

Pigmentation Biology

Melanoma Biology and the Promise of Zebrafish

Craig J. Ceol,^{1,*} Yariv Houvras,^{1,2,*} Richard M. White,^{1,*} and Leonard I. Zon¹

Abstract

Advantageous organismal and technical attributes of the zebrafish are being increasingly applied to study cancer biology. Along with other tumor models, zebrafish that develop melanomas have been generated. In both genetics and phenotype, zebrafish melanomas are strikingly similar to their human counterparts. For this reason, studies in the zebrafish are poised to make significant contributions to melanoma biology. In this review, we summarize important features of human melanoma and discuss how the zebrafish can be used to address many questions that remain unanswered about this devastating disease.

Introduction

MELANOMA IS THE MOST AGGRESSIVE and lethal form of skin cancer. Annually, melanoma is responsible for an estimated 8110 deaths in the United States and at least 48,000 deaths worldwide.^{1,2} Whereas advances in detection and treatment have reduced rates of many cancers, the incidence of melanoma has risen sharply in recent decades.^{3,4} There is a high mortality rate for metastatic melanoma, in part because these tumors are radioresistant and refractory to available chemotherapies.⁵ However, early stage melanomas, those primary tumors that have not spread to lymph nodes, can be excised with little risk of recurrence. For these reasons it is important to identify and monitor individuals genetically at risk for melanoma and better recognize premetastatic lesions in these and other patients. More effective treatments of metastatic disease are also necessary.

Melanoma arises from melanocytes, which are the pigment-producing cells of human skin. Melanocytes can proliferate and give rise to various types of benign nevi, which are commonly referred to as moles. Transformed melanocytes can yield melanomas that generally begin growth radially in the epidermis. Radial growth then transitions to a vertical growth phase that involves invasion through the basement membrane into the underlying dermis. Metastasis follows thereafter.

There are many areas of melanoma biology that are underexplored. For example, the steps by which a normal melanocyte becomes or generates a melanoma cell are largely unknown. How the disease subverts properties of normal melanocytes and their neural crest progenitors has been a topic

of recent investigations, and this will be a fertile area of research in the future. Lastly, while great strides have been made in identifying genetic defects that contribute to melanoma,⁶ there are clearly many more genes that remain to be tied to this disease.

The zebrafish is becoming increasingly utilized to address some of these issues. Certain attributes of zebrafish make it useful for the study of melanocyte biology and melanoma. Zebrafish melanocytes (also referred to as melanophores) are externally visible, and single cells can be visualized in a living animal. In mammals, melanin pigment-containing melanosomes are transported to neighboring keratinocytes. However, zebrafish melanocytes retain melanin, which consequently serves as a reliable and useful cell-type marker. The development of melanocytes from the embryonic neural crest is well characterized (Fig. 1). Further, a wealth of zebrafish pigmentation mutants that affect melanocyte specification, differentiation, and function are available, and the genes defective in these mutants have conserved roles in mammals.^{7,8} The goal of this review is to highlight important findings in melanoma research and discuss issues that may be tractably addressed using zebrafish.

Melanocyte Development and Melanoma

Given the highly migratory nature of human melanoma, it is perhaps not surprising that many of the genes that are important for melanocyte development and emergence from the neural crest have pathogenic roles in cancer. An understanding of the developmental origins of the melanocyte lineage is likely to yield useful information regarding the cellular

¹Stem Cell Program and Division of Hematology/Oncology, Children's Hospital Boston and Dana-Farber Cancer Institute, Howard Hughes Medical Institute, Harvard Stem Cell Institute, Harvard Medical School, Boston, Massachusetts.

²Massachusetts General Hospital Cancer Center, Boston, Massachusetts.

*These authors contributed equally to this work.

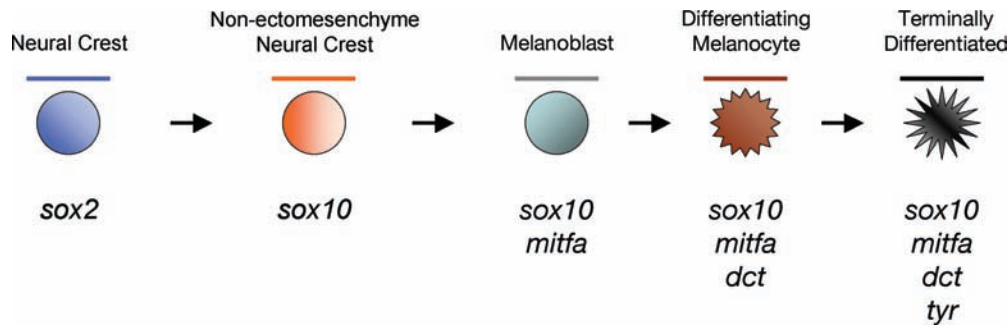


FIG. 1. Factors involved in the development of the melanocyte lineage in zebrafish. Embryonic precursors of the melanocyte lineage can be identified by specific markers. *sox2* is expressed in the developing ectoderm, and becomes down-regulated at the edge of the neural plate from where the crest cells emerge. *sox10* is an early marker of specified neural crest cells of the pigment lineage, and is not seen in nonectomesenchymal crest derivatives (i.e., craniofacial skeleton). The committed melanoblast temporally expresses *sox10* then *mitfa*, followed by markers of differentiation such as dopachrome tautomerase (*dct*) and tyrosinase (*tyr*).

functions and corresponding genetic pathways that may be usurped in melanoma. The repertoire of genes involved in the development of the melanocyte lineage has been fully reviewed elsewhere, so this section will focus on several factors that have been implicated as having a pathogenic role in melanoma. These pathways may offer rational therapeutic targets in this otherwise chemoresistant disease.

Melanocytes are derived from the neural crest

Vertebrates exhibit as many as six pigmented cell types, referred to collectively as chromatophores, although most species only contain a subset of these. In the zebrafish, three primary pigment cells are commonly found: the black-brown melanocyte, the yellow xanthophore, and the reflective iridophore. All of these cell types are derived from the highly migratory embryonic neural crest. The neural crest is induced during gastrulation, at the border zone between the neural and nonneural ectoderm.⁹ The early events in neural crest induction depend upon intact BMP signaling, as the *Bmp2* knockout mouse is essentially devoid of neural crest derivatives.¹⁰ Fate decisions of early progenitors toward the neural crest lineage are dependent upon intact Notch signaling, likely via DeltaA-dependent lateral inhibition.¹¹ Before migration, neural crest cells undergo an epithelial–mesenchymal transition (EMT), in which cells undergo loss of adhesion to neighboring cells and acquire migratory and invasive properties. This is, in part, dependent upon the Snail/Slug family of transcription factors, due to their ability to transcriptionally repress *E-cadherin*.¹² Onset of *Snail* transcription temporally coincides with neural crest migration.

In the zebrafish, fate mapping studies indicate that most neural crest cells fated to become chromatophores become lineage-restricted before migration.¹³ The potency of each premigratory neural crest cell ranges from a single lineage (i.e., melanocytes) to trilineage (i.e., melanocytes, iridophores, and xanthophores), although the latter is somewhat rare. Although the different types of chromatophores are closely related by lineage, a range of zebrafish mutants preferentially affects only one cell type. For example, the *mitfa* (a.k.a. *nacre*) mutant is primarily deficient in melanocytes,¹⁴ and the *fms* (a.k.a. *panther*) mutant is more severely affected in the xanthophore than the melanocyte lineage.¹⁵ These data indicate

different genetic requirements for each chromatophore type. There are also mutants in which multiple lineages are affected (e.g., *colorless* zebrafish, which are mutant in *sox10*, show loss of all three pigment cell types¹⁶), supporting the notion that other genes are either required for specification of a multipotent cell type or necessary for a postspecification function common to these cell types. There is only a single chromatophore lineage in mammals, the melanocyte, and below we focus on the genes required for development of the melanocyte lineage, with particular emphasis on those genes implicated in melanoma.

Melanocyte-specific genes involved in melanoma

Mitf. The central role of Mitf in melanocyte development has been demonstrated by loss-of-function studies in model organisms and by the pigmentary deficiencies evident in *MITF* mutant Waardenburg syndrome type IIa (WS2a) patients.^{17,18} Mouse mutants with pigment abnormalities caused by an *Mitf* mutation were originally described in 1942.¹⁹ Mouse *Mitf* mutants commonly lack pigmentation, are deaf, and have a reduction in eye size known as microphthalmia. In the zebrafish, the *mitfa* mutant, which lacks all neural crest–derived embryonic and adult melanocytes, provides clear evidence that this gene plays a critical role in specification of the lineage. Mitf is a myc-family basic helix–loop–helix/leucine zipper protein,²⁰ which is well conserved among vertebrate species, and closely related genes are present in invertebrates such as *Caenorhabditis elegans* as well.²¹ Abnormalities observed in WS2a patients and many *Mitf* mutant mouse strains are genetically dominant and are often produced by point mutations that are thought to disrupt MITF DNA or protein interactions. Whereas WS2a patients are often deaf and mouse *Mitf* mutants suffer from other defects such as microphthalmia and deafness, no such defects have been noted in the fish, possibly due to redundancies provided by the paralogous *mitfb* gene²² or to species-specific requirements of Mitf-dependent melanocytes in eye and ear development.

Molecularly, Mitf sits at a critical juncture between neural crest specification and promotion of melanogenesis.²³ It is transcriptionally regulated by several other genes that are known to promote melanocyte development from the neural

crest. Tcf/LEF binding sites, which are the mediators of canonical Wnt signaling, are found within the *MITF* promoters in humans and zebrafish.²⁴ Sox10 is an sry-box transcription factor necessary for multiple neural crest derivatives that can bind to the *Mitf* promoter and activate its transcription.^{25–29} In mammals, recent evidence indicates that PAX3 can upregulate transcription of *MITF*.³⁰ Finally, MITF can be post-translationally modified.^{31,32} Activation of c-KIT by its ligand, SCF, promotes phosphorylation of MITF at serine-73 (via ERK2) and serine-409 (via p90RSK), which upregulates transactivation by MITF through recruitment of p300. In addition, MITF regulates the melanocyte lineage by transcriptionally activating several genes involved in melanin synthesis, including *TYROSINASE*,³³ *TYRP1*,³⁴ and *MC1R*.³⁵ *Mitf* promotes survival of the melanocyte lineage by upregulation of *Bcl2*,³⁶ which likely explains why some murine *Mitf* mutants specify melanocytes that ultimately die prematurely via apoptosis. More recently, an oncogenic role of MITF has been discovered.³⁷ *MITF* is amplified and overexpressed in a subset of human melanomas, and overexpression may facilitate inappropriate cell cycle progression and survival of tumor cells. One area that awaits further clarification regards the importance of MITF in the melanocyte stem cell. Given increasing evidence that such stem cells exist, at least in murine hair follicles,³⁸ it will be important to understand whether MITF is required for properties such as self-renewal and multipotency, particularly in the setting of tumor development. Allelic variants of *Mitfa* in the zebrafish, in combination with overexpression under inducible promoters, will help to define the precise role of MITF in adult melanocyte homeostasis and during tumor progression.

Kit. Kit is a type III receptor tyrosine kinase that is required for melanocyte development in mammals and fish. Humans with heterozygous mutations of the *c-KIT* gene have the pigment disorder piebaldism, and mouse mutants for either *c-Kit* or its ligand, Scf/Steel, exhibit varying degrees of pigmentary, hematopoietic, and germ cell abnormalities.^{39–41} In contrast to mouse mutants, the zebrafish *kit* (a.k.a. *sparse*) mutant exhibits defects only in melanocytes.⁴² In *kit* mutant embryos melanocyte specification occurs properly, but these cells fail to migrate and subsequently undergo apoptosis. The *kit* mutant also exhibits defects in adult melanocytes, in that they fail to form dermal stripe melanocytes that arise early in the larval-to-adult metamorphic period and all of the epidermal, scale-associated melanocytes.

The role of *c-KIT* in human melanoma has come under renewed interest in the past few years. *c-KIT* is expressed in early melanocytic lesions, but downregulation of expression is typically seen with advancing stages of melanoma.^{43,44} Three initial trials of the c-KIT inhibitor imatinib mesylate in melanoma were disappointing, with essentially negative results.^{45–47} However, it has recently been demonstrated that a significant proportion of atypical melanomas, arising in areas such as mucosal surfaces, palms, and soles, harbor activating mutations of the *c-KIT* gene.^{48,49} Preliminary data from an ongoing phase II trial have reported a dramatic response to imatinib in one such patient,⁵⁰ suggesting that inhibition of c-KIT might be useful in well-selected populations of patients. Some caution, however, is warranted in the use of this inhibitor in unselected patients (i.e., those without *c-KIT* mutations), because loss of *c-KIT* expression may be associated with invasion and/or

metastatic progression. Because of the clinical importance of the invasion/metastasis question, it will be critical to define the role of c-KIT in adult melanocyte migration and self-renewal, and how this is altered in the oncogenic setting. The amenability to chemical approaches in the zebrafish melanoma model, along with the availability of numerous c-KIT inhibitors, may provide a unique opportunity to define the role of c-KIT inhibition in early and late stages of disease.

Endothelins. The endothelins are a family of vasoactive peptides (ET1, ET2, and ET3) that bind to the G-protein-coupled receptors EDNRA or EDNRB. The specific role of the endothelins in pigment cell biology was recognized over a decade ago, when knockout mice for either *Ednrb* or *Et3* were found to have melanocyte defects.^{51,52} Defects in this pathway are also found in human patients with Waardenburg-Shah syndrome, which combines features of Hirschsprung's disease (aganglionic megacolon due to loss of neural crest-derived enteric neurons) and Waardenburg syndrome (white hair forelock, heterochromia irides, and vitiligo). Using the inducible tet-system in mice, *Ednrb* has been shown to be required during a narrow window of neural crest development, and its loss during this period leads to almost complete loss of the lineage.⁵³ This indicates that the endothelins are necessary for the dispersal and survival of already-specified melanoblasts that are present in the neural crest during this window. The zebrafish *ednrb1* (a.k.a. *rose*) mutant too has a melanocyte deficiency, although it is not as severe as in other species. Whereas mouse mutants have defects in embryonic melanocytes, *ednrb1* mutants have normal embryonic and early stripe melanocytes. The pigment defect in *ednrb1* mutants does not become apparent until mid-to-late metamorphosis, when severe defects in the late stripe melanocytes are observed.^{54,55}

EDNRB is highly expressed in most human melanoma cell lines and some primary human melanomas as well.^{56,57} Treatment of melanoma cultures with the specific EDNRB antagonist BQ788 causes the cells to cease proliferation, and in some cases leads to apoptosis.⁵⁸ Some evidence suggests that endothelins signal through the MAP kinase pathway.⁵⁹ Endothelin signaling can also occur independently of MAP kinase, and it is expected that signaling by endothelins may synergize with *BRAF* activation, an important event in many melanomas. In support of this parallel pathway hypothesis, knockdown of oncogenic *BRAF* in melanoma cells led to a decrease in proliferation and colony formation, an effect that could be somewhat rescued by exposure to ET1.⁶⁰ The endothelins may also promote melanoma cell invasiveness by transcriptionally upregulating the expression of Snail, which leads to a loss of E-cadherin and a recapitulation of the embryonic EMT program.⁶¹ These data led to a small phase II trial of the nonselective EDNRA/EDNRB antagonist bosentan in human melanoma. Disease stabilization was noted in 6/32 patients, which is typical for other small molecule inhibitors used on unselected patients.⁶² Whether endothelin blockade will synergize with *BRAF* inhibition in human melanoma remains an unanswered question. Little data currently exist as to the role of endothelin-receptor subtypes (i.e., EDNRA vs. EDNRB) in stages of melanoma development, and some data would suggest that EDNRB expression is lost with melanocytic transformation.⁶³ Given the availability of EDNRA/EDNRB mutant zebrafish, and chemicals that specifically block each receptor type, zebrafish melanoma models will provide a

powerful tool for dissecting the role of these genes during tumor evolution.

Snail/Slug. The Snail family of DNA binding proteins is encoded by several highly conserved genes in species from *Drosophila* to humans. In zebrafish and *Xenopus*, the Snail family gene *slug* is expressed in specified neural crest cells, and represents one of the earliest markers of this lineage.⁶⁴ There is a reciprocal relationship within a group of neural crest specifiers that includes *Slug*, *Sox10*, and *Foxd3*. Morpholino knockdown of *Sox10* leads to a loss of *Slug* expression, and overexpression of *Sox10* leads to an expansion of the *Slug* expression domain.⁶⁵ The converse is also true: *Slug* knockdown with a dominant negative form represses *Sox10*, and ectopic *Slug* activates expression of *Sox10*. The mechanism of this reciprocity is unclear; the *Slug* protein may require secondary intermediates rather than acting as a direct transcriptional regulator of *Sox10*. *Slug* is required for the onset of neural crest migration, likely via its ability to transcriptionally repress *E-cadherin* and activate EMT.¹² Upon cessation of migration, when neural crest cells have reached their final destination in the embryo, expression of *Slug* is lost.⁶⁶

Using a xenotransplantation approach, Weinberg and colleagues uncovered a central role for *slug* in the invasive and metastatic nature of human melanoma as well.⁶⁷ In this study, primary human melanocytes were transformed with a defined set of factors (*SV40ER*, *hTERT*, and *RAS*) and gave rise to metastatic melanoma nodules in a mouse xenografts. In contrast, similarly transformed fibroblasts formed primary, but no metastatic tumors, suggesting that programs inherent to the melanocyte lineage mediated the migratory nature of these transformed cells. *Slug* may enable these programs to be used, as knockdown of *SLUG* in melanoma cells led to a small decrease in primary tumor growth in xenotransplants, but a marked reduction of metastatic potential of these cells. These data indicate that lineage-specific genes may play a broader role in the clinical behavior of melanoma, because early metastatic dissemination is one of the hallmarks of this disease. Advances in imaging techniques using transparent zebrafish adults in the setting of transgenic fluorescent lines, along with the possibility of *in vivo* gene targeting using morpholinos,⁶⁸ will allow for a more complete understanding of which step in the invasion cascade (e.g., extravasation, extracellular matrix disruption, and endothelial adhesion) *Slug* transcription is needed.

Melanoma Genetics

Gene dysfunction contributes to the development of many cancers, including melanoma. A number of genes have been implicated in melanoma formation through the identification of somatic genetic defects in benign and malignant melanocytic lesions and germline defects in melanoma-prone families.^{6,69} Some of the common genetic lesions are discussed below.

Receptor tyrosine kinases including c-KIT

Receptor tyrosine kinase (RTK) overactivation has been linked to melanoma pathogenesis. Seminal work on melanomas that form in *Xiphophorus* platyfish interspecific hybrids^{70–72} led to the discovery that increased activity of *Xmrk*, an RTK related to mammalian epidermal growth factor receptors,

promotes tumorigenesis.⁷³ Various RTKs in mammals have been implicated in melanoma, most notably *c-KIT*. Point mutations that are thought to constitutively activate *c-KIT* signaling are found in nearly 30% of acral and mucosal melanomas.⁴⁹ Tumors in these locations are quite rare, but the availability of the *c-KIT* inhibitor imatinib mesylate is likely to provide substantial benefit to this small patient cohort.⁵⁰ There is an unresolved paradox regarding the role of *c-KIT* in melanoma: while the aforementioned rare melanomas are dependent on *c-KIT* overactivation, a majority of advanced melanomas lose *c-KIT* expression.⁴⁴ The conservation of *c-KIT* function and availability of mutants offer an avenue to address this paradox in zebrafish.

Ras family GTPases

Ras family GTPases are among the most commonly mutated genes in cancers.⁷⁴ In general, mutations increase the ratio of active GTP-bound to inactive GDP-bound Ras, causing constitutive downstream signaling. Although mutant *HRAS* is important in the formation of benign Spitz nevi⁷⁵ and can promote melanoma formation in mice,⁷⁶ *NRAS* is most relevant to human melanoma. *NRAS* activating mutations are present in roughly 20–30% of primary and metastatic melanomas.^{77,78} Ras family members signal through multiple downstream effectors, and a determination of the pathways important for *NRAS* oncogenic activity has been an area of considerable interest. Zebrafish and other models of melanoma, in which additional mutations can be overlaid on a defined genetic background, provide an excellent means of pathway analysis.

BRAF

A landmark cancer resequencing effort identified *BRAF* mutations in melanoma.⁷⁹ Although the percentages vary from one study to another, roughly 50–60% of melanomas from intermittently sun-exposed skin, the most common site of disease, have mutations that activate *BRAF*. These *BRAF* mutations are somatically acquired as wild-type *BRAF* is found in normal tissue from melanoma patients. A V600E substitution is by far the most prevalent mutation, and it results in a 700-fold overactivation of intrinsic *BRAF* kinase activity.⁸⁰ *BRAF* is an Ras effector that signals through ERKs to affect cell division, survival, and other processes. *BRAF* mutations are found not only in melanomas but also in nevi,⁸¹ indicating that mutant *BRAF* cooperates with other genetic lesions in tumor formation. Because mutant *BRAF* can induce senescence in cultured melanocytes and nevi,⁸² it is likely that some of these genetic lesions are needed to bypass a senescent arrest. *BRAF* and *NRAS* mutations are mutually exclusive in melanomas,^{79,83} suggesting that mutation in just one of these genes is enough to overactivate downstream ERK signaling. However, *NRAS* and *BRAF* mutations are not equivalent. Whereas *BRAF* signals primarily through ERKs, Ras activity is transduced through PI3 kinase and other pathways. The involvement of multiple Ras effector pathways is suggested by the finding that *PTEN* allelic loss, which is predicted to upregulate PI3 kinase activity, occurs in about 40% of *BRAF* mutant melanomas.⁷⁸

The utility of zebrafish in melanoma research was strikingly demonstrated by studies in which human oncogenic *BRAF*^{V600E} was expressed under the control of the melanocyte

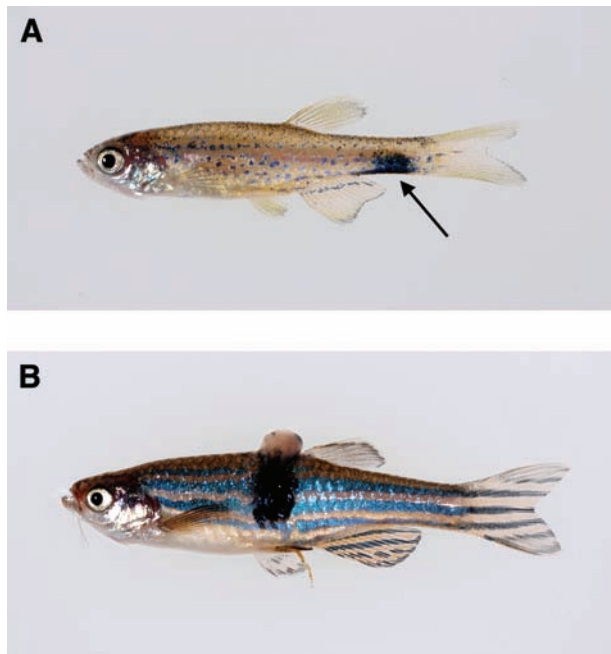


FIG. 2. Melanocytic lesions in zebrafish expressing $BRAF^{V600E}$. (A) Spotted leopard zebrafish expressing human $BRAF^{V600E}$ under the control of the melanocyte $mitfa$ promoter develop clusters of melanocytes akin to human nevi (arrow). (B) $p53$ Mutant zebrafish expressing the same $mitfa:BRAF^{V600E}$ transgene develop melanomas.

$mitfa$ promoter.⁸⁴ Wild-type zebrafish expressing $BRAF^{V600E}$ developed nevi that, like their human counterparts, were highly pigmented melanocyte clusters confined to the epidermis (Fig. 2A). $BRAF^{V600E}$ expression in $p53$ mutant fish led to the formation of melanomas (Fig. 2B) that histologically resembled human melanomas. Melanocytes expressing $BRAF^{V600E}$ and mutant for $p53$ did not always form melanomas, suggesting that $BRAF^{V600E}$ mutations may be necessary but are not sufficient for melanoma formation. This study was instrumental in validating that $BRAF$ mutations play a causative role in melanoma formation and are not simply bystander events in human tumors.

MITF

MITF represents a lineage-specific oncogene in human melanoma. Integrative analysis of copy number and expression arrays demonstrated that *MITF* is amplified in a subset of human melanomas, and cooperates with oncogenic $BRAF^{V600E}$ to transform normal melanocytes.³⁷ The oncogenic potential of *mitf* was strictly dependent upon activated MAP kinase signaling, suggesting that a combination of driver mutations (i.e., $BRAF$) plus lineage specification genes (*MITF*) is needed for the complete cancer phenotype. The mechanisms by which *MITF* acts as an oncogene are not completely resolved, but may involve dysregulation of the cell cycle⁸⁵ and apoptotic response.³⁶

CDKN2A

Studies of genomic alterations in melanoma-prone families led to the identification of mutations that inactivate the *CDKN2A* locus.^{86,87} *CDKN2A* is a complex locus that encodes

the *INK4A* and *ARF* tumor suppressor genes.⁸⁸ Each of these genes begins with its own coding sequence, but downstream exons are shared. Rather uniquely, the shared RNA coding sequence is translated in alternate reading frames (hence *ARF* for alternate reading frame), resulting in proteins with distinct, unrelated amino acid sequences. The *INK4A* protein inhibits cell cycle progression by negatively regulating CyclinD/CDK4,6 activity. Inhibition of CyclinD/CDK4,6 prevents its hyperphosphorylation and inactivation of the Retinoblastoma (RB) tumor suppressor protein. The *ARF* protein inhibits MDM2-mediated ubiquitylation and degradation of p53. Because of its unusual genetic structure, a single lesion that affects the *CDKN2A* locus can extinguish both *RB* and *p53* pathways, impacting melanocyte cell cycle regulation, apoptosis, senescence, and other processes.

p53

p53 Mutations are present in about half of all solid tumors but are relatively rare (10–25%) in melanomas.⁸⁹ However, a variety of data suggest that loss of *p53* pathway activity is a critical contributor to melanoma pathogenesis. As noted above, *ARF* deficiency or silencing is common in melanomas, and dysfunction of *ARF* negatively impacts *p53* activity. Further, general assessments of the *p53* activity, for example by microarray monitoring of the DNA damage response, support a loss of pathway function in many melanomas.⁹⁰ These data suggest that animal models of melanoma utilizing *p53* loss-of-function mutations can accurately reflect human tumors.

Clinical Challenges

Although some progress has been made in understanding the basis of melanoma, significant challenges in the clinical management of this disease remain. These challenges are reflected in the fact that the overall prognosis for patients with advanced melanoma is very poor, largely because there are no effective therapies.⁹¹ Translational research that uses fundamental knowledge of a disease to develop therapeutic strategies holds promise for improving melanoma treatment. We believe that a zebrafish melanoma model has unique attributes that may lead to important insights at the interface of melanocyte biology and clinical medicine. Below we outline some of the areas in which zebrafish melanoma research may be most helpful.

Defining relevant target genes in human melanoma

Identifying the right proteins and pathways to target in melanoma is critical for developing successful therapies. We have seen substantial progress in other malignancies after drugs were developed to target critical genes.⁹² How can a zebrafish model of melanoma identify novel genes that might be good drug targets?

Genetic screens have proven invaluable in linking novel genes to a phenotype of interest.⁹³ When this phenotype involves tissues, for example, melanocytes, and pathologies, for example, cancer, that are vertebrate-specific, the zebrafish can be a powerful platform. Screens in the zebrafish have typically focused on embryonic phenotypes, primarily because of practical advantages. However, it is possible to screen for an adult cancer phenotype, especially one that is externally

visible. Although there are technical considerations that make this approach challenging, there are screen designs that could identify enhancers and suppressors of melanoma.

Validating candidate target genes in zebrafish melanomas

From a therapeutic perspective we are interested in drug targets that cancers rely on for their growth and metastasis.⁹⁴ Human genetics and bioinformatics have created a rich body of data that can be mined for such targets. RNAi screening is a powerful approach for performing loss-of-function genetic analysis in cells and model organisms.⁹⁵ We believe that it will be possible to develop a zebrafish melanoma model system in which RNAi can be used to target potential oncogenes. This approach could be used to determine which genes are necessary for continued tumor growth and do so in a setting, much like that of human tumors, with vasculature and cellular heterogeneity.

Which human precancerous lesions have the highest potential for malignancy?

Some melanomas arise from premalignant nevi, but most nevi do not give rise to melanomas. A similar situation exists in *BRAF*^{V600E} transgenic zebrafish. Nevi in these animals are accessible by biopsy and may be subjected to gene expression and other analyses. By studying the same lesions as it progresses from a nevus to and early melanoma then, later, a more aggressive tumor, it will be possible to characterize the ontogeny of tumor development over time. This analysis could identify markers that are useful in the clinic in predicting whether certain nevi have high or low malignant potential. In addition, identifying markers that refine staging criteria of early melanomas to reflect propensity toward invasion and metastasis could be clinically useful.⁹⁶

Transparent adult fish as tools for understanding metastasis

Recent work from our laboratory has described a relatively transparent zebrafish strain we refer to as *casper*.⁹⁷ This fish, mutant in both the *roy* and *mitfa* genes, is devoid of all embryonic and adult melanocytes and iridophores, allowing for detailed visualization of internal structures. With this platform, migration and invasion of transplanted tumor cells (using either melanin or GFP as a marker) can be easily visualized and quantified at the single-cell level. This system may serve as an attractive tool for chemical or genetic screens to identify novel modifiers of the metastatic melanoma phenotype.

Testing compounds for their ability to prevent melanoma onset

Chemoprevention refers to the treatment of healthy subjects in an effort to prevent disease. For example, in a large human cohort study men who took aspirin regularly had lower rates of colorectal cancer than subjects who did not.⁹⁸ It would be useful to have a model system in which compounds could be screened for their abilities to prevent tumor formation. We believe that it would be possible to use a zebrafish model of melanoma to screen compounds for efficacy in

preventing melanoma. Mechanistically, the compounds might target tumor vasculature, metabolism, pathways that regulate growth and differentiation, or apoptosis.

Identifying new compounds to target melanoma

There is a great need for chemotherapies that are effective against melanoma. Large-scale chemical screening is possible with zebrafish^{99–101} and, when coupled with a melanoma phenotype, may enable the identification of such therapies. Chemical screening with limiting amounts of drug would be best performed on small zebrafish embryos or larvae, and tumor shrinkage and disappearance could serve as a readout. Ideally, an early onset, highly penetrant melanoma model can be developed for screening. Alternatively, it may be possible to screen for inappropriate melanocyte proliferation or survival in a zebrafish embryo. An advantage of performing chemical screens on a cancer phenotype in the zebrafish will be distinguishing between drugs that kill melanoma versus tumor stromal cells and testing how various genetic backgrounds affect drug efficacy. These experimental approaches make the zebrafish an attractive preclinical model for drug discovery and development.

Conclusions

Advanced melanoma is a devastating and lethal cancer. Significant progress in understanding the basis for this disease has been made. Further research, particularly the kind that translates knowledge of the disease into treatment options, will be required to improve the prognosis for melanoma patients. With great ease of studying melanocytes and a proven melanoma model, the zebrafish is poised to make great contributions toward this goal.

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Disclosure Statement

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Address reprint requests to:
Leonard I. Zon, M.D.
Karp Research Laboratories
Children's Hospital Boston
300 Longwood Ave., 7th Floor
Boston, MA 02115

E-mail: zon@enders.tch.harvard.edu

