Molecular Pathways: Induction of Polyploidy as a Novel Differentiation Therapy for Leukemia

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Abstract

Differentiation therapy has emerged as a powerful way to target specific hematologic malignancies. One of the best examples is the use of all-trans retinoic acid (ATRA) in acute promyelocytic leukemia (APL), which has significantly improved the outcome for patients with this specific form of acute myeloid leukemia (AML). In considering how differentiation therapy could be used in other forms of AML, we predicted that compounds that induce terminal differentiation of megakaryocytes would be effective therapies for the megakaryocytic form of AML, named acute megakaryocytic leukemia (AMKL). We also speculated that such agents would reduce the burden of abnormal hematopoietic cells in primary myelofibrosis and alter the differentiation of megakaryocytes in myelodysplastic syndromes. Using a high-throughput chemical screening approach, we identified small molecules that promoted many features of terminal megakaryocyte differentiation, including the induction of polyploidization, the process by which cells accumulate DNA to 32N or greater. As the induction of polyploidization is an irreversible process, cells that enter this form of the cell cycle do not divide again. Thus, this would be an effective way to reduce the tumor burden. Clinical studies with polyploidy inducers, such as aurora kinase A inhibitors, are under way for a wide variety of malignancies, whereas trials specifically for AMKL and PMF are in development. This novel form of differentiation therapy may be clinically available in the not-too-distant future. Clin Cancer Res; 19(22); 1–5. ©2013 AACR.

Background

Polyploidization of megakaryocytes

A small number of cell types in humans undergo accumulation of multiple copies of their DNA (polyploidization) as they differentiate (Fig. 1). There are two different mechanisms by which polyploidization can occur, cell fusion and DNA division without cytokinesis, which is called endomitosis. Osteoclasts, for example, become polyploid by fusion of 2N cells to form large phagocytic cells with multiple separate nuclei. In contrast, megakaryocytes become polyploid by undergoing repeated rounds of DNA replication without completing cell division, resulting in very large mature cells that usually contain a single multilobed nucleus with DNA contents up to 128N (1). Polyploidization of megakaryocytes is essential for efficient platelet production, in part due to increased cytoplasmic volume and also due to upregulation of differentiation genes (2, 3). In acute megakaryocytic leukemia (AMKL), low ploidy megakaryoblasts predominate. This lack of polyploid megakaryocytes is a consequence of a block in polyploidization and differentiation of the rapidly proliferating leukemic blasts.

The mechanisms that control endomitosis and the ways that it differs from the normal proliferative cell cycle have been investigated at the cellular and molecular levels. During a proliferative cell division, chromosomes are bound by the chromosome passenger complex, which is composed of the proteins survivin, INCENP, aurora kinase B (AURKB), and borealin. Chromosomes are tethered to bipolar spindles, sites that accumulate aurora kinase A (AURKA), by microtubules and line up at a central region termed the midzone. As mitosis progresses, pairs of chromosomes are separated to opposite poles and a cleavage furrow, the region in which cells are separated that gradually closes like a purse string during cytokinesis, forms. During normal cytokinesis, RhoA activation at the site of initiation of cleavage furrow formation is orchestrated by the guanine exchange factors (GEF) ECT2 and GEF-H1 in coordination with proteins at the midzone to establish the actomyosin ring at the cleavage furrow. This ring generates the contraction required for ultimate cell separation (abscission; refs. 4, 5). Activated RhoA and its functional effectors (e.g., ROCK, mDia) need to be localized to the cleavage furrow for cytokinesis to occur (6–10).

Studies using time-lapse microscopy to observe megakaryocytes undergoing endomitosis suggest that the initial endomitotic cleavage event in which cells progress from 2N to 4N occurs due to failure very late in cytokinesis with normal cleavage furrow formation followed by, instead of
processes, including actin cytoskeleton reorganization, Cdc42) are molecular switches that regulate many cellular
ular to the midzone; refs. 12, 15). Figure 1. Megakaryocyte progenitors must decide whether to divide to give rise to two daughter cells (top) or instead to commit to terminal differentiation and polyploidization (bottom). The switch from the proliferative cell cycle to the endomitotic one involves changes in activity and expression of several genes, including RhoA, ECT2, GEF-H1, and MYH10. Most notably, the reduction in RhoA activity and MYH10 prevents the formation and action of the contractile ring and the completion of cytokinesis. Inhibition of AURKA has been found to promote the endomitotic process over proliferation. Note that, at late stages of anaphase, AURKA localizes to the central spindle, whereas active RhoA is associated with the cleavage furrow.

abscession, furrow regression (11–14). These endomitotic megakaryocytes form an apparently intact midzone with normal localization of essential components, including survivin, AURKB, INCENP, PRC1 (protein-regulating cytokinesis 1), MKLP1 and MKLP2 (mitotic kinesin-like protein), MgcRacGAP, and microtubules (12, 15). However, RhoA localization to the midzone and/or RhoA activation may be inhibited in endomitotic megakaryocytes. In contrast with normal cytokinesis, the contractile ring of megakaryocytes undergoing endomitosis contains decreased levels of RhoA at the 2N-to-4N transition. In higher ploidy cells (greater than 4N), little to no cleavage furrow formation occurs during anaphase, and RhoA is not detectable at sites where cleavage furrow would be expected (perpendicular to the midzone; refs. 12, 15).

Rho family small GTPases (e.g., RhoA, Rac1, and Cdc42) are molecular switches that regulate many cellular processes, including actin cytoskeleton reorganization, microtubule dynamics, cell-cycle progression, and cytokinesis (16). Activated RhoA facilitates actin polymerization via mDia and promotes contraction of the actin myosin ring by activating ROCK to phosphorylate and thus activate myosin. The myosins involved in cytokinesis are the nonmuscle myosins MYH9 and MYH10 (non-muscle myosins IIA and IIb, respectively). Myosins generate the force needed to close the contractile ring, which leads to the final separation of the cells. In dividing 2N megakaryoblasts, MYH10 predominates in the cleavage furrow. However, in megakaryocytes undergoing endomitosis, neither MYH10 nor MYH9 is present. The lack of MYH10 at the 2N-to-4N endomitotic stage may be due to repression of MYH10 mRNA transcription by Runx1, a transcription factor that is known to be required for both polypliodization and subsequent cytoplasmic maturation. However, lack of MYH10 may not be the only reason for failure of complete closure of the cleavage furrow in endomitotic megakaryocytes.

Mitosis and endomitosis are also controlled by different guanine exchange factors, which facilitate Rho GTPase switching from the inactive GDP-bound state to the active GTP-bound state. The Dbl family guanine nucleotide-exchange factors (GEF) have a tandem Dbl homology (DH)-Pleckstrin homology (PH) domain, in which the DH domain contains GDP/GTP exchange activity (17). GEFs are involved in RhoA localization and activation during different stages of cytokinesis. Two such GEFs, ECT2 and GEFH1, are involved in different stages of cytokinesis. The GEFECT2 (epithelial cell transforming sequence 2) is required for initiation of cleavage furrow formation and GEFH1 is required for the final stages of cleavage furrow closure and abscission. To initiate cleavage furrow formation perpendicular to the midzone after breakdown of the nuclear envelope during mitosis, ECT2 is recruited to the central spindle by the central spindlin complex during late anaphase, where it then promotes cleavage furrow formation (18, 19). ECT2 is an oncogene that resides on chromosome 3q26, a region frequently targeted for chromosomal alterations in human tumors and overexpressed in many primary human tumors (20, 21). RNA interference knockdown of ECT2 results in mitotic failure and binucleate cells due to the lack of cleavage furrow ingression (22). We have found that downregulation of ECT2 expression occurs during megakaryocyte maturation and is required for later stages of endomitosis (starting when 4N cells become 8N) in which little to no cleavage furrow is apparent. In contrast, at the 2N-to-4N stage of endomitosis, the cleavage furrow forms and anaphase is almost complete before the furrow regresses and the two separated nuclei rejoin. For abortion of cytokinesis at this late stage of anaphase, in addition to a lack of MYH10, we have found that GEFH1 is entirely absent.
from the central spindle and the cleavage furrow. The molecular regulation of GEFH1 requires expression of MKL1, a transcriptional cofactor that is mutated as part of the t(1;22) translocation that occurs in non–Down syndrome–associated AMKL (DS-AMKL). In AMKL cells with the t(1;22), GEFH1 levels are extremely high and the cells do not undergo polyploidization most likely due to the presence of GEFH1, which localizes and activates RhoA to promote closure of the cleavage furrow and complete abscission.

Malignant megakaryocytes are defective in polyploidization

In the neoplastic process, tumor cells acquire alterations that result in excessive proliferation and impaired differentiation. Similar to APL, in which leukemic blasts continue to divide instead of differentiate (23), immature megakaryocytes in AMKL continue to progress through mitosis and fail to enter the endomitotic cell cycle. In myelodysplastic syndrome (MDS), a disease that involves both abnormal hematopoietic stem cell self-renewal and a deficiency in terminal differentiation of mature myeloid cells, and in primary myelofibrosis (PMF), a clonal disorder characterized by abnormal hematopoiesis and myelofibrosis, megakaryocytes also show impaired polyploidization. On the basis of this observed block in polyploidization, two laboratories envisioned the development of “polyploidy inducing agents” as potential therapies for MDS, PMF, or AMKL. In the first instance, Lannutti and colleagues discovered that the small-molecule SU6656 could potently induce polyploidization of a variety of cell lines and megakaryocytes collected from the bone marrows of individuals with MDS (24). SU6656 was found to inhibit multiple kinases, including members of the Src as well as the Aurora kinase families. Such a compound was proposed as a new therapy for MDS.

In the second study, we screened for small molecules that could drive polyploidization and induce features of terminal differentiation of malignant megakaryocytes (25). Using the CMK cell line, which was derived from an individual with DS-AMKL, we identified 205 small molecules that induced statistically significant polyploidization of the cells. Of these, the compound with the strongest polyploidy and differentiation activity was the kinase inhibitor dimethylfasudil (diMF).

AURKA was discovered as a major target of diMF in megakaryocytes. We showed that diMF and the highly selective AURKA inhibitor, MLN8237 (alisertib; ref. 26), showed potent anti-AMKL activity both in vitro and in vivo. For example, diMF significantly increased survival of mice engrafted with an AMKL cell line, with a 40% long-term survival rate in the group treated with 66 mg/kg (P = 0.003) and 30% with 33 mg/kg (P = 0.004; ref. 25). Furthermore, MLN8237 led to significant reductions in tumor burden of NSG immunocompromised mice engrafted with primary human non-DS blasts (27). On the basis of these results, we propose that polyploidy inducers are a novel therapeutic strategy for AMKL. Furthermore, induction of polyploidization may also be effective in the treatment of other diseases of megakaryocytes. For example, it is believed that immature megakaryocytes directly contribute to fibrosis in PMF, induction of differentiation and polyploidization is likely to benefit these patients as well.

Given that megakaryocytes are poised to become polyploid, we predicted that polyploidy inducers would not affect other cell types, at least at the doses that promote megakaryocyte polyploidization. Indeed, treatment of both human and mouse primary cell cultures with diMF led to increased polyploidy of the CD41-positive megakaryocytes, but no change in the CD41-negative fraction that includes all other bone marrow cells (25). Furthermore, we did not observe any defects in other hematopoietic cells in healthy mice treated with either diMF or MLN8237 (25). However, in many cases, once a cell becomes 4N, it cannot undergo DNA synthesis due to cell-cycle checkpoints. Thus, if highly proliferative leukemia cells of other lineages could be induced to undergo polyploidization with these inhibitors, they might also be susceptible to such treatments.

Clinical–Translational Advances

AMKL is a rare form of acute myeloid leukemia (AML) that affects both children and adults. In pediatric cases, the leukemia is most often seen in children with Down syndrome (DS), who are at several hundred-fold increased risk for AMKL compared with children without Down syndrome (28, 29). DS-AMKL is characterized by trisomy 21 and mutations in the essential transcription factor gene GATA1 (30). AMKL is also observed in young children without Down syndrome, but in these cases, there are no mutations in GATA1. Instead, the tumors tend to acquire chromosomal translocations, such as t(1;22) (31), which results in expression of the RBM15-MKL1 fusion protein (32, 33) or Inv(16) (p13.3q24.3), which leads to expression of the CBFA2T3-GLIS2 fusion (27, 34). Recurrent translocations involving Jarid1A (KDM5A) on chromosome 12 have also been reported for non-DS-AMKL (27, 35). Finally, AMKL is also seen in elderly patients. The genetic basis for this leukemia is largely undefined, although mutations in JAK2, JAK3, and FLT3 have been detected in rare cases (36–38).

Among these subtypes, children with Down syndrome have the best prognosis. Studies by the Children’s Oncology Group found a 5-year event-free survival (EFS) rate of 79% and an overall survival rate of 84% (39). Similar results were seen in the Pediatric Oncology Group (POG) 9241 trial (40). Despite the excellent outcome, there is room for improvement in this subtype. Pediatric non-DS-AMKL is an aggressive malignancy with inferior outcome relative to DS-AMKL. The POG9241 study found that non-DS-AMKL patients had a 36.3% 5-year EFS rate despite intensive chemotherapy (41). Although this POG study indicated that children with t(1;22) had an excellent outcome, a different study reported a median survival duration of only 8 months (42). In contrast, the Inv(16)(p13.3q24.3) cases have an inferior outcome compared with other non-DS-AMKL cases (34). The COG 2891 study found that children with AMKL had significantly
inferior overall survival compared with children with M0–M5 subtypes of AML (43). Similarly poor outcomes for non-DS-AMKL were seen in a St. Jude study (44). Finally, AMKL in adults has a poor prognosis. The vast majority of patients undergo remission but relapse. Two separate studies reported an extremely poor outcome, with a median survival of 40 weeks (45, 46). Clearly, new therapeutic options are needed for patients with AMKL.

On the basis of work with polyploidy inducers, we propose that these agents, alone or more likely in combination with conventional chemotherapy, would provide sustained remission. MLN8237 is under investigation for a wide variety of tumors, including multiple hematologic malignancies such as myeloma, diffuse large B-cell lymphoma, non–Hodgkin lymphoma, and other advanced hematologic malignancies and solid tumors. In a phase II study of MLN8237 in advanced AML or intermediate-2/high-risk MDS, use of MLN9237 as a single agent showed a 13% overall response rate (47). Due to the rarity of AMKL, however, it is likely that few if any patients with this subtype were included in this or other clinical studies of MLN8237.

In addition to single-agent studies, MLN8237 has been tested in combination with a variety of conventional chemotherapies. For example, in preclinical studies of multiple myeloma, combining MLN8237 with dexamethasone, doxorubicin, or bortezomib induced a synergistic/additive response (48). In preclinical studies of MLN8237 in esophageal adenocarcinoma, the addition of cisplatin enhanced the antitumor effect (49). With respect to AMKL, the addition of conventional chemotherapies, such as cytarabine and anthracyclines, to polyploidy agents may not be required, as the MLN8237 and dimF efficiently target leukemia blasts. In contrast, combining polyploidy inducers with new therapies that eradicate leukemia stem cells may improve efficacy.

Conclusions

Acute megakaryoblastic leukemia remains an intractable disease with poor outcome. Similarly, MDS and PMF are disorders that require new therapeutic strategies. Novel therapies that specifically target the immature megakaryocytes and override the blocks to polyploidization provide a new strategy to induce sustained remission and increased overall survival. Moreover, continued advances in our understanding of the process of polyploidization and how it differs from the proliferative cell cycle will provide additional targets for therapeutic intervention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Writing, review, and/or revision of the manuscript: D.S. Krause, J. Crispino

Grant Support

This review was supported in part by grants from the Samuel Waxman Cancer Research Foundation, the NHLBI (HL112792) and the NCI (CA101774) to J.D. Crispino and from the NIH (DK086267) and state of CT (10SC803) to D.S. Krause.

Received May 10, 2013; revised July 2, 2013; accepted July 10, 2013; published OnlineFirst August 19, 2013.

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doi:10.1158/1078-0432.CCR-12-2604

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