Cre* to delete the enzyme in granulocyte-macrophage progenitors (GMPs). *Cebpa-Cre;Dicer1$^{fl/fl}$* mice died shortly after birth, likely due to defective *Cebpa*-dependent induction of the lung epithelium in late gestation,[10] similar to mice with conditional deletion of *Dicer1* driven by a Sonic Hedgehog transgene.[11]

To circumvent the perinatal lethality observed in this model, Alemdehy et al transplanted fetal liver cells from mutant and control embryos into lethally irradiated recipient mice. No quantitative differences in Lin-Sca-1^{neg}Kit^{pos}, common myeloid progenitors (CMPs), GMPs, or megakaryocyte erythroid progenitors (MEPs) were observed in *Dicer1*-deficient mice. However, there was a 50% reduction in colony-forming unit–granulocyte macrophages (CFUGMs) from *Dicer1* mutants. Culture of Lin^{-};Dicer1$^{\Delta/\Delta}$ progenitors in GM-CSF resulted in myeloid cells with a hyposegmented nucleus, typical of Pelger-Huet Anomaly. Hyposegmented and bilobed neutrophils were also observed in vivo. This disease has been correlated with mutations in the Lamin B receptor.[12] No changes in Lamin B receptor expression was observed in *Dicer1*-null GMPs. Whether Dicer1 affects other genes in the Lamin B receptor pathway or whether additional genes play a role in Pelger-Huet Anomaly remains to be resolved.

Dicer1-deficient neutrophils also appear to have defect migration as Ly-6G^{pos}Dicer1$^{\Delta/\Delta}$ granulocytes were decreased in the peripheral blood and spleen. However, unlike the *Osterix-Dicer1fl/fl* mice,[8] no myeloproliferative disease or acute myeloid leukemia was observed.

The effect of *Dicer1* deletion on gene expression in GMPs was examined by expression profiling. There were 300 significantly up-regulated transcripts including Bim, K-Ras, Hmga2, Hoxa9, and p21. Gene dosage appeared to also play a role as loss of 1 allele of *Dicer1* affected transcript levels.

While it is not surprising that Dicer1 plays a critical role in many cellular lineages, there is remarkable specificity in phenotypes observed with the conditional alleles of *Dicer1* reported to date. Whether individual lineages have a unique miRNA target as has been shown with inducible Mx1-Cre remains to be explored.[9] Several miRNAs play critical roles in the regulation of hematologic malignancies when gain-of-function or loss-of-function mouse modeling experiments are analyzed.[13] To identify the precise targets involved in lineage-specific determination, miRNA add-back screens should be devised. It will be interesting to determine whether single miRNAs will regulate specific cell progenitors as has been demonstrated for miR-125a.[9] Gaining an understanding of the underlying biology of miRNAs will help validate this interesting group of macromolecules for therapeutic modulation.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● VASCULAR BIOLOGY

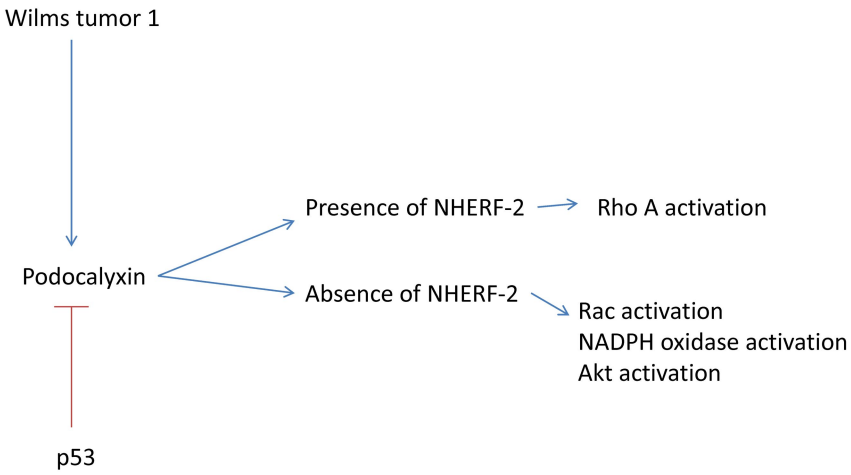
Comment on Bhattacharya et al, page 4798

NHERF-2 silences the silencers

Jack L. Arbiser EMORY UNIVERSITY SCHOOL OF MEDICINE

Hyperresponsiveness to growth factors underlies a wide variety of human diseases, including hemangiomas, the most common tumor of childhood. Hemangiomas have been found to be clonal neoplasms of endothelial cells, with somatic mutations in unknown genes likely to be responsible for their development.

In this issue of *Blood*, Bhattacharya et al discover a novel mechanism of endothelial quiescence mediated by the Na+/H+ exchanger regulatory factor-2 (NHERF-2) gene, which might play a role in regulating responses to exogenous growth factors.[1]



NHERF-2 is a molecular switch that controls endothelial and tumor migration. In the presence of NHERF-2, rho A activation predominates, while in the absence of NHERF-2, a WT-1/podocalyxin/rac/reactive oxygen signaling pathway is activated.

Specifically, they describe that loss of NHERF-2 leads to a loss of endothelial quiescence and an enhanced response to exogenous growth factors. This is of clinical interest because previous studies have shown that hemangiomas do not make large amounts of endogenous VEGF. A major source of VEGF in hemangiomas is paracrine, namely overlying keratinocytes and stromal cells. Thus, controlling responsiveness to exogenous growth factors is a key to the pathogenesis and treatment of hemangiomas.

Bhattacharya et al discovered that NHERF-2 expression is relatively enriched in endothelial cells compared with the closely related gene NHERF-1, which exhibits a wider tissue distribution.[1] SIRNA-mediated knockdown of NHERF-2 resulted in a significant increase in trititated thymidine uptake in cultured endothelial cells. Intriguingly, knockdown of NHERF-2 did not affect the phosphorylation of canonical VEGFR2 sites, including Y951, Y1059, and Y1175, and appears to down-regulate phosphorylation of Y416 Src. MAPK phosphorylation does not appear to be appreciably elevated as well. What, then, mediates the hyperresponsiveness of endothelial cells to VEGF in the absence of NHERF-2? Stimulation of downstream pathways may provide an answer. Levels of the proliferative oncogene C-myc are up-regulated in the absence of NHERF-2 and are further potentiated by exogenous VEGF. Finally, the cell-cycle inhibitor p27 is down-regulated by NHERF-2 knockdown. Significantly, down-regulation of NHERF-2 in the bend3 model of hemangioma leads to augmented growth in vivo. Thus, NHERF-2 loss leads to activation of a C-myc–p27 pathway, with the functional consequence of augmenting endothelial proliferation in vitro and in vivo.

Informatics on the oncogenes and tumor suppressors downstream of NHERF-2 loss reveals that beta-catenin can stimulate C-myc transcription through a TCF-dependent mechanism.[2] In a tumor setting, enhanced C-myc signaling is associated with Skp2-mediated degradation of p27. NHERF-2 was found to be a binding partner, and possibly a negative regulator, of beta-catenin. The question of whether NHERF-2 prevents nuclear localization of beta-catenin is worthy of further study, given that nuclear localized beta-catenin is common in many solid tumors.

The physiologic function of NHERF-2 is not completely understood. NHERF-2 has been demonstrated to be a regulator of G protein receptor signaling, involving the parathyroid receptor, lysophosphatidic acid receptors, and the cystic fibrosis transporter (CFTR).[3] NHERF-2 has been knocked out, giving viable and fertile animals with a subtle phenotype in the gut. Thus, none of these findings can fully explain the angioproliferative phenotype of NHERF-2 loss. Intriguingly, the closely related NHERF-1 protein binds the tumor suppressor PTEN, resulting in down-regulation of Akt.[4] Loss of PTEN is associated with the genetic abnormalities Cowden disease and Bannayan-Zonanna syndrome, which are associated with hemangiomas and vascular malformations.[5] Thus, it is possible that loss of NHERF-2 might cause activation of Akt. The lack of other signaling events seen with NHERF-2 knockdown (no effect on VEGFR2 receptor phosphorylation or MAPK), make Akt activation a very plausible and easily testable hypothesis, especially the presence of NHERF-2 overexpressing endothelial cells. One would predict that increased NHERF-2 would result in decreased Akt expression.

Hemangiomas are the original members of a class of tumors known as the reactive oxygen–driven tumor.[6] These neoplasms are characterized by elevated Akt, which prevents apoptosis because of reactive oxygen, and at the same time have high levels of superoxide, which oxidizes wild-type p53, IkB, and PTEN. The functional consequences of this are wild-type inactive p53, activation of NFkB, and phosphorylation of Akt. These tumors can be treated by inhibitors of superoxide and hydrogen peroxide, which as single agents have a major impact on these pathways. NADPH oxidase 1 and 2, which produces superoxide, and NADPH oxidase 4, which produces hydrogen peroxide, have been implicated in hemangioma growth, as inhibitors of these NADPH oxidases have been highly efficacious against murine hemangioma models as well as human hemangiomas.[7] A final question is whether NHERF-2 is functionally equivalent to inhibition of NADPH oxidases.

NHERF-2 is also prominent as a partner of the polarity-related protein podocalyxin. Podocalyxin, a transcriptional target of the Wilms tumor 1 gene (WT-1), was initially described as a protein involved in renal podosomes, but has been more recently implicated in tumor migration. High-level expression of podocalyxin has been associated with increased metastatic ability and poor outcome in renal cell carcinoma.[8] The high-level expression of podocalyxin is associated with elevated rac activation.[8] Given that rac activation is required for generation of superoxide through NADPH oxidases, it may be that the presence of NHERF-2 represents a molecular switch, in that the presence of NHERF-2 causes a decrease in rac activation and an increase in rhoA activation[9] (see figure). The loss of NHERF-2 may lead to the reverse phenotype, rac activation, increased podocalyxin, and increased reactive oxygen, thus leading to increased actin polymerization and increased motility of both endothelial and tumor cells.[10] Interestingly, WT-1, a major activator of podocalyxin, is highly expressed in hemangioma cells, as well as highly invasive tumors (ie, glioblastoma).[11] Thus, NHERF-2 represents a novel target for pharmacologic therapy, and drugs

that act to reduce reactive oxygen could potentially phenocopy NHERF-2.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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