CELL CYCLE
2016, VOL. 15, NO. 6, 772–774
http://dx.doi.org/10.1080/15384101.2015.1137712

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EDITORIALS: CELL CYCLE FEATURES

LIN28B: an orchestrator of oncogenic signaling in neuroblastoma

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ARTICLE HISTORY Received 21 December 2015; Accepted 29 December 2015

The aggressive nature of many pediatric malignancies appears to be shaped by a relatively simple somatic genome, especially in comparison to adult tumors. However, when one considers the genome of the host from which these tumors arise, the landscape grows more complex. Our laboratory has focused on neuroblastoma, an aggressive childhood cancer of the developing nervous system. We have been guided by the hypothesis that germline variation in critical oncogenes and tumor suppressors leads to deregulated signaling that both initiates and sustains this malignancy. Through an ongoing genome-wide association study (GWAS), we have identified associations between neuroblastoma susceptibility and germline variation in several genes, including Lin-28 Homolog B (*LIN28B*) (Fig. 1).[1]

LIN28B is an RNA binding protein that blocks the maturation of let-7, a diverse microRNA family known to inhibit tumorigenesis. LIN28B also binds directly to mRNAs, likely influencing transcription and translation. We previously demonstrated that neuroblastoma cells harboring the germline risk-allele at the *LIN28B* locus showed high *LIN28B* expression and increased cell proliferation.[1] In addition, we showed that high *LIN28B* expression is associated with poor patient survival.[1] Providing further evidence that *LIN28B* drives tumorigenesis, mice overexpressing LIN28B in the developing adrenal gland developed neuroblastoma.[2]

As multiple lines of investigation strongly supported an oncogenic role for LIN28B in neuroblastoma, we recently aimed to define key LIN28B-directed signaling networks.[3] We first analyzed gene expression data from neuroblastoma primary tumors and identified a strong positive correlation between *LIN28B* expression and RAN (RAS-related nuclear protein) signaling. *RAN*, a member of the Ras family of small GTPases, is well known for its role in nuclear trafficking, is overexpressed in diverse malignancies, and promotes AURKA (Aurora kinase A) phosphorylation. *RAN* is located at chromosome 12q24, a region of recurrent DNA copy number gain we and others have observed in neuroblastoma. We demonstrated that neuroblastomas with 12q gain were associated with increased *RAN* expression, suggesting that somatic gain might represent another means of driving *RAN* expression. We also showed that downregulation of RAN led to decreased cellular proliferation, mimicking LIN28B downregulation, and that *RAN* expression was correlated with advanced stage neuroblastoma and decreased overall survival.

Next, we demonstrated that LIN28B directly induced the expression of RAN, identifying 2 distinct mechanisms by which it does so (Fig. 1). First, we showed that RAN Binding Protein 2 (RANBP2) is a direct let-7 target. As RANBP2 had been shown to stabilize the expression of various proteins, including RAN, within the murine retina,[4] we hypothesized that it played a similar role in neuroblastoma and indeed demonstrated that RANBP2 knockdown led to decreased RAN protein levels. Next, we found that LIN28B binds *RAN* mRNA, constituting a second and direct mechanism of regulation. Finally, we demonstrated a convergence of LIN28B and RAN signaling on AURKA. LIN28B promoted RAN expression, which in turn induced higher levels of phosphorylated AURKA, leading to kinase activation. Unexpectedly, we also observed higher levels of total AURKA, implying that there might be RAN-independent mechanisms by which LIN28B influenced AURKA. We discovered that AURKA was a direct let-7 target, explaining the effect of LIN28B on total AURKA levels. As AURKA stabilizes MYCN at the level of the protein,[5] these findings reveal an intricate and novel signaling cascade connecting LIN28B, RAN, AURKA, and MYCN.

Our findings illustrate the ability of genome-wide association studies to inform our understanding of aberrant oncogenic signaling in neuroblastoma. Thus, *LIN28B* joins *LMO1* and *BARD1*, as well as the long noncoding RNA *CASC15-S*, as factors in both initiating and sustaining neuroblastoma tumorigenesis. With the contribution of 12q24 gain to *RAN* expression in neuroblastoma subsets, the interplay of both germline and somatic variation in shaping the oncogenic phenotype is also illustrated. Of note, although we focused on the interactions between LIN28B and RAN itself, our analyses revealed strong correlations between LIN28B and additional RAN pathway members. These RAN

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Feature to: Schnepp RW, et al. Cancer Cell. 2015; 28(5): 599-609; PMID: 26481147; http//dx.doi.org/10.1016/j.ccell.2015.09.012

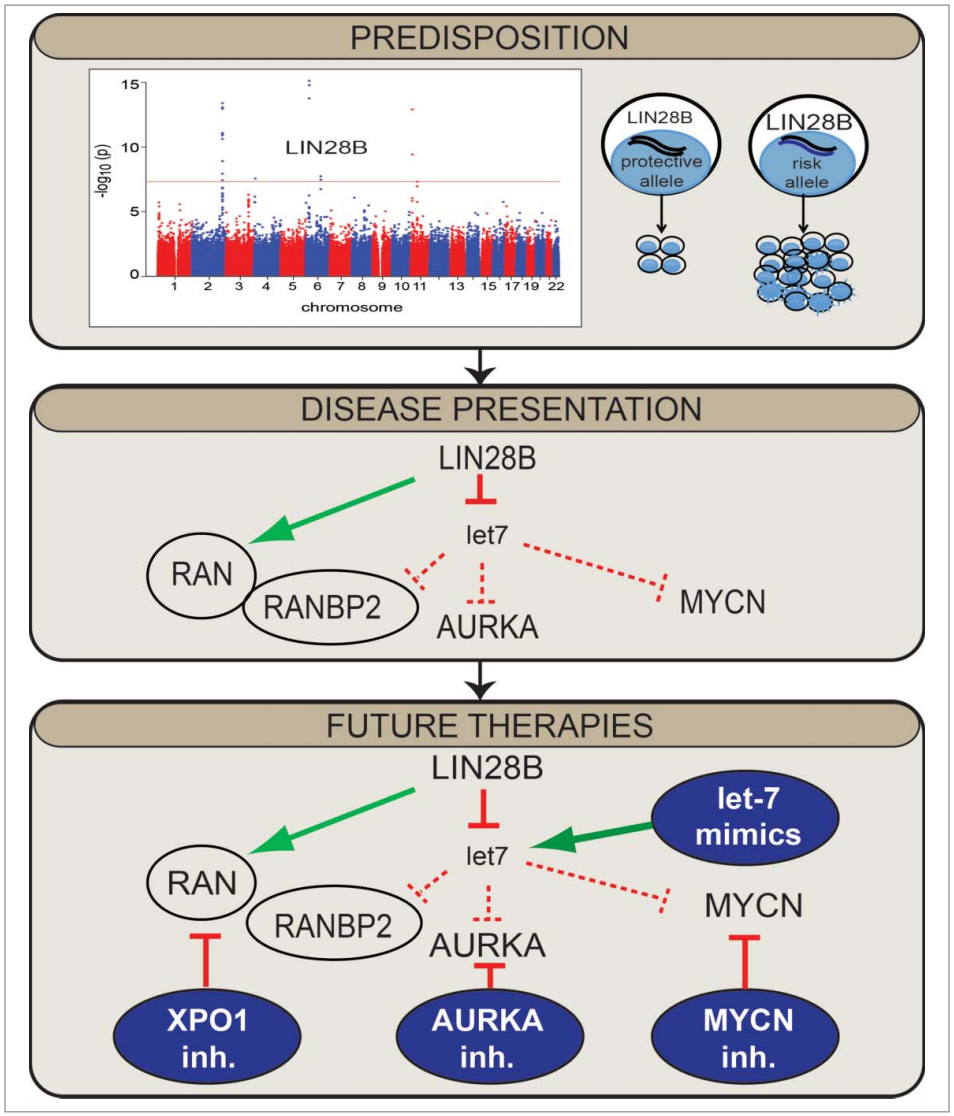

Figure 1. LIN28B influences neuroblastoma susceptibility and disease presentation via a complex oncogenic signaling cascade offering possibilities for future therapeutic intervention. Shown under "Predisposition" is a Manhattan plot depicting the discovery of germline variants within LIN28B associated with neuroblastoma; cells harboring the risk allele express high levels of LIN28B and increased cell proliferation.[1] "Disease Presentation" illustrates a complex signaling cascade in neuroblastoma driven by LIN28B that involves RAN, AURKA, and MYCN.[3] Targeting of downstream LIN28B signaling with novel combinatorial approaches may improve the treatment of patients with high-risk neuroblastoma, as depicted in "Future Therapies."

pathway associated genes encode proteins that play key roles in the regulation of the cell cycle and apoptosis, nuclear trafficking, invasion, and metastasis. It will be interesting to dissect the influence of LIN28B on these genes and determine how these genes impact the phenotype of neuroblastoma.

From a translational genomics perspective, the elucidation of this network suggests potential novel combinatorial approaches that could target downstream LIN28B signaling and improve the treatment of patients with high-risk neuroblastoma (Fig. 1). While there are currently no therapies targeting RAN, inhibitors against XPO1/CRM1 (a protein that directly binds RAN and, like RAN, participates in nuclear export) are under investigation for both liquid and solid malignancies. Moreover, AURKA inhibitors, in combination with chemotherapy, are in Phase 2 trials for neuroblastoma[6] and Bromodomain and extraterminal (BET) inhibitors that target the MYC family, including MYCN, have demonstrated some efficacy in neuroblastoma preclinical models.[7] Novel-novel approaches inhibiting RAN/XPO1, AURKA, and MYCN, and promoting let-7 expression could provide a rational approach to target the LIN28B network, although further work to determine optimal combinations is needed. Given the diverse cancers in which deregulation of LIN28B is seen, it will be intriguing to determine whether this LIN28B-mediated network is seen in the context of other malignancies and whether modulating this pathway might improve the treatment of cancer.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

[1] Diskin SJ, et al. Nat Genet 2012; 44(10):p. 1126-30; PMID:22941191; http://dx.doi.org/10.1038/ng.2387

[2] Molenaar JJ, et al. Nat Genet 2012; 44(11):p. 1199-206; PMID:23042116; http://dx.doi.org/10.1038/ng.2436
[3] Schnepp RW, et al. Cancer Cell 2015; 28(5):p. 599-609; PMID:26481147; http://dx.doi.org/10.1016/j.ccell.2015.09.012
[4] Patil H, et al. J Biol Chem 2014; 289(43):p. 29767-89; PMID:25187515; http://dx.doi.org/10.1074/jbc.M114.586834
[5] Otto T, et al. Cancer Cell 2009; 15(1):p. 67-78; PMID:19111882; http://dx.doi.org/10.1016/j.ccr.2008.12.005
[6] Mosse YP, et al. Clin Cancer Res 2012; 18(21):p. 6058-64; PMID:22988055; http://dx.doi.org/10.1158/1078-0432.CCR-11-3251
[7] Puissant A, et al. Cancer Discov 2013; 3(3):p. 308-23; PMID:23430699; http://dx.doi.org/10.1158/2159-8290.CD-12-0418