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REVIEW

Fishing the targets of myeloid malignancies in the era of next generation sequencing

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ABSTRACT

Recent advent in next generation sequencing (NGS) and bioinformatics has generated an unprecedented amount of genetic information in myeloid malignancies. This information may shed lights to the pathogenesis, diagnosis and prognostication of these diseases and provide potential targets for therapeutic intervention. However, the rapid emergence of genetic information will quickly outpace their functional validation by conventional laboratory platforms. Foundational knowledge about zebrafish hematopoiesis accumulated over the past two decades and novel genome editing technologies and research strategies in this model organism have made it a unique and timely research tool for the study of human blood diseases. Recent studies modeling human myeloid malignancies in zebrafish have also highlighted the technical feasibility and clinical relevance of these models. Careful validation of experimental protocols and standardization among laboratories will further enhance the application of zebrafish in the scientific communities and provide important insights to the personalized treatment of myeloid malignancies.

1. Introduction

Human myeloid malignancies encompass a very heterogeneous group of diseases with diverse clinicopathologic characteristics including chronic myelogenous leukemia (CML), acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and myeloproliferative neoplasms (MPN). Characterization of the cytogenetic and genetic aberrations of these diseases has shed important lights to their pathogenesis, classification and prognostication. For instance, identification of a gain-of-function mutation in JH2 domain of Janus Kinase 2 (*JAK2*V617F) has led to novel concepts of MPN pathogenesis and demonstration of such mutation has now become an essential diagnostic criterion for polycythemia vera [1]; mutations of Nucleophosmin1 (*NPM1*) [2] and CCAAT-enhancer binding protein α (*CEBP/α*) [3] have not only revised the classification of AML but led to better risk stratification at first complete remission (CR1) [4,5]. Furthermore, internal tandem duplication (ITD) of Fms-like Tyrosine Kinase 3 (*FLT3*) in AML has provided an important prognostic marker and molecular targets for therapeutic intervention [6].

With the advent of next generation sequencing (NGS), new genetic information is being accrued at an unprecedented rate. Since the first report of AML genome in 2008, novel mutations in myeloid malignancies have been identified rapidly. These gene mutations are diverse in molecular functions, including DNA methylation, chromatin and histone modification, transcription and signal transduction regulation, RNA splicing, cohesin complex and tumor suppression (Table 1). Mutations involving DNA Methyltransferase 3A (*DNMT3A*) [7] and Isocitrate Dehydrogenases 1/2 (*IDH1/2*) in AML [8] as well as Calreticulin (*CALR*) in MPN [9,10] have begun to shed new light on the pathogenesis, classification and prognostication of these heterogeneous diseases. The new genetic information in AML arising from NGS has also provided important leads for clinical trials that evaluated the therapeutic potential of agents targeting these recurrent mutations.

With the increased availability of NGS technology and expected decrease in operation cost, the exponential increase in genetic and transcriptomic data will soon outpace our ability to validate their pathogenetic significance using conventional laboratory techniques and mouse models. Furthermore, gene mutations often exhibit cooperativity [11] and mutual exclusivity [12] in a context dependent fashion and neoplastic clones carrying distinct mutational profiles exhibit dynamic changes in the course of treatment [13,14], adding to the complexity of these diseases. The genetic heterogeneity and intricate interactions between diverse set of gene mutations are clinically relevant as they underscore the heterogeneity of disease mechanisms and the importance of a personalized rather than regimental approach to treatment. The zebrafish model is uniquely suitable to address these hitherto complicated issues.

1.1. Why Zebrafish?

Zebrafish was first identified in the Ganges River in East India and Burma in 1822. They typically live in shallow and slow water stream

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Table 1
Mutations identified by NGS in human myeloid malignancies.

Gene Function	Gene	Recurrent Mutations	Malignancies
DNA Methylation	*DNMT3A* [7]	R882H/C	AML MDS MPN
	IDH1 [111]	R132H/C	AML MDS MPN
	TET2 [112]	Various	AML MDS MPN
Chromatin/Histone Modification	*EZH2* [113]	Various	AML MDS MPN
	NSD1 [114]	Various	AML
Transcription Factor	*BCOR* [115] [116]	Various	AML MDS
	BCORL1 [116]	Various	MDS
	ETV6 [117]	Various	MDS
	SETBP1 [118]	D868N, G870S	MDS
Signal Transduction	*CALR* [9]	Exon 9	MPN
	FLT3 [119]	N676K	AML
	SHKBP1 [120]	Various	AML
RNA Splicing	*PRPF40B* [121]	Various	MDS
	SF1 [121]	Various	MDS
	SF3A1 [121]	Various	MDS
	SF3B1 [121] [122]	K700E	MDS
	SRSF2 [121]	P95H	MDS
	U2AF1 [121] [123]	S34F	MDS
	U2AF65 [121]	Various	MDS
	ZRSR2 [121]	Various	MDS
Cohesin Complex	*RAD21* [124] [125] [126]	Various	AML MDS
	SMC1A [124] [126]	Various	AML
	SMC3 [124] [126]	Various	AML MDS
	STAG1 [124] [126]	Various	AML MDS
	STAG2 [124] [126]	Various	AML MDS
Tumor Suppressor	*TLE4* [120]	Various	AML
Others	*ATP2A2* [114]	Various	AML
	CCND3 [114]	Various	AML
	C10orf2 [114]	Various	AML
	FAM5C [127] [128]	Various	AML
	GNAS [117]	Various	MDS
	HNRNPK [127]	Various	AML

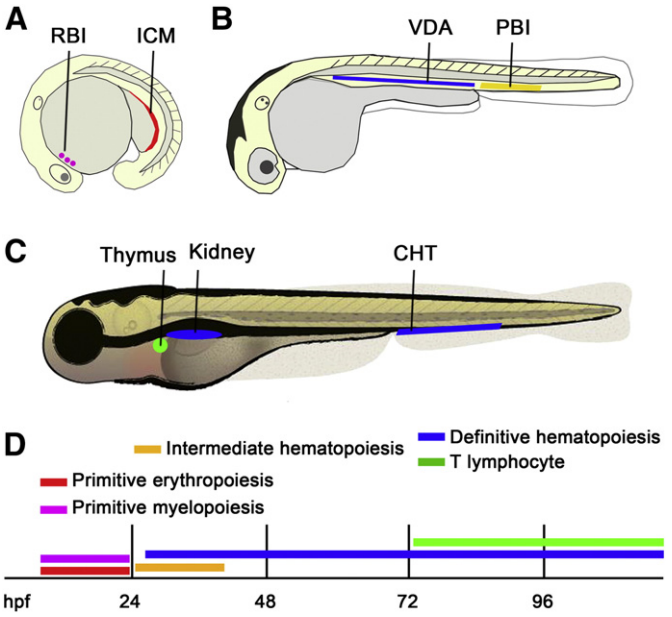

Fig. 1. Primitive and definitive hematopoiesis in zebrafish embryo. (A) The primitive wave comprises primitive myelopoiesis and erythropoiesis that arise in the rostral blood island (RBI) and intermediate cell mass (ICM), respectively. (B) A transient intermediate wave generates the erythromyeloid progenitors (EMPs) in the posterior blood island (PBI). Definitive hematopoietic stem and progenitor cells (HSPCs) arise in the ventral wall of dorsal aorta (VDA) then migrate to the caudal hematopoietic tissue (CHT). (C) HSPCs migrate from CHT to kidney, where life-long hematopoiesis occurs. They also seed the thymus, where they differentiate and mature into T lymphocytes (green). (D) Timeline of zebrafish hematopoiesis.

in India and Southeast Asia. The organism has been studied for developmental biology since 1930. Dr. George Streisinger in Oregon pioneered the study of zebrafish genetics in 1960 and the organism became one of the most popular biomedical models worldwide [15]. As a model for the study of hematopoiesis, the organism is unique in its high fecundity and optical transparency at embryonic stage. The embryos can survive without functional circulation as they obtain oxygen from ambient water by simple diffusion. Genes with hitherto indispensable function in hematopoiesis can be perturbed without causing embryonic lethality. Importantly, the zebrafish reference genome has been published [16], facilitating the design of tools for gene deletion or knock-down. Transgenic zebrafish lines with tissue specific fluorescent reporters are readily available, enabling direct tracking and quantitative evaluation of specific hematopoietic lineages. Zebrafish research in the field of cancer biology has been reviewed recently [17–19]. This article focuses on the technologies and strategies that are currently available to laboratory scientists and practicing hematologists who are interested in harnessing this model for the study of human myeloid malignancies.

1.2. Zebrafish hematopoiesis

Embryonic hematopoiesis in vertebrates occurs in two successive waves known as primitive and definitive hematopoiesis. The migratory pattern of different waves of hematopoiesis is remarkably conserved between mammals and zebrafish [20]. The primitive wave comprises myelopoiesis and erythropoiesis (Fig. 1). In primitive myelopoiesis, *pu.1* is first expressed at 12 hpf (hours-post-fertilization) in the anterior lateral plate mesoderm (ALPM) where it drives differentiation of hematopoietic precursors towards myeloid fate. After formation of the rostral blood island (RBI) at 16 hpf, the *pu.1* + myeloid progenitor cells begin to spread on the yolk sac [21], and subsequently switch on expression of pan-leukocyte gene *l-plastin* [22–24], as well as those genes associated with macrophage (*csf1ra*, *mfap4*, *cxcr3.2*, *mpeg1* and *ptpn6*) [25] and neutrophil lineages (*lyc* and *mpo*) [22]. Primitive erythropoiesis begins in the posterior lateral plate mesoderm (PLPM) at 10 hpf that migrate medially and fuse to become the intermediate cell mass (ICM) at 18 hpf. It is characterized by the expression of *gata1* and embryonic hemoglobin. The erythromyeloid progenitors (EMPs) that arise autonomously in the posterior blood island (PBI) also generate a wave of transient bi-potential hematopoietic cells characterized by *gata1* and *pu.1* expression at 30 hpf [26]. Definitive hematopoiesis arises from the ventral wall of dorsal aorta (VDA, equivalent to aorta-gonad-mesonephros of mammalian embryos) shortly after 24 hpf and endothelial hematopoietic transition occurs between 32 to 60 hpf as evidenced by the expression of *c-myb* and *runx1* [26,27]. These definitive hematopoietic stem and progenitor cells (HSPCs) subsequently migrate to the caudal hematopoietic tissue (CHT), thence the kidney marrow, where life-long hematopoiesis occurs [28]. Zebrafish thrombocytes appear in the VDA at 40 hpf and the circulation at 48 hpf and are characterized by CD41 expression ($CD41^{bright}$ in transgenic CD41:GFP embryos) [29]. Adult zebrafish kidney marrow is equivalent to mammalian bone marrow, where HSPCs give rise to multiple hematopoietic lineages, including erythroid, myeloid and lymphoid cells. Flow cytometric analysis of kidney marrow has resulted in characterization and quantification of these lineages [30]. The remarkable similarities between zebrafish and mammalian blood systems in morphology, molecular signaling and genetics have made zebrafish a unique model for the study of human hematopoiesis.

1.3. Technology development in zebrafish

Advances in transgenesis, genome editing and transplantation methodologies in zebrafish have greatly facilitated its application in biomedical research, particularly in the field of cancer biology and hematology. The rapid development of embryonic hematopoiesis in zebrafish enables high throughput screening of emerging “driver” from “bystander” mutations and identification of potential chemical