Empowering host immunity by kinase-targeting in LSC

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Using a series of well-designed set of experiments involving transgenic, bone marrow transplantation and patient-derived xenograft (PDX) mouse models, recent study of Perry et al in *Nature Cell Biology* (NCB) showed that a conventional anthracycline chemo-agent doxorubicin (DXR) can be repurposed at low dose as an immune therapy to eliminate leukemia stem cells (LSCs) via targeting Akt-mediated Wnt–β-catenin activity.[1] These findings elegantly bridge several apparently unrelated concepts from cancer biology and clinical medicine onto a focal point of chemo-resistant LSC. From the perspective of acute myeloid leukemia (AML) therapy, traditional chemotherapy, kinase-targeting agent as well as immune therapy are independently developed based on distinct biological mechanism. Perry's study blurred these distinctions and took on the unique advantage of various therapies to converge on a conceptually novel strategy: a low dose chemo-agent can extrinsically empower immune cells (such as CD8+ T cells), as well as intrinsically inhibit LSCs activity by targeting Akt's enzymatic ability to phosphorylate β-catenin. Interestingly, the multiple effects of DXR on LSCs was mechanistically rooted in a novel discovery made by authors that β-catenin can simultaneously regulate self-renewal as well as immune-checkpoint, pointing to an intriguing connection between normal hematopoietic stem cell and immune protection.

LSCs underlie cancer therapy resistance and eradication of those cells has been proven to be extremely difficult. Therapies are developed to either attack the intrinsic LSC properties or enhance antitumor immune function without specifically targeting the LSC. Several therapies become available for clinical evaluation based on the aberrant biological properties of LSCs, such as the BCL-2 inhibitor venetoclax[2] to disrupt mitochondrial activity, gemtuzumab ozogamicin[3] utilizing an anti-CD33 antibody conjugated to the antitumor antibiotic calicheamicin to create a targeted delivery vehicle. Immune-based treatments with adoptive-cell therapy (CAR-T, etc.), immune checkpoint blockers have emerged as a promising treatment option for acute leukemia. However, adverse side-effects related to CAR-T therapy have been extensively reported with frequent events of cytokines storm.[4] Immune checkpoint blockade therapies, such as the inhibition of single target PD-1/PD-L1, is obstructed by the low response rate in cancer patients.[5]

Through high-throughput screening of a FDA-approved small-molecule library, the authors unexpectedly developed a stem cell-specific immune therapy by targeting an intrinsic biological property of LSC. The cooperative role of the Wnt–β-catenin and PI3K-Akt pathways in resistance to multiple anticancer therapies was reported.[6] The cooperative activation of these two pathways was enriched in LSCs ($Lin^{-}CD3^{+}c\text{-}Kit^{Mid}$) while infrequently detected in normal hematopoietic stem/progenitor cells (HSPC) cells of the patients. To target this vulnerability unique to LSC, researchers took advantage of their previous findings that the cooperation via Akt-mediated phosphorylation of serine residue 552 of β-catenin plays a critical role in intestinal and normal hematopoietic stem cells. Perry et al discovered that interfering phosphorylation of serine residue 552 of β-catenin by low dose anthracycline DXR can promote antitumor immunity by down-regulating multiple immune checkpoints (PD-L1, CD24, etc.) in LSC. This is a novel function of the cooperation between these two pathways beyond its well-known role in self-renewal and expansion of stem cells. This study demonstrated that LSC hijacked stem cell property in immune checkpoint regulation to promote immune evasion. Remarkably, anthracyclines favored the restoration of antitumor immune responses not only through altering the intrinsic immunogenicity of LSC but also preserving or even enhancing CD8+ killer T cells and other immune cells. Redirecting the patient's own immune system to eliminate tumor cells through modulating of multiple checkpoints is a highly attractive approach that aims to effectively enhance anti-LSC immune activity.

It is worth noting that effective targeting of LSC by DXR was only achieved at a low dose (one fortieth of the clinical dose), as high dose DXR significantly compromised the T cell population required for LSC clearance. Interestingly, several "low dose" chemotherapy regimens have been reported to treat acute leukemia. Reduced-intensity chemotherapy combined with tyrosine kinase inhibitor imatinib in adults with Ph-positive acute lymphoblastic leukemia (ALL) can lower early toxicity without decreasing the long term survival[7]; low-dose chemotherapy, in conjunction with granulocyte stimulating factor (G-CSF), attains comparable mutation clearance and overall 4-year survival in pediatric AML as standard-dose chemotherapy while significantly reducing hematological toxicity.[8] The observed clinical efficacy may be attributable to the fact that G-CSF can

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mobilize LSC and drive cell cycle progression for increased sensitivity to chemo-agents, in addition to its effect on immune regulation.[9] These studies challenge the conventional rationale in chemotherapy in which maximum tolerated dose (MTD) are given and has significantly improved survival of human leukemia. However, MTD strategy had shown little success in treating refractory and relapsed diseases, as well as in eradicating LSCs while inevitably suppressing immune function and increasing morbidity and mortality.[10] With an increased understanding of molecular networks in cancer biology, specific vulnerability of the tumor cell can be targeted. By harnessing distinct property of tumors cells or in combination with bioactive cytokine, low dose chemotherapy may serve as an attractive alternative to not only effectively inhibit the survival of leukemia cells, but preserve the host immunity for improved tumor clearance and quality of life.

Therapeutically, Akt-activated β-catenin can serve as a biomarker indicating patients who might benefit from low-dose anthracycline treatment. Further studies are needed to determine whether the Akt–β-catenin interaction is broadly active in acute leukemia or preferentially present in specific subtypes. However, even in applicable T-ALL and refractory/relapse AML patients examined by the authors, pS552-β-cat+ cells only accounted for approximate 50% of chemo-resistant LSCs in patient MRD (measurable/minimal residual disease) samples. Therefore, it remains to be determined the clinical benefit of this therapy on patient outcomes. Finally, low-dose DXR specifically reduced the number of LSCs but not blast cells in mouse model. As both standard induction regimens for AML and ALL employ hematoxic high-dose chemo-agents including anthracycline, direct combination with low-dose DXR/DNR is not applicable. Standard induction chemo-regimen can be followed by low-dose anthracycline treatment once normal hematopoiesis is largely restored during induction therapy.[11,12] Alternatively, low-dose DXR can be used as a maintenance therapy for MRD+ patients with Akt-activated β-catenin activity. The mouse model, preclinical, and early clinical data presented by Perry et al pave the way for a novel and promising LSC-targeted immuno-therapy in treating acute leukemia patients.

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