ALK and MYCN: When Two Oncogenes Are Better than One

Zhihui Liu[1] and Carol J. Thiele[1,*]
[1]Pediatric Oncology Branch, Center for Cancer Research, National Cancer Institute, 9000 Rockville Pike, Bethesda, MD 20892, USA
*Correspondence: ct47a@nih.gov
DOI 10.1016/j.ccr.2012.03.004

Mutations of *ALK* are frequently observed in *MYCN*-amplified neuroblastomas and correlate with poor clinical outcome, but how these oncogenes cooperate in neuroblastoma development remains unclear. In this issue of *Cancer Cell,* Zhu et al. describe a mechanism by which ALK and MYCN synergistically induce neuroblastoma in the zebrafish model system.

Neuroblastoma (NB) is the most common childhood malignant solid tumor of the sympathetic nervous system (Maris, 2010). Numerous chromosomal abnormalities, including deletions of chromosome arms 1p, 11q, gain of 17q, and amplification of *MYCN* on chromosome 2p, are associated with NB. The overall prevalence of *MYCN* amplification is about 20% and is a genetic marker of poor prognosis that has been used for treatment stratification of NB patients since the late 1980s (Maris, 2010). During the past 40 years, the outcome of children with high-risk NB has shown marked improvements. However, the 5 year event-free survival is only 50% for these patients, and there are significant toxicities associated with current treatments. Until recently, no recurrent mutations in druggable targets had been identified. The discovery of activating mutations in the *ALK* oncogene in both hereditary (80%) and sporadic (7%–8%) NB provides an opportunity for targeted therapy (reviewed in Azarova et al., 2011).

The *ALK* oncogene was identified initially as a gene that fused with *NPM* through 2;5 chromosome translocation in cases of anaplastic large-cell lymphoma (Morris et al., 1994). Although the wild-type (WT) ALK is preferentially expressed in the central and peripheral nervous systems, its physiologic role, ligands, and signaling pathways remain largely obscure (Azarova et al., 2011). In a series of elegant studies, activating mutations in ALK were identified in primary NB tumors, and their oncogenic potential was demonstrated. WT ALK was also thought to possess oncogenic activity in NB cells when its expression level surpasses a threshold (Azarova et al., 2011).

A question in the field has been whether *ALK* mutations alone are sufficient to cause neuroblastoma. The finding that *ALK* mutations occur in equal frequencies across all genomic NB subtypes and in both low- and high-risk tumors seemed to be inconsistent with *ALK* being the sole "driver" oncogene in NB. One clue came with the finding that a common mutation *ALK F1174L* is observed at a higher frequency in *MYCN*-amplified tumors (Azarova et al., 2011). Moreover, overexpression of WT or mutant ALK stimulated *MYCN* transcription in neuroblastoma cell lines, and coexpressing activated ALK and MYCN increased NIH 3T3 cell transformation in vitro (Schönherr et al., 2012). Amplification of *ALK* also occurs in about 2%–3% of NB, but almost exclusively in those with *MYCN*-amplification (Azarova et al., 2011). Taken together, these studies raised the possibility of a positive cooperative effect between the dysregulation of both ALK and MYCN.

In this issue of *Cancer Cell*, Zhu et al. (2012) explore these questions by generating a series of transgenic zebrafish models to assess the tumorigenicity of ALK, ALK F1174L, and MYCN alone or together. Using the dopamine-β-hydroxylase promoter to drive EGFP-MYCN expression, Zhu et al. (2012) first investigated whether MYCN transgenic zebrafish can develop NB as had been shown in mice (Weiss et al., 1997). They showed that MYCN-induced zebrafish NB at a low frequency (17.3%), with these tumors sharing histologic, immunocytochemical, and ultrastructural features with human NB. Zebrafish NB arise from neuroblasts that migrate into the interrenal gland (equivalent to the human adrenal gland) late in development (~21 days post fertilization), and the sympathoadrenal precursors coexpress neuronal-specific Hu proteins and the catecholaminergic enzymes TH and DβH.

Zebrafish expressing ALK or ALK F1174L transgene alone did not develop NB. To address whether ALK and MYCN genetically interact, Zhu et al. (2012) generated transgenic zebrafishes that expressed the human ALK, or ALK F1174L, together with MYCN. Tumor onset was accelerated in fish expressing both MYCN and ALK F1174L compared to those expressing MCYN alone, and the penetrance in the MYCN/ALK F1174L fish was 3-fold higher than MYCN fish. Like the tumors in the MYCN fishes, tumors in MYCN/ALK F1174L fish arise in the interrenal gland and resemble human NB. This suggests that ALK mutations may be "initiating" events and need a "second hit", such as dysregulated MYCN, to fully transform cells. This provides a context which might explain the findings of *ALK* mutations in the tumors of low risk, good prognosis NB patients.

So how does ALK accelerate tumor onset and increase penetrance in MYCN transgenic fish? Zhu et al. (2012) found that the number of Hu^+ neuroblasts was significantly increased in MYCN transgenic fish compared with controls at 3–5 weeks post fertilization (wpf) (Figure 1). These MYCN-overexpressing neuroblasts fail to differentiate, resulting in reduced numbers of chromaffin cells. Moreover, at 5–7 wpf, the number of Hu^+ neuroblasts in the interrenal gland decreased significantly due to apoptotic cell death. These findings indicate that overexpression of MYCN causes expansion of sympathoadrenal neuroblasts

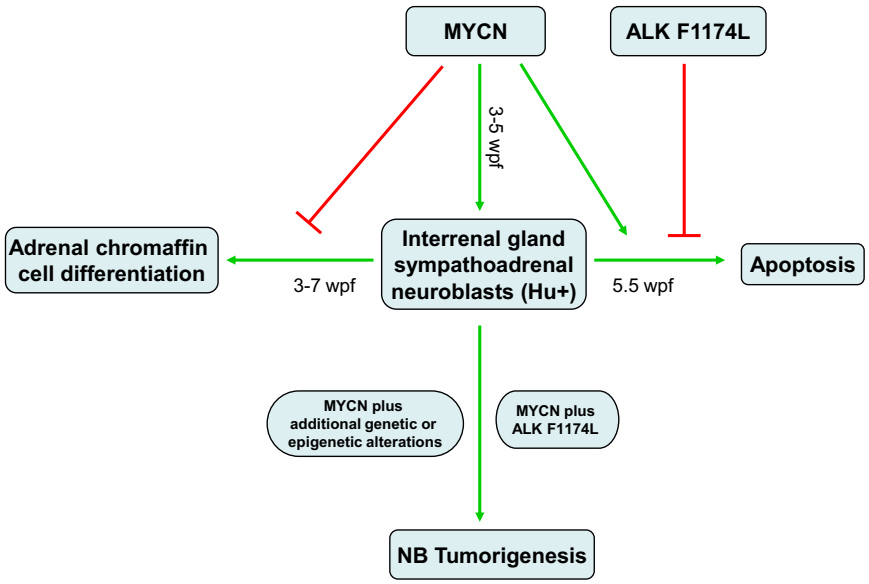

Figure 1. MYCN and ALK F1174L Synergistically Impact Neuroblastoma Tumorigenesis
In zebrafish, the MYCN overexpression causes expansion of the sympathoadrenal neuroblasts from 3 to 5 wpf, and the MYCN overexpressing neuroblasts fail to differentiate into chromaffin cells. Only a small group of zebrafish (17%) with MYCN overexpression developed neuroblastoma; others won't because MYCN overexpression also triggers an apoptotic response at 5.5 wpf. The activated ALK F1174L provides a cell survival signal that blocks the apoptotic response of MYCN-overexpressing neuroblasts at this juncture in development, so the tumor penetrance in the MYCN/ALK F1174L coexpressing transgenic fish is 3-fold higher (56%).

and prevents their differentiation, yet it also induces a developmentally-timed apoptotic response (Figure 1). However, in the presence of activated ALK, these cells survive but fail to differentiate resulting in the continued accumulation of Hu^+ neuroblasts and the development of highly penetrant, fully transformed NB (Figure 1). For the MYCN-only transgenic fish that develop tumors, it is possible that additional genetic alterations cooperate with MYCN to contribute to NB formation. Zhu et al. (2012) ruled out mutations in the tyrosine kinase domain of the zebrafish *alk* gene and loss of capsase-8 expression that is known to occur in *MYCN*-amplified human NB. Thus, other genetic mutations or epigenetic events that activate prosurvival pathways may occur with MYCN overexpression in these tumors. In fact, recent studies showed that the Myc family (including MYCN) directly upregulates the transcription of all core components of the Polycomb Repressive Complex 2 (PRC2) to maintain embryonic stem cell pluripotency (Neri et al., 2012). Dysregulation of EZH2 (a subunit of PRC2) has been shown to silence differentiation-associated genes with tumor suppressor activity in undifferentiated human NB (Wang et al., 2012). In this zebrafish model system, WT or activated ALK expression alone is not sufficient for the development of NB but requires overexpression of MYCN. It remains to be clarified if increased expression of ALK via the use of a stronger promoter or protein stabilization will result in tumorigenesis on its own.

The results from Zhu et al. (2012) demonstrate that ALK and MYCN collaboratively contribute to NB development, with ALK F1174L attenuating MYCN-induced apoptosis perhaps by activating pro-survival pathways. A clinical Phase I/II trial targeting ALK is underway in children with solid tumors, including those with NB using Crizotinib, the FDA-approved ALK/MET inhibitor. Given the clinical experience with targeted agents and the development of drug resistance, it is unlikely that targeting ALK alone will lead to durable responses. Multi-modality therapeutic approaches will be needed. The general view has been that transcription factors such as MYCN are less amenable to targeted therapeutic approaches. However, the recent identification of inhibitors of BET (bromodomain and extraterminal subfamily of human bromodomain proteins), which decrease MYCN mRNA levels in *MYCN*-amplified NB cell lines (Mertz et al., 2011) and strategies to destabilize MYCN protein (Hogarty and Maris, 2012), indicate it may be feasible to target MYCN. The zebrafish MYCN-ALK F1174L NB tumor model serves not only to understand developmental alterations leading to tumor formation but it can also serve as a platform for evaluating unbiased drug screens as well as the next generation of targeted approaches.

REFERENCES

Azarova, A.M., Gautam, G., and George, R.E. (2011). Semin. Cancer Biol. *21*, 267–275.

Hogarty, M.D., and Maris, J.M. (2012). Cancer Cell *21*, 145–147.

Maris, J.M. (2010). N. Engl. J. Med. *362*, 2202–2211.

Mertz, J.A., Conery, A.R., Bryant, B.M., Sandy, P., Balasubramanian, S., Mele, D.A., Bergeron, L., and Sims, R.J., 3rd. (2011). Proc. Natl. Acad. Sci. USA *108*, 16669–16674.

Morris, S.W., Kirstein, M.N., Valentine, M.B., Dittmer, K.G., Shapiro, D.N., Saltman, D.L., and Look, A.T. (1994). Science *263*, 1281–1284.

Neri, F., Zippo, A., Krepelova, A., Cherubini, A., Rocchigiani, M., and Oliviero, S. (2012). Mol. Cell. Biol. *32*, 840–851.

Schönherr, C., Ruuth, K., Kamaraj, S., Wang, C.L., Yang, H.L., Combaret, V., Djos, A., Martinsson, T., Christensen, J.G., Palmer, R.H., and Hallberg, B. (2012). Oncogene. Published online January 30, 2012. 10.1038/onc.2012.12.

Wang, C., Liu, Z., Woo, C.W., Li, Z., Wang, L., Wei, J.S., Marquez, V.E., Bates, S.E., Jin, Q., Khan, J., et al. (2012). Cancer Res. *72*, 315–324.

Weiss, W.A., Aldape, K., Mohapatra, G., Feuerstein, B.G., and Bishop, J.M. (1997). EMBO J. *16*, 2985–2995.

Zhu, S., Lee, J.S., Guo, F., Shin, J., Perez-Atayde, A.R., Kutok, J.L., Rodig, S.J., Neuberg, D.S., Helman, D., Feng, H., et al. (2012). Cancer Cell *21*, this issue, 362–373.