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Unraveling Neuroblastoma Pathogenesis with the Zebrafish

Erin N. Dankert[a], A. Thomas Look,[b] and Shizhen Zhu[a]

[a]Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Mayo Clinic Cancer Center, Rochester, MN, USA; [b]Department of Pediatric Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

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Ten years of genome-wide association studies (GWAS) by many investigators have produced a series of remarkable discoveries based on large numbers of patients with diverse diseases, leading to optimism that insights gained through GWAS will increasingly be translated into new diagnostics and therapeutics [1]. The future of GWAS appears especially bright given the recent widespread application of whole genome sequencing and CRISPR-Cas9 genome editing technology, which promise to more precisely elucidate molecular pathogenesis and provide genomic strategies for therapeutic intervention.

Neuroblastoma GWAS studies led by J. Maris have been among the most successful in human cancer, leading to the identification of multiple inherited associations involving specific genomic regions that significantly impact the risk of a child developing either low risk or high risk disease [2]. Among the GWAS hits reported by the Maris group, single nucleotide polymorphisms (SNPs) associated with the LIM-domain-only-1 gene (*LMO1*) are perhaps the most predictive and are associated with increased expression of *LMO1* in neuroblastoma patients with high-risk features: metastatic disease, advanced age, and an unfavorable pathologic tumor grade [3]. One of these SNPs resides in a noncoding region and is necessary for the formation of a large super-enhancer that drives the high levels of *LMO1* expression required for neuroblastoma development [4]. This property illustrates the general finding that disease-associated SNPs are often located within super-enhancers that control cell state by regulating the expression of key lineage-associated developmental regulatory genes [4].

To substantiate the significance of *LMO1* in neuroblastoma pathogenesis *in vivo*, S. Zhu and A.T. Look, in collaboration with J. Maris, generated stable transgenic zebrafish lines that overexpress the wild-type *LMO1* gene in the peripheral sympathetic nervous system (PSNS) under control of the dopamine-β-hydroxylase (*dβh*) promoter. We found that transgenic fish overexpressing *LMO1* alone do not develop neuroblastoma over 6 months of monitoring, consistent with the hypothesis that *LMO1* cooperates with other genetic abnormalities to promote neuroblastomagenesis [5]. Indeed, in this study, overexpressed *LMO1* synergized with high levels of *MYCN* expression to accelerate neuroblastoma onset and increase disease penetrance [5] (Figure 1), providing the first direct *in vivo* support for the GWAS prediction that *LMO1* overexpression might promote the initiation of neuroblastoma.

As reported by Wang *et al.*, SNPs that correlate with high levels of *LMO1* expression are specifically enriched in high-risk neuroblastoma patients with widespread metastasis [3]. Strikingly, we detected distant metastasis of neuroblastoma in transgenic fish overexpressing both *MYCN* and *LMO1* at 6 months of age, which was not observed in fish of the same age that overexpressed *MYCN* alone [5]. Thus, because of the dire prognosis carried by wildly disseminated neuroblastoma, we sought to better understand the mechanisms underlying the propensity of this tumor for metastatic spread.

The zebrafish model system provides clear advantages for elucidating the basis of neuroblastoma metastasis. It faithfully recapitulates all stages of tumor metastasis *in vivo*, and its relative transparency permits real-time imaging of tumor initiation, progression and dissemination over time. Using this model and neuroblastoma cell lines both overexpressing *LMO1*, we demonstrated enhanced expression of a panel of genes affecting the tumor cell-extracellular matrix interactions, including *loxl3*, *itga2b*, *itga3*, and *itga5*, and correlation of their upregulation with neuroblastoma cell invasion and migration [5] (Figure 1). A very recent study using an avian model of metastatic neuroblastoma [6] supports these findings, contributing to a rational for wider use of the zebrafish model to unravel critical cell-cell and cell-microenvironment interactions that drive neuroblastoma metastasis.

We also suggest that the zebrafish would provide a valuable resource for evaluating new small-molecule inhibitors or combination therapeutics against metastatic tumor cells. We have shown that suppression of LOX (lysyl oxidase) enzymatic activity with aminopropionitrile (BAPN), a small-molecule inhibitor of the LOX family, can successfully abolish the enhanced invasive and migratory properties induced by *LMO1* overexpression in the BE2C neuroblastoma cell line [5]. In addition, cilengitide,

CONTACT Shizhen Zhu Zhu.shizhen@mayo.edu 221 4th Ave SW, Guggenheim 13-21C, Rochester, MN, 55902.
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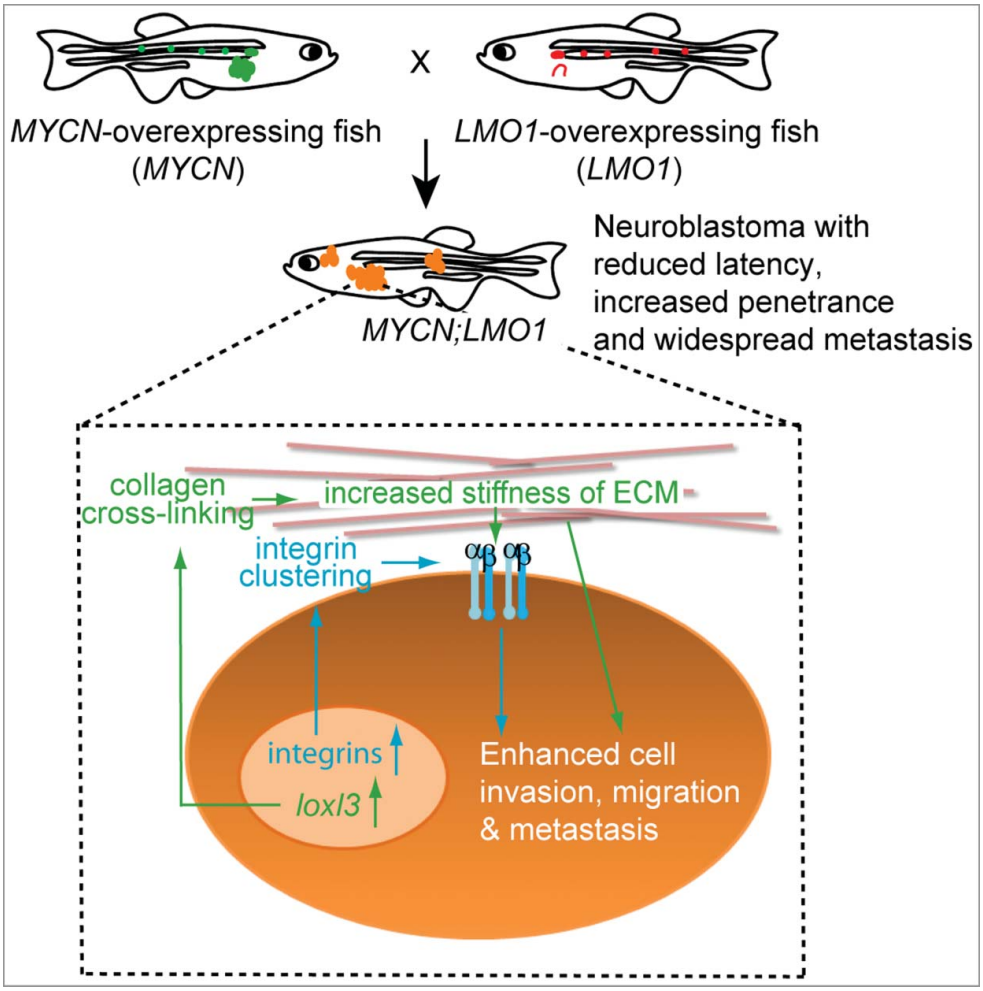

Figure 1. Zebrafish model of neuroblastoma showing that overexpression of *LMO1* collaborates with *MYCN* overexpression to promote more rapid initiation of neuroblastoma at a higher penetrance [5]. *LMO1* overexpression also promotes widespread metastasis, by upregulating the expression of genes involved in collagen cross-linking, leading to increased stiffness of the extracellular matrix (ECM), and integrin clustering, leading to enhanced tumor cell-ECM interactions.

a cyclic peptide inhibitor of $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins, has recently been considered as an agent that specifically targets metastatic cells [7]. Because the expression levels of members of the *LOX* and *integrin* gene families are significantly elevated by LMO1 activity and contribute to tumor cell dissemination (Figure 1), the zebrafish model offers an ideal tool with which to evaluate the efficacy of single agents or combinations of inhibitors designed to target members of these gene families.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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