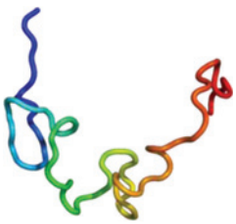
Breaking Advances
Highlights from Recent Cancer Literature

LIN28B as a Susceptibility Gene and Oncogenic Driver in Neuroblastoma



LIN28 (LIN28A) and its homologue LIN28B are small RNA binding proteins, are amplified in solid tumors and in leukemia, and can contribute to the reprogramming of differentiated cells into pluripotent stem cells. LIN28 and LIN28B are negative regulators of miRNA processing and cooperate to block processing of the *Let-7* miRNA precursor into its mature form. The family of seven *Let-7* miRNAs (*MIRLET7*) function as tumor suppressors, silencing oncogenes including MYC, MYCN, RAS, and CDK6. Reporting in *Nature Genetics*, two groups have now described a role for LIN28B in childhood neuroblastoma, one of the most common solid tumors of childhood. Through analysis of an expanded, ongoing, and highly productive genome-wide analysis study of 2800 tumors and 4200 controls, Diskin and colleagues identified two new SNPs at 6q16. One of these mapped to an intron in *LIN28B* and the other to *HACE1*, a HECT domain– and ankyrin repeat–containing E3 ubiquitin protein ligase 1, and a candidate tumor suppressor implicated in Wilms and in GI tumors. Analysis of human neuroblastoma tumors showed a correlation of low expression of *HACE1* and high expression of *LIN28B* with risk and poor survival. In a related but independent study, Molenaar and colleagues identified a rare 6q21 amplicon in 3 of 263 sporadic human neuroblastoma tumors, with four genes including *LIN28B* mapping to this amplicon. They subsequently showed that *LIN28B* was overexpressed in high-risk neuroblastoma in three independent datasets, with *LIN28B* expression again correlating with poor survival. Both papers provided functional validation in neuroblastoma cell lines, including the demonstration that high levels of *LIN28B* led to decreased levels of *Let-7* miRNA. Diskin and colleagues showed correlation of *LIN28B* and *MYCN*, the latter, a proto-oncogene that is frequently amplified in high-risk neuroblastoma. Interestingly, the Molenaar group showed that knockdown of *LIN28B* led to high levels of *MYCN* but not to similarly high levels of *RAS* or *CDK6*. Molenaar and colleagues then generated a transgenic mouse model that directed misexpression of murine *Lin28b* to the neural crest. These mice developed neuroblastoma-like tumors in appropriate anatomic locations. Tumors showed high levels of both Lin28b and Mycn proteins and low levels of Let-7 (*Mirlet7*). Analysis of mRNA arrays from *Mycn*-driven and *Lin28b*-driven murine neuroblastoma tumors showed considerable overlap. Taken together, these studies suggest that both *HACE1* and *LIN28B* influence susceptibility in neuroblastoma, and that *LIN28B* drives transformation in neuroblastoma through enhanced expression of *MYCN*.

Diskin SJ, Capasso M, Schnepp RW, Cole KA, Attiyeh EF, Hou C, et al., Common variation at 6q16 within HACE1 and LIN28B influences susceptibility to neuroblastoma. Nat Genet 2012;44:1126–30.

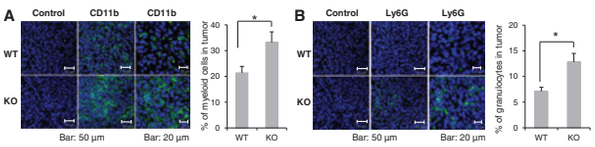
Molenaar JJ, Domingo-Fernández R, Ebus ME, Lindner S, Koster J, Drabek K, et al., LIN28B induces neuroblastoma and enhances MYCN levels via let-7 suppression. Nat Genet 2012;44:1199–206.

The Unfolded Protein Response as a Target in MYC-Driven Dancer

The proto-oncogene MYC is a transcription factor that is hyperactivated in myriad human cancers, including 100% of Burkitt's lymphomas. MYC promotes tumorigenesis by driving the expression of genes regulating cell growth, metabolism, and cell cycle, among others. The most overrepresented class of MYC transcriptional targets regulates ribosome biogenesis and protein synthesis. An emerging field of study has focused on the critical role for increased protein synthesis downstream of MYC in tumorigenesis. Using genetic tools to restore increased protein synthesis to normal levels in MYC-overexpressing cells lead to prolonged lifespan in mouse models of *Myc*-driven cancer. These findings suggest that drugs targeting translation downstream of MYC may render this once 'undruggable' oncogene susceptible to targeted therapeutics. Indeed, novel therapeutics are currently being developed to use this susceptibility in MYC-overexpressing cells. While it is now obvious that increased protein synthesis is required for MYC-driven tumorigenesis, the precise mechanisms by which increased protein synthesis leads to cancer downstream of MYC remain poorly understood. In this study, Hart and colleagues show one mechanism by which MYC-overexpressing cells cope with the increased rate of protein synthesis: induction of endoplasmic reticulum (ER) stress signaling pathways induced as a result of the accumulation of unfolded proteins in the lumen of the ER. The authors show that the unfolded protein response (UPR) plays a critical role in promoting the survival of cells experiencing ER stress due to MYC-driven increases in protein synthesis rates. Using tissue culture and *in vivo* models of acute and chronic MYC activation, the authors show that the ER stress-sensing kinase PERK (EIF2AK3) becomes activated in response to the accumulation of excess protein. Strikingly, genetic ablation of PERK activity leads to dramatic increases in apoptosis of MYC-overexpressing cells, showing a cytoprotective role for the UPR in this context. Mechanistically, loss of PERK signaling in MYC-overexpressing cells prevented the cytoprotective ER stress-mediated induction of autophagy that occurred in cells with intact PERK signaling. Importantly, pharmacologic or genetic inhibition of the autophagic response led to increased MYC-dependent apoptosis. The authors used a mouse minute mutant ($Rpl24^{+/-}$) to restore MYC-driven increases in protein synthesis rates to normal levels in premalignant MYC-overexpressing B cells *in vivo,* which alleviated the UPR response and abrogated PERK signaling and autophagy induction. The study also showed by expression profiling analysis that the UPR response is a hallmark of MYC-overexpressing human lymphomas, which highlights the potential therapeutic benefit of targeting PERK for inhibition in these cancers. Altogether, this work highlights an important cell autonomous escape mechanism by which cells harboring MYC oncogenic lesions can evade apoptosis and form tumors. Most importantly, this work suggests that inhibiting UPR may represent a novel therapy against MYC-promoted cancers.

Hart LS, Cunningham JT, Datta T, Dey S, Tameire F, Lehman SL, et al. ER stress-mediated autophagy promotes Myc-dependent transformation and tumor growth. J Clin Invest 2012;122: 4621–34.

FPR2 Regulates Macrophage Polarization and Tumor Progression



A role for type-II cytokine production in the tumor-promoting activity of tumor-associated-macrophages (TAM, M2 type) is now becoming evident. In addition to expressing various chemokine G-protein-coupled-receptors (GPCR), macrophages also express a chemokine GPCR known as formyl peptide receptor 2 (*FPR2* in humans, *Fpr2* in mice). *Fpr2* has been implicated in both innate and adaptive immune responses. The present study by Liu and colleagues examined the precise role of FPR2 in antitumor host response. At first, the authors observed significantly higher tumor growth and metastasis with reduced survival in *Fpr2*-KO mice implanted subcutaneously or receiving intravenous injection with Lewis Lung Carcinoma (LLC) cells as compared to the wild type (WT) mice. In contrast, subcutaneously implanted LLC in *Fpr2*-transgenic mice overexpressing *Fpr2* grew more slowly than those in WT littermates. These observations suggested a potential role of *Fpr2* in regulating tumor progression. It was noticed that a significantly higher number of TAMs along with a variety of myeloid-derived cells infiltrated the LLC tumors in the *Fpr2*-KO mice as compared to the WT mice. The profound infiltration of the TAMs was a result of the selective expression of the macrophage chemoattractant CCL2 by the LLC cells. CCL2 appeared to use CCR2 and CCR4, receptors for mediating the chemotaxis response of the TAMs. Further studies showed that the *Fpr2*-KO mice-derived TAMs were defective in eliciting or polarizing towards an M1 phenotype in response to the "factors" released by the tumors. In contrast, the *Fpr2*-KO mice-derived TAMs were found to polarize towards a M2 phenotype and expressed specific M2 markers. On the other hand, the M1 polarization and phenotype was measurable in the WT mice as they expressed specific M1 markers. The definitive role of Fpr2 in regulating the M1/M2 phenotype was further confirmed by using specific antagonists against Fpr2 in the WT mice, which increased the expression of CCL2, CCR4, and ARG1, signature molecular expression markers associated with M2 polarization observed in the *Fpr2*-KO mice. This study indicates a definitive role of Fpr2 in maintaining the M1 phenotype of the TAMs with more potent antitumor activities and has potential to develop immunotherapy and monitoring strategies.

Liu Y, Chen K, Wang C, Gong W, Yoshimura T, Liu M, Wang JM. Cell surface receptor FPR2 promotes anti-tumor host defense by limiting M2 polarization of macrophages. Can Res; Published OnlineFirst November 8, 2012: doi:10.1158/0008-5472.CAN-12-2290.

VEGF-A Levels and Response to Bevacizumab in Solid Tumors

The development of antiangiogenic strategies to treat cancer underlies the need to identify which patients would be predicted to benefit from these therapies. Bevacizumab, the humanized anti-VEGF-A antibody, is the most widely tested antiangiogenic in clinical trials. Given that it is specifically targeted to VEGF-A, the authors asked whether VEGF-A levels in patients are predictive of response to bevacizumab in clinical trials. They evaluated baseline VEGF-A levels from the serum of patients enrolled in clinical trials using bevacizumab in 4 phase III trials involving lung cancer, colorectal cancer, and renal cell carcinoma. The proportion of patients whose serum was available ranged from 19% to 85% depending on the trial, and a total of 1,816 samples were analyzed. In addition, 132 tumor samples from 2 of the 4 clinical trials were available for study. VEGF-A levels were generally higher in the serum of patients from all 4 trials when compared to healthy control subjects. Interestingly, no correlation between circulating VEGF-A and tumor VEGF-A levels could be identified for those patients with matched samples. In the evaluation of the effect of a therapy in a clinical trial, the authors distinguished a prognostic effect (relationship of VEGF-A to outcome independent of treatment) from a predictive effect (relationship of VEGF-A specifically to bevacizumab therapy). To assess a prognostic relationship, the authors compared VEGF-A levels with outcome in the patients who did not receive bevacizumab and found a significant prognostic relationship in 3 of the 4 clinical trials (higher VEGF-A levels being associated with worse outcome). To assess a predictive effect, the authors examined hazard ratios from patients in the bevacizumab-treated arm, comparing those with low VEGF-A versus those with high VEGF-A. If the VEGF-A is, in fact, a predictive marker for bevacizumab, then patients in the high-VEGF-A group would be expected to derive greater benefit from the drug. However, no such effect was observed. The authors acknowledge several limitations of this study, including its retrospective nature, the fact that conclusions can only be reached for the specific cancer types, and also that conclusions should be drawn only in the context of any additional therapies that were combined with bevacizumab in these studies. However, this study does point out that the mere presence of elevated levels of the bevacizumab target should not be assumed to necessarily be predictive of response to this therapy. At minimum, this study prompts the search for additional biomarkers that may in the future identify patient subsets that would be likely to receive the most benefit from antiangiogenic therapies.

Hegde PS, Jubb AM, Chen D, Li NF, Meng G, Bernaards C, et al. Predictive impact of circulating vascular growth factor in 4 phase III trials evaluating bevacizimab. Clin Cancer Res; Published OnlineFirst November 20, 2012; doi:10.1158/1078-0432.CCR-12-2535.

Note: Breaking Advances are written by *Cancer Research* Editors. Readers are encouraged to consult the articles referred to in each item for full details on the findings described.

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Cancer Res 2012;72:6317-6318.