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REVIEW

Targeting leukemia stem cells: The new goal of therapy in adult acute myeloid leukemia

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Abstract

The most popular view of hematopoietic cell lineage organization is that of complex reactive or adaptative systems. Leukemia contains a subpopulation of cells that display characteristics of stem cells. These cells maintain tumor growth. The properties of leukemia stem cells indicate that current conventional chemotherapy, directed against the bulk of the tumor, will not be effective. Leukemia stem cells are quiescent and do not respond to cell cycle-specific cytotoxic agents used to treat leukemia and thus contribute to treatment failure. New strategies are required that specifically target this malignant stem cell population.

Key words: Acute myeloid leukemia; Leukemia stem cells; Targeted therapy; Prognosis

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INTRODUCTION

Acute myeloid leukemia (AML) is a paradigm of cancer stem cells with a hierarchy analogous to that seen in normal hematopoiesis. Leukemia stem cells have long-term repopulating potential and the ability to propagate and maintain the AML phenotype. By their ability to hijack homeostatic mechanisms and take refuge within the sanctuary of the bone marrow microenvironment, they consequently contribute to disease resistance. Thus, targeting this stem cell population and its microenvironment is a new goal for therapy of adult AML.

THE LEUKEMIA STEM CELL HYPOTHESIS

Leukemia, as with all malignant diseases, undergoes a series of genetic events that result in the activation or overexpression of genes promoting proliferation, the silencing of genes involved in the inhibition of proliferation, and the development of the ability to elude apoptosis. However, this does not explain self-renewal, clonal expansion, and additional mutations. Over the past few years, it has been recognized that malignant diseases contain a particular subpopulation of cells with biological features that are reminiscent of stem cells[1]. The modern concept of "cancer stem cell" was promoted by John Dick and colleagues, who showed that cells with the ability to transfer human AML to NOD/SCID mice are frequently found exclusively in the $CD34^+$ $CD38^-$ compartment[2,3]. Stem cells modulate tissue formation, and maintenance and repair, based on a complex interaction of cell-autonomous and cell-non autonomous regulatory mechanisms[4]. In the hematopoietic system, there are three different populations of multipotent progenitors: (1) stem cells with a capacity for long-term renewal; (2) stem cells with a capacity for short-term renewal; and (3) and multipotent progenitors that cannot renew but differentiate into the varied lineage and undergo rapid division, allowing them to populate the bone marrow[5]. Classically, a slow cycling fraction of cells is generating a fast cycling fraction. However, an

alternative hypothesis predicts that all tumor cells have the potential to self-renew and recapitulate the tumor, but with a low probability that any tumor cell enters the cell cycle and finds a permissive environment[5].

THE LEUKEMIA THERAPEUTIC CHALLENGE

AML is one of the most common leukemias in adults. The outcome of therapy for AML has improved over recent years, mainly in younger patients. However, the challenges in this area remain considerable. The incidence of AML increases with age. AML is therefore primarily a disease of the elderly. This patient population has a very poor prognosis, which is attributed to having a disease that is inherently more resistant to current standard cytotoxic agents in relationship with acquired genetic characteristics of the leukemia, and/or relatively poor tolerance of these agents because of comorbidity and reduced tolerance of adverse effects. In contrast to the molecular mechanisms of leukemogenesis in children and younger adults, recent studies indicate that the majority of cases of AML in the elderly have quite distinct biological and molecular genetic features[6]. These features include trilineage dysplasia, complex unfavorable cytogenetic abnormalities (involving chromosomes 5 and/or 7, del(5q), del(7q), abnormalities of 11q, inv(3), and complex or multiple abnormalities), the potential for clonal remissions and reversion to an MDS marrow picture at remission. There is also a high incidence of drug-resistant phenotypes (mediated by MDR-1/P-glycoprotein or other members of the ABC transporter family). Treatment options for the older AML patient population (≥ 65 years) are limited. The traditional chemotherapeutic approach to a patient with AML has been based on treatment with a combination of an anthracycline (or anthracenedione) with cytarabine. The unmet therapeutic need is therefore greatest among older patients with AML, in whom response rates are comparatively low (50% for those over 60 years old), relapse rates are exceedingly high (more than 85%), and long-term survival rates are less than 10%[7,8]. Current chemotherapeutic options provide essentially no chance for durable remission, and toxicity of the treatment is significant. Nearly all patients relapse, and the median survival is approximately nine months. Consequently, many older patients with AML are not offered, or choose to decline, traditional intensive chemotherapy and receive supportive care only. More effective therapies to provide durable remissions in a significant proportion of patients and less toxic therapies, which could be offered to more patients, are desperately needed for the treatment of AML in the elderly. Biological insights into the mechanisms of defective molecular pathways in malignant cells have recently resulted in the identification of novel targets for drug development. New drugs are currently in early clinical development with the aim of circumventing chemotherapy resistance.

The possible existence of a rare stem cell-like population of cells within a much larger pool of malignant cells has presented new questions as to the biology of leukemia relapse and resistance. Most stem cells are assumed to be quiescent at steady state, and to express a number of membrane transporters with broad specificity linked to drug resistance. Assuming that leukemia stem cells recapitulate these two aspects of stem cells, quiescence and inherent drug resistance are likely to make the leukemia stem cell population the most difficult to eradicate fraction.

MODELS OF LEUKEMOGENESIS

Two models of leukemogenesis have long been proposed. The "stem cell model" or the "hierarchy model", suggesting that leukemias originate from stem or progenitor cells through deregulation of self-renewal pathways. Theoretically, the leukemia stem cell model is based on the idea that pluripotency and maturation are mutually exclusive. Leukemia stem cells maintain themselves and the clone by self-renewal, and they mature to generate progeny that lack stem cell properties. In contrast, in the "stochastic model", any cell could be the target of leukemogenesis. This model predicts that a tumor is biologically homogeneous and the behavior of malignant cells is influenced by intrinsic or extrinsic factors. Transformation results from random mutation and subsequent clonal selection[9,10]. All leukemia cells are equally sensitive to such stochastic influences and can revert from one state to another. Recently, a third model was proposed. In this model, leukemia cells can dedifferentiate and regain leukemia stem cell capacity, thereby sustaining the disease[11]. In all cases, both bone marrow and stromal cells may have abilities to differentiate into different cell types, suggesting that pluripotency and maturation might be influenced by the micro environmental stimuli[12]. This is encapsulated in the concept of the "niche" in the bone marrow that is required to maintain the status of the bone marrow stem cells[13].

Although hematopoiesis has been considered hierarchical in nature, recent data suggest that the system is functionally quite plastic[14]. Rather than a hierarchical transition from stem to progenitor cell, it appears that a fluctuating continuum exists, in which the phenotype of primitive marrow cells shifts from one state to another and back again. A primitive progenitor cell can actually make very different lineage choices during one cell cycle transit[15]. It has also been suggested that hematopoietic stem cells are functioning concurrently, continuously cycling and contributing to blood cell production. This suggests that hematopoietic stem cells are not completely dormant, cell quiescence being relative[16]. Cell cycle passage could determine the fate of cells derived from stem cell division and renew stem cell multipotency[17]. Cell cycle transit is associated with a continually changing stem cell phenotype[14]. The identity of the stem cell could be masked at certain points in the cycle. In these models, stem cells would show asymmetric division. The other alternatives would lead to either hyperproliferation or stem cell exhaustion. In some models, less primitive cells

could give rise to more primitive cells[18]. Some daughter cells could have greater pluripotency than the parent cells. Here the stem cell population is viewed as a continuum, rather than being composed of discrete states.

These last models are more compatible with the view of the cell lineages representing complex reactive or adaptive systems[19], in which self-organization arises on the macro-scale from micro-scale interactions of the individuals that constitute the system. Complex adaptive systems often have multiple ground states or points of equilibrium, and transitions between these states may lead to small or large instabilities, including system collapse. Mathematical modeling of stem cell lineage systems, taking into account a limited number of parameters, such as affinity for a growth environment (the stem cell niche) and cycling status of the cell, produces clonal fluctuation patterns that are a precise match for those seen experimentally in human leukemias[20]. This model includes stochastic elements. In the setting of cell lineages as complex reactive or adaptive systems, fluctuations are necessary for self-organizing systems to explore new states.

IMPLICATIONS FOR THERAPY

The concept of leukemia stem cells has implications in leukemia therapy, most particularly for the development of targeted therapies. Based on the understanding of the molecular basis of cancer, current therapeutic strategies focus on inhibiting the molecular drivers of cancer. Research should therefore focus more on leukemia stem cells than on the bulk cells that makes up the majority of the tumor. Characterization of the biological features that initiate and maintain leukemia is an essential step in the development of novel effective agents. The challenge is to identify proapoptotic stimuli that spare the normal hematopoietic stem cells while exerting the desired effect on leukemia stem cells[21]. Malignant stem cells have a number of biological features that are different from their normal-tissue counterparts and these might be exploited for therapeutic benefits. The identification of genes that regulate self-renewal might provide rational targets for therapeutic intervention, particularly if their requirement is more critical for self-renewal in leukemia stem cells than hematopoietic stem cells. New agents, such as kinase inhibitors, histone deacetylase inhibitors, cyclin D kinase inhibitors, nuclear factor κB (NF-κB) inhibitors, methylation inhibitors, heat shock protein inhibitors, farnesyltransferase inhibitors, and proteasome inhibitors, showing specific mechanisms that target leukemia cells, are now available. However, there is little or no evidence that inhibiting these different pathways is relevant to inhibiting proliferating leukemia stem cells.

Targeting drug efflux pumps

A therapeutic approach could be to target drug efflux pumps. ATP-dependent drug efflux has been linked to the increased expression of ABC transporter proteins[22]. At least 22 ABC transporters have been identified in leukemia stem cells, and all were expressed at lower levels in $CD34^+$ $CD38^+$ cells in comparison with the $CD34^+$ $CD38^-$ cells[23]. Several agents effective in overcoming the inherent drug efflux pumps have been studied, but found to have limited efficacy because of the high expression of the targeted receptors in normal hematopoietic stem cells, making them equally susceptible to the inhibitors[24,25]. Third-generation multidrug resistance modulators that are more powerful are currently under clinical investigations[26].

Targeting cell cycle

Another approach could be to target the self-renewal machinery of leukemia stem cells, by inducing the quiescent leukemia stem cells into the cell cycle. Leukemia stem cells respond to depletion of the leukemia cell mass that occurs when antiproliferative drugs are administered to leukemia patients. Thus, one way to eliminate dormant leukemia stem cells would be to find the window when they cycle and kill them at that point. Unfortunately, little is known of the biology of these cells. It has been proposed that recruitment of leukemia stem cells from G0 to the S phase of the cell cycle might contribute to their eradication by cell cycle-specific agents.

A priming strategy might improve the efficacy of cell cycle-dependent cytotoxic agents. Recent clinical trials have shown that sensitization of leukemia cells and their progenitors by granulocytic growth factors can improve the outcome of patients with newly diagnosed AML[27,28]. Inhibition of CXCR4 (the receptor for bone marrow stroma derived SDF-1) has been shown to overcome resistance to numerous drugs in leukemia/stromal co-cultures *in vitro*[29]. CXCR4 inhibition also affects CXCR4-mediated signaling events that are induced by leukemia/stroma co-culture conditions. In a recent clinical trial using an anti-CXCR4 in patients with AML in complete remission, massive mobilization (up to 80%) of leukemic cells was observed when hematopoietic growth factor application was followed by anti-CXCR4[30]. It is expected that mobilization of leukemic stem cells with CSF and anti-CXCR4, accompanied by chemotherapy, will result in increased anti-leukemic effects. Thus the mobilization of leukemic stem cells is a concept that is presently being revisited.

Targeting molecular pathways including PTEN, p21, and PML might also be an attractive proposition[31]. The *BMI1* oncogene-driven pathway is one of the key regulatory mechanisms of pluripotency. The polycomb group gene *BMI1* influences the proliferative and self-renewal potential of normal and leukemia stem cells[32].

Conversely, prolonging the quiescent phase could also be beneficial. The existence of patients with indolent forms of AML and the wide variation in the duration of relapse-free interval among patients can sustain this hypothesis.

Targeting cell surface antigens

Although both leukemia stem cells and normal stem cells express CD34 but not CD38, there are differences

between their surface phenotype that could be useful for targeting leukemia stem cells. Recent data suggest that the majority of leukemia stem cells express CD33, the target of gemtuzumab ozogamicin[33]. However, the expression is not specific to leukemia stem cells. Conversely, CD123 (IL3α receptor) is expressed on most leukemia stem cells, but not on normal stem cells[33,34]. A specific fusion of IL3 and a diphtheria toxin has therefore been generated showing interesting results in NOD-SCID mice[35] and encouraging data in a phase I / II clinical trial in patients with relapsed or refractory AML[36]. An anti-IL3 receptor alpha chain (CD123)-neutralizing antibody (7G3) has been shown to target AML leukemia stem cells, impairing homing to bone marrow and activating innate immunity in NOD/SCID mice[37]. Studies also reported that leukemia stem cells could be targeted with monoclonal antibodies to CD44, CLL-1, or CXCR4[38-40].

Targeting NF-κB activity

Recent studies have described means of differential activation of apoptosis mechanisms in leukemia stem cells[41-43]. The transcription NF-κB has been found to be constitutively activated in leukemia stem cells but not in normal hematopoietic stem cells. Molecules able to inhibit NF-κB activity might selectively target the leukemia stem cell. The combination of idarubicin with proteasome inhibitors has been shown to mediate selective apoptosis in leukemia stem cells while sparing normal cells[43]. Recently, parthenolide, a sesquiterpene lactone with potent NF-κB inhibitory activity, was found to kill AML progenitors selectively while sparing normal progenitors[42]. Unfortunately, it is not water-soluble and is not, therefore, a candidate for pharmacologic development. However, the development of soluble analogs is ongoing[44]. Another molecule, TDZD-8 (4-benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione),which also has NF-κB inhibitory activity, showed promising activity on AML progenitor cells expressing $CD34^+$ $CD38^-$[44].

Targeting other pathways involved in self-renewal

Other pathways are involved in self-renewal. Pathways such as the HOX gene, WNT/β-catenin, PTEN, Hedgehog, and BMI-1 are frequently mutated and could be selectively targeted in leukemia stem cells. Another transcriptional pathway that appears to alter self-renewal is that associated with the AP-1 transcriptional factor, JunB[45]. The Notch pathway might also be deregulated in leukemia stem cells. Inhibition of γ-secretase (which is necessary for Jagged and Notch signaling) by N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT) has been shown to inhibit leukemia stem cell growth[46]. The phosphatidylinositide-3 kinase (PI3K) pathway is the major signaling pathway downstream of oncogenic tyrosine kinases, and is activated in AML[47]. Inhibition by LY294002 of the activation of the PI3K also leads to a dose-dependent decrease in survival of leukemia stem cells[48]. The use of mTOR inhibitors such as rapamycin and its derivative has demonstrated a loss of clonogenic potential of AML blasts, while sparing normal progenitors[49]. Phases I and II studies of mTOR inhibitors, in combination with standard chemotherapy, are ongoing[50,51].

Targeting cell differentiation

A differentiation block is a main feature of AML. Malignancy can be suppressed in certain types of leukemia stem cells by inducing differentiation with cytokines that regulate normal hematopoiesis, or with other compounds that use alternative differentiation pathways. The suppression of malignancy by inducing differentiation can bypass genetic abnormalities that give rise to malignancy and shows that leukemia stem cells can be genetically reprogrammed. CD44, a mediator of the stem cell/niche interaction, also represents a potential target for differentiation of leukemia stem cells. Targeting CD44 with an activating monoclonal antibody has led to eradication of human leukemia stem cells in NOD/SCID mice[38]. This could lead to a new therapeutic approach targeting the leukemia stem cell/niche interaction.

Targeting leukemia stem cells *via* active specific immunization

Targeting leukemia stem cells *via* active specific immunization has also been proposed, based on the development of immunoconjugates with toxic moieties. Immunotherapy, aiming at the generation of anti-leukemia T-cell responses, could provide a new therapeutic approach in eliminating minimal residual disease cells in leukemia. Leukemia stem cells could be targeted with a $CD8^+$ cytotoxic T lymphocyte clone specific for minor histocompatibility antigens; an approach that might be useful in relapsing AML patients after allogeneic transplant[52]. The presence of cytotoxic T lymphocytes directed against leukemia blasts emphasizes their suitability as immunological targets. Increased immunogenicity can also be achieved by the differentiation of leukemia blasts into leukemia dendritic cells, which have been demonstrated to induce antileukemic T-cell responses *in vitro*. However, it remains to be established whether AML-dendritic cells are able to elicit profound immune responses *in vivo*[53].

CONCLUSION

In conclusion, most cytotoxic therapeutic strategies currently used for leukemia therapy damage DNA or disrupt mitosis to induce cell death in highly proliferative cells that represent the bulk of malignant cell populations. Stem cells tend to be more resistant to chemotherapy. This involves the presence of multidrug resistance, antiapoptotic proteins, and DNA repair mechanisms. Furthermore, most current therapies do not target the signaling pathways that regulate self-renewal. Selectivity of targeting leukemia stem cells over normal stem cells is needed to avoid systemic toxicity. An important endpoint will therefore involve assessing changes in the size of the leukemia stem cell population. With the development

of clinical trials involving more targeted therapies, time progression or patient survival will thus become the ultimate clinical endpoints. However, a survey of markers for leukemia stem cells, *via* micro array or proteomic profiling, will also be important. The development of mathematical modeling could also be useful to understand responses to treatments that target malignant stem cell complexes in reactive or adaptative systems[54,55].

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