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Fish pharming: zebrafish antileukemia screening

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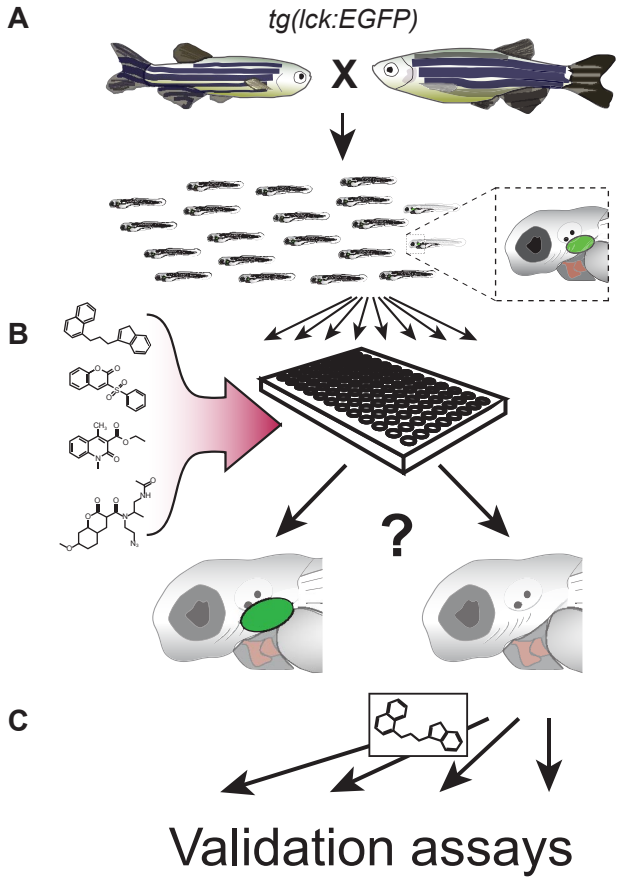
In this issue of *Blood*, Ridges et al report the first successful use of a medium throughput zebrafish screen to identify novel compounds effective against human leukemia.[1]

The zebrafish, historically a robust developmental patterning model, has in recent years begun to show its strength as a useful disease model, particularly for cancer.[2] One of the great promises of this model is its potential for use in drug discovery.[3] For example, whole transgenic embryos carrying oncogenes and fluorescent reporters can be arrayed in 96-well plates and used to screen small molecule libraries for novel compounds that reduce tumor load. Unlike cell-based screens, whole-animal screens can yield valuable additional information such as pharmacokinetic and organ-toxicity data, but up to now no antileukemia lead compound has been successfully identified by medium throughput screening. These new results augur the general use of zebrafish as a platform for cancer drug discovery.

Zebrafish and humans show striking similarity in hematopoietic development, and zebrafish have cognates of all human adult blood lineages, including T and B lymphocytes.[4] For these reasons, it is conceivable that drugs that show hematopoietic effects in zebrafish might have similar effects on human cells. Indeed, a pharmacologic screen for drugs that could cause alterations in hematopoietic stem cell (HSC) numbers during embryonic specification revealed that prostaglandins can expand HSC populations,[5] and protocols based on this observation are now in clinical trials for use in expanding human HSCs for transplantation therapy (http://clinicaltrials.gov/ct2/show/results/NCT00890500).

In their groundbreaking work, Ridges and colleagues reasoned that because T-acute lymphoblastic leukemia (T-ALL) generally involve immature blasts, looking for compounds that prevent T-cell maturation during embryonic development in zebrafish might identify compounds that would have similar effects on the immature blasts found in T-ALL. They took advantage of a transgenic zebrafish line (*lck:EGFP*) carrying an *EGFP* transgene under the control of the T cell–specific *lck* promoter and treated embryos with library compounds to determine whether any had the capacity to block T-cell development, readily visualized by fluorescence in *lck:EGFP* fish (figure panel A). Their survey of 25 000 small molecules (figure panel B) revealed that a compound with previously unappreciated biologic activity, 1H-indole-3-carbaldehyde 8-quinolinylhydrazone, which they termed Lenaldekar (LDK), is able to specifically ablate immature T cells.

In follow-up studies, Ridges et al verified LDK antileukemic activity in a variety of settings (figure panel C). LDK showed activity against a zebrafish T-ALL model carrying a human *cMYC* oncogene under the control of the *rag2* promoter, with 15% of treated fish displaying stable tumor load and 85% showing significant decreases. Remarkably, while all untreated animals had succumbed to their cancer by 40 days, the LDK-treated group remained in long-term remission months after the 14-day treatment course had ended. Likewise, 4-week LDK treatment yielded 4-fold lower tumor loads in mice xenografted with a human T-ALL cell line. Finally, LDK showed activity not only against multiple T-ALL cell lines and primary tumors in vitro, but also against diverse additional leukemias,



Zebrafish screen for potential T-ALL therapeutics. (A) Adults carrying a transgene that fluorescently labels T cells (*lck:EGFP*) are mated. Resulting embryos have fluorescent T cells in the thymus (green oval in magnified embryo schematic). Immature T cells in embryos are posited to be similar to malignant lymphoblasts. (B) Five-day-old embryos are arrayed into 96-well plates and incubated with different compounds from a small molecule library. Forty-eight hours later, embryos are observed to determine general health, and examined for normal (left embryo) or decreased (right embryo) T-cell numbers, visible as reduced fluorescence. (C) Effective compounds are further verified and tested in follow-up assays.

including T315I mutated, BCR-ABL positive, therapy-refractory B-ALL and CML samples. Thus, LDK shows promise for a variety of hematologic malignancies.

Ideally, anticancer therapeutics should specifically target unwanted proliferative cells, with few off-target effects. LDK activity appears to be highly specific. In cell culture, LDK, compared with an AKT inhibitor, showed equivalent toxicity toward malignant lymphoblasts. But unlike the AKT drug, LDK was not nearly as lethal toward mature T cells in peripheral blood at the lymphoblast-lethal dose. LDK appears to work in 2 ways: by inhibition of the PI3K/AKT/mTOR pathway and by cell-cycle delay in G2/M. Interestingly, cell-cycle effects appeared to be relatively specific for lymphoblasts, because cell-cycle defects were not observed in LDK-treated zebrafish embryos during development, before the emergence of lymphoblasts. Moreover, because the original screen was performed in whole animals, LDK was preselected for its lack of general toxicity. This tolerability was confirmed in mammals where injection or oral administration produced reasonably long-lasting serum levels of drug, but had no obvious organ toxicity in a variety of assays. Thus LDK appears to be a highly effective antileukemic agent, with few off-target effects.

The data presented by Ridges and colleagues demonstrate that zebrafish are an exceptional cancer and drug discovery model. Previously it has been shown that zebrafish get cancer,[2] that human oncogenes cause malignancy in zebrafish,[2] that novel oncogenes can be discovered by mutagenesis,[6] and that known human cancer therapeutics can be effective in fish.[7] High throughput means of transplanting, injecting, and screening embryos are rapidly developing.[3,8] Here, Ridges et al show that novel human anticancer therapeutics can be discovered de novo using small molecule screening in zebrafish.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

REFERENCES

1. Ridges S, Heaton WL, Joshi D, et al. Zebrafish screen identifies novel compound with selective toxicity against leukemia. *Blood*. 2012;119(24):5621-5631.

2. Mione MC, Trede NS. The zebrafish as a model for cancer. *Dis Model Mech*. 2010;3(9-10):517-523.

3. Zon LI, Peterson RT. In vivo drug discovery in the zebrafish. *Nat Rev Drug Discov*. 2005;4(1):35-44.

4. Stachura DL, Traver D. Cellular dissection of zebrafish hematopoiesis. *Methods Cell Biol*. 2011;101:75-110.

5. North TE, Goessling W, Walkley CR, et al. Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. *Nature*. 2007;447(7147):1007-1011.

6. Frazer JK, Meeker ND, Rudner L, et al. Heritable T-cell malignancy models established in a zebrafish phenotypic screen. *Leukemia*. 2009;23(10):1825-1835.

7. Mizgirev IV, Revskoy S. A new zebrafish model for experimental leukemia therapy. *Cancer Biol Ther*. 2010;9(11):895-902.

8. Carvalho R, de Sonneville J, Stockhammer OW, et al. A high-throughput screen for tuberculosis progression. *PLoS One*. 2011;6(2):e16779.

Comment on Ribeiro et al, page 5824

Mutant *DNMT3A*: teaming up to transform

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The report by Ribeiro et al in this issue of *Blood* confirms the evolving data that *DNMT3A* mutations represent another common alteration in adult acute myeloid leukemia (AML) and are an important modulator of outcome.[1]

Within the past 2 years, the invention of next-generation sequencing (NGS) has revealed a plethora of previously undescribed genetic abnormalities affecting several different pathways, including mutations in *IDH1* and *IDH2*,[2] and *DNMT3A*[3,4] as well as numerous less common or even patient-specific abnormalities. Clarification of the prevalence and the prognostic impact of these changes has become a critical issue in how to identify the driver lesions and in deciding which factors should be added to the set of molecular abnormalities routinely tested.

In a large and well-characterized population of adult patients with AML, Ribeiro et al investigated the prevalence and prognostic impact of *DNMT3A* mutations. In this series, 96 of 415 patients (23%) carried a mutation of the gene, which ranks it among the most common changes in adult AML. *DNMT3A* mutations were predominantly found in patients with French-American-British (FAB) M4 and M5 morphology and were significantly associated with increased white cell counts at diagnosis. More importantly and in agreement with most studies published so far, patients with *DNMT3A* mutations were a median 10 years older than patients with wt-*DNMT3A*. *DNMT3A* mutations were mostly found in patients with cytogenetically normal (CN)–AML and occurred together with certain abnormalities enriched in this group, most importantly *NPM1* and *FLT3-ITD*. Although *DNMT3A* mutations were not associated with a specific mRNA gene expression profile (GEP), the authors could show an enrichment of *DNMT3A* mutant samples in a cluster associated with a specific methylation pattern; however, this cluster was mostly characterized by *NPM1* mutations.

When analyzing the reported data for *DNMT3A* in adult AML (see table),[1,3,5-10] several interesting aspects become evident. The mutation is found in approximately 15% to 25% of series reported from Europe and the United States[1,3,5,9]; however, the prevalence is somewhat lower in the 2 large unselected studies from Asia (7% and 14%),[8,10] potentially indicating an effect of ethnic background. Given the association with CN-AML observed in all studies, it is not astonishing that the highest prevalence was reported in the 2 series focusing on CN-AML (29%–36%).[6,7] Most reports agree in certain clinical aspects (increased patient age, high WBC) and all confirmed the association with FAB M4/M5-morphology. The important association with increased patient age is also evident in most series, which is in line with the very low prevalence in pediatric AML.[11] In addition, the majority of studies could confirm the association with outcome, indicating that patients with *DNMT3A* mutations have a significantly shorter overall and disease-free survival. This appears not to be an effect of a decreased rate of complete remission, but reflects an increase in disease recurrence. On the biologic side, the correlation with other molecular changes (*NPM1*, *FLT3-ITD*) and the mutual exclusive mutational spectrum with genes directly involved in the regulation of DNA methylation (ie, *TET2* and *ASXL1*) further strengthens the importance of epigenetics as a key molecular pathway for leukemic transformation (see figure). Because the majority of these mutations are associated with inferior outcome,

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