and processed ex vivo in ambient air oxygen (~21% O_2) or cultured in ambient air. Ambient air O_2 levels are not the same as physoxia (lowered O_2 levels present in vivo that are relevant to in vivo physiology). Collecting and processing cells in ambient air results in a phenomenon termed extra physiological shock/stress (EPHOSS).[12] EPHOSS is associated with enhanced mitochondrial ROS, and is linked with P53, opening of the mitochondrial permeability transition pore, ROS, hypoxia inducing factor-1α, and the hypoximir miR210. Mitigating EPHOSS by collecting and processing cells at a lowered O_2 allows for detection of increased HSCs and decreased slow- cycling HPCs.[12,13] Hence, it is appropriate to reevaluate the effects of radiation and RIBEs by collecting/processing and culturing cells at lowered O_2/physoxia to remove the confounding influences of EPHOSS on collected cells. This approach will provide a more accurate reading of the results and may be especially important for evaluation of the effects of radiation and RIBEs on cells of aged mice[13] and humans.

Another area that could use reanalysis in this context is that of RIBE-induced cytokines, chemokines, and growth factors and whether these molecules, produced or released in response to radiation, are in a full-length or truncated form. Dipeptidylpeptidase4 (DPP-4) is an enzyme that truncates selected proteins with an alanine, proline, or other amino acid at the penultimate N terminus.[14] Full-length and DPP-4–truncated proteins do not have similar functional activities. In certain situations, DPP4-truncated proteins (such as granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, IL-3, TPO, and EPO) have no or less activity than their full-length forms, but can block activities of their full-length forms.[14]

In their article, Hu et al add to and bring us closer to a more in-depth analysis of the impact of RIBEs on human HSCs and HPCs in the context of HCT. It is clear that there is much more to be learned about health benefits involving radiation (see bottom of figure). This is a virgin field, ready for continued rigorous evaluation in terms of cell responses and in-depth mechanistic insight.

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REFERENCES

1. Hu L, Yin X, Zhang Y, et al. Radiation-induced bystander effects impair transplanted human hematopoietic stem cells via oxidative DNA damage. *Blood*. 2021;137(24):3339-3350.
2. Nagasawa H, Little JB. Induction of sister chromatid exchanges by extremely low doses of alpha-particles. *Cancer Res*. 1992;52(22):6394-6396.
3. Najafi M, Fardid R, Hadadi G, Fardid M. The mechanisms of radiation-induced bystander effect. *J Biomed Phys Eng*. 2014;4(4):163-172.
4. Pouget JP, Georgakilas AG, Ravanat JL. Targeted and off-target (bystander and abscopal) effects of radiation therapy: redox mechanisms and risk/benefit analysis. *Antioxid Redox Signal*. 2018;29(15):1447-1487.
5. Wikipedia. Bystander effect (radiobiology). https://en.wikipedia.org/wiki/Bystander_effect_(radiobiology). Accessed 17 February 2021.
6. Wikipedia. Health threat from cosmic rays. https://en.m.wikipedia.org/wiki/Health_threat_from_cosmic_rays. Accessed 17 February 2021.
7. Shen H, Yu H, Liang PH, et al. An acute negative bystander effect of γ-irradiated recipients on transplanted hematopoietic stem cells. *Blood*. 2012;119(15):3629-3637.
8. Broxmeyer HE, Galbraith PR, Baker FL. Relationship of colony-stimulating activity to apparent kill of human colony-forming cells by irradiation and hydroxyurea. *Blood*. 1976;47(3):403-411.
9. World Health Organization. Ionizing radiation health effect and protective measures. https://www.who.int/news-room/fact-sheets/detail/ionizing-radiation-health-effects-and-protective-measures. Accessed 17 February 2021.
10. International Atomic Energy Agency. Radiation in everyday life. https://www.iaea.org/Publications/Factsheets/English/radlife. Accessed 17 February 2021.
11. Kirolikar S, Prasannan P, Raghuram GV, et al. Prevention of radiation-induced bystander effects by agents that inactivate cell-free chromatin released from irradiated dying cells. *Cell Death Dis*. 2018;9(12):1142-1157.
12. Mantel CR, O'Leary HA, Chitteti BR, et al. Enhancing hematopoietic stem cell transplantation efficacy by mitigating oxygen shock. *Cell*. 2015;161(7):1553-1565.
13. Capitano ML, Mohamad SF, Cooper S, et al. Mitigating oxygen stress enhances aged mouse hematopoietic stem cell numbers and function. *J Clin Invest*. 2021;131(1):e140177.
14. Ropa J, Broxmeyer HE. An expanded role for dipeptidyl peptidase 4 in cell regulation. *Curr Opin Hematol*. 2020;27(4):215-224.

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LYMPHOID NEOPLASIA

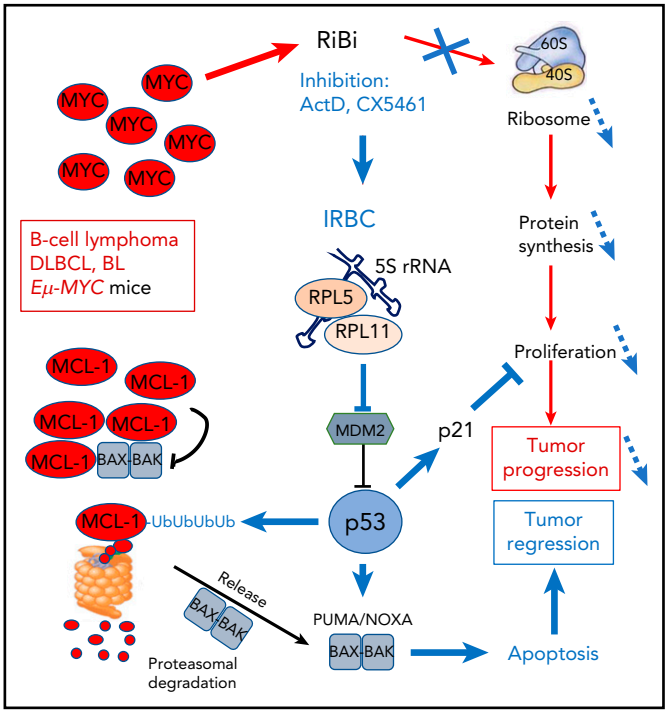
Comment on Domostegui et al, page 3351

Blocking RiBi to suppress MYC lymphomagenesis

Jean-Francois Peyron | INSERM

In this issue of *Blood*, Domostegui and colleagues demonstrate that in MYC-driven B-cell lymphoma, activation of the ribosome biogenesis (RiBi) checkpoint triggers an apoptotic response, through the p53-induced, proteasome-dependent degradation of MCL-1.[1]

The MYC bHLH (basic Helix-Loop-Helix) transcription factor may be the most deregulated oncogene in all of human cancers, where it promotes increased cell metabolism and growth, enhanced survival, and abnormal proliferation.[2] In particular, MYC is a strong stimulator of RiBi and is unique by its simultaneous enhancing effect on the activity of the RNA polymerases pol-I, pol-II, and pol-III. They respectively enhance transcription of the 47S precursor ribosomal RNA (rRNA), of messenger RNAs (mRNAs) for the 80 ribosomal proteins (RPs), and of the 5S rRNA, in order to boost the production of ribosomes.[3] MYC is the hallmark oncogene amplified in aggressive diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL). Myc-driven lymphomas can be modeled in *Eμ-MYC* mice, where *MYC* is overexpressed in B lymphocytes under the control of the strong immunoglobulin heavy chain enhancer. Not surprisingly, it was observed that *Eμ-MYC* lymphomas are addicted to RiBi and protein synthesis. Indeed, the survival of *Eμ-MYC* mice was greatly increased by inducing a haploinsufficiency of either of the genes coding for the L24/RPL24 or L38/RPL38 RPs, correcting the enhanced protein



The amplified MYC oncogene in DLBCL and BL lymphoma strongly stimulates RiBi that supports cell proliferation and tumor progression through enhanced protein synthesis. Blocking RiBi with ActD or the pol-I inhibitor CX5461 decreases ribosome numbers impacting abnormal proliferation. In parallel, the IRBC comprising 5S rRNA, RPL5, and RPL11 is formed that blocks mdm2 to activate p53. p53 interferes with proliferation through induction of p21 and stimulates ubiquitination of the antiapoptotic BCL-2 family member MCL-1 that is frequently amplified in MYC-driven lymphomas. Ubiquitinated MCL-1 is then degraded in the proteasome to free BAK-BAX dimers that trigger mitochondrial membrane disruption and apoptosis, leading to tumor regression.

synthesis rate of *Eμ-MYC* lymphoma cells back to homeostatic values.[4] These results were in accordance with the ribosome being considered an active player in cancerous transformation and also an attractive anticancer target. Blocking either RiBi or the ribosome directly has shown promising effects[5] and homoharringtonine, a protein synthesis inhibitor that directly blocks the ribosome, has been approved for clinical use in resistant chronic myeloid leukemia and is being evaluated in acute myeloid leukemia in various clinical trials. In a different direction, the depletion of the *RPL24* gene triggers a p53-dependent cell-cycle arrest.[6] Moreover, a multimolecular complex, the impaired ribosome biogenesis checkpoint (IRBC), consisting of 5S rRNA, RPL5, and RPL11, was then identified as a checkpoint of efficient RiBi. Upon RiBi inhibition, 5S rRNA, RPL5, and RPL11 are released from the preribosomal complex to form the IRBC that interacts with and blocks HDM2/mdm2 to induce p53 stabilization, acting as a tumor suppressor.[7]

The current study addresses the contribution of decreased global translation vs activation of IRBC/p53 in the anticancer effects of targeting RiBi. The authors used cell lines derived from *Tp53*$^{+/+}$; *Eμ-MYC* mice. They show that the inducible short hairpin RNA-depletion of RPL7a or RPL11 both decreased protein synthesis. However, only the depletion of RPL7a, which does not affect the IRBC, could trigger a p53-dependent apoptotic response.

The MCL-1 antiapoptotic protein of the BCL-2 family is frequently coamplified with MYC to cooperate for transformation. Domostegui et al showed that after interference with RiBi, p53 induced polyubiquitination of MCL-1. Ubiquitinated MCL-1 is then degraded by the proteasome, leading to apoptotic cell death (see figure). This was not observed in a p53-mutated background.

The authors also show that low doses of actinomycin D (ActD), that blocks the transcription of rDNA by RNA polymerase I, induced IRBC and apoptosis of p53$^{+/+}$ lymphoma cells. Their analysis of the Genomics of Drug Sensitivity in Cancer database revealed that cancer cell lines originating from blood cells are more sensitive to ActD than other tumor types, and among them the p53 status is predictive of the response to ActD. This was confirmed in vitro on a panel of BL and DLBCL cell lines. This important result supports the potential use of ActD in treating wt-TP53 hematological malignancies. ActD is already used, albeit at higher doses, for some pediatric tumors such as Wilms tumors, rhabdomyosarcoma, and Ewing sarcoma. Interestingly, it has been realized that interference with RiBi was a property of many classical antineoplastic drugs, such as anthracyclines.[8] Also, oxaliplatin was recently shown to act through a RiBi stress response, in contrast to the other members of the cisplatin drug family that induce DNA damage.[9] It would be interesting to explore the implications of this study on IRBC on the mode of action of these classical drugs.

Genetic defects that alter RiBi or ribosome function/production are at the roots of ribosomopathies, such as Diamond-Blackfan anemia or Schwachman-Diamond syndrome, that are congenital diseases associated with a high risk of developing cancer, particularly leukemia. One of the hypotheses to explain the malignant transformation is that the defects in RiBi/ribosome trigger during a hypoproliferative stage associated with inefficient protein synthesis, a salvage activation of p53 in an attempt to eliminate defective cells. However, the occurrence of a secondary genetic event that blocks the p53 pathway allows the cells to escape apoptotic elimination and to proceed to malignant transformation.[10] Here also it could be interesting to analyze the potential role of IRBC and of MCL-1 in the support of the survival of RiBi defective cells. Finally, the work of Domostegui et al suggests that direct interference of MCL-1 function with specific BH3 mimetics could be an alternative approach in treating p53 mutated lymphomas, in association with RiBi inhibition.

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REFERENCES

1. Domostegui A, Peddigari S, Mercer CA, et al. Impaired ribosome biogenesis checkpoint activation induces p53-dependent MCL-1 degradation and MYC-driven lymphoma death. *Blood*. 2021;137(24):3351-3364.

2. Stine ZE, Walton ZE, Altman BJ, Hsieh AL, Dang CV. MYC, metabolism, and cancer. *Cancer Discov*. 2015;5(10):1024-1039.

3. van Riggelen J, Yetil A, Felsher DW. MYC as a regulator of ribosome biogenesis and

protein synthesis. *Nat Rev Cancer*. 2010; 10(4):301-309.

4. Barna M, Pusic A, Zollo O, et al. Suppression of Myc oncogenic activity by ribosomal protein haploinsufficiency. *Nature*. 2008;456(7224): 971-975.

5. Gilles A, Frechin L, Natchiar K, et al. Targeting the human 80S ribosome in cancer: from structure to function and drug design for innovative adjuvant therapeutic strategies. *Cells*. 2020;9(3):629-651.

6. Barkić M, Crnomarković S, Grabusić K, et al. The p53 tumor suppressor causes congenital malformations in Rpl24-deficient mice and promotes their survival. *Mol Cell Biol*. 2009; 29(10):2489-2504.

7. Donati G, Peddigari S, Mercer CA, Thomas G. 5S ribosomal RNA is an essential component of a nascent ribosomal precursor complex that regulates the Hdm2-p53 checkpoint. *Cell Rep*. 2013;4(1):87-98.

8. Burger K, Mühl B, Harasim T, et al. Chemotherapeutic drugs inhibit ribosome biogenesis at various levels. *J Biol Chem*. 2010;285(16):12416-12425.

9. Bruno PM, Liu Y, Park GY, et al. A subset of platinum-containing chemotherapeutic agents kills cells by inducing ribosome biogenesis stress. *Nat Med*. 2017;23(4): 461-471.

10. De Keersmaecker K, Sulima SO, Dinman JD. Ribosomopathies and the paradox of cellular hypo- to hyperproliferation. *Blood*. 2015; 125(9):1377-1382.

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LYMPHOID NEOPLASIA

Comment on Vaisitti et al, page 3365

A new option for Richter syndrome

Martin R. Müller | KRH Klinikum Siloah Hannover

In this issue of *Blood*, Vaisitti et al[1] demonstrate that the receptor tyrosine kinase–like orphan receptor 1 (ROR1), which is expressed on the cells of various hematological malignancies and solid tumors, but not on normal adult tissues, is a potential therapeutic target for treatment of Richter transformation. Using a xenograft mouse model, they showed a significant therapeutic effect using the ROR1-directed antibody drug conjugate (ADC) VLS-101 (see figure).

RS refers to the development of an aggressive lymphoma in patients with chronic lymphocytic leukemia (CLL).[2] While diffuse large B cell lymphoma (DLBCL) is by far the most common histology, Hodgkin lymphoma and T-cell lymphomas can also occur. DLBCL-like RS carries a particularly dismal prognosis. The clonal relationship between the underlying CLL and the DLBCL provides the most important prognostic information. Patients with a clonally related DLBCL transformation show a median survival of only 14 months, whereas patients with clonally unrelated transformation have a significantly better prognosis, with a median survival of 5 years.[3] Approximately 80% of patients with DLBCL-like RS have clonally related disease and, thus, a bad prognosis. Transformation while on therapy with targeted agents like ibrutinib or venetoclax seems to be associated with an even poorer outcome, with a median survival of only 3 months.[4]

The mainstay of therapy for RS is anthracycline-containing chemotherapy plus rituximab, with R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone) and dose-adjusted EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin plus rituximab) being the preferred regimens. Nonmyeloablative allogeneic hematopoietic stem cell transplantation is an option for a subpopulation of patients and significantly improves the clinical outcome when performed during the first remission.[5] A number of novel agents (ibrutinib, venetoclax, pembrolizumab, nivolumab, and selinexor) have shown clinical activity but with disappointing remission durations. The optimal combination of classical immunochemotherapy and novel agents is currently being investigated in several clinical trials. Two recently reported studies have demonstrated promising results with chimeric antigen receptor T cells.[6] Overall, the therapeutic options especially for clonally related RS remain very limited, and new therapeutic options are desperately needed.

ADCs are a novel set of anticancer agents with excellent activity in a variety of malignancies. They typically consist of a monoclonal antibody directed against a specific antigen on malignant cells that is linked to several molecules of a cytotoxic drug. Upon binding their respective antigen, ADCs are rapidly internalized and eventually release the cytotoxic agent into the cytosol of the tumor cell. A number of ADCs are currently approved for cancer therapy. Brentuximab vedotin is a CD30-directed ADC approved for the treatment of Hodgkin disease and systemic anaplastic large cell lymphoma.[7] Trastuzumab emtansine is used in the treatment of Her-2/neu–positive breast cancer.[8] Inotuzumab ozogamicin,[9] which is directed against CD22, is approved for the treatment of relapsed and refractory acute lymphoblastic leukemia, whereas gemtuzumab ozogamicin, which targets CD33, is used in the first-line treatment of acute myeloid leukemia. Polatuzumab vedotin[10] is a CD79b-directed ADC that significantly improves the clinical outcome in patients with DLBCL when used in combination with bendamustine and rituximab.

Vaisitti et al now report that ROR1 is expressed on the surface of RS cells and can be efficiently targeted using the ADC VLS-101, resulting in significant remissions and improvement of overall survival in a mouse model. The authors employed a xenotransplant mouse model to investigate tumors derived from patients with DLBCL-like RS with different expression levels of ROR1. In chronic lymphocytic leukemia cells, ROR1 binds to the noncanonical Wnt signaling member Wnt5a, resulting in the activation of different guanine exchange factors and subsequently phosphatidylinositol-3 kinase and Janus kinase signaling cascades, culminating in cell proliferation. A phase 1 study with naked antibody to inhibit the ROR1 pathway failed to show significant clinical activity. This prompted the group to create the ROR1-directed ADC VLS-101, which was used in this study. While the ROR1 pathway does not appear to be activated in RS cells, the investigators show that ROR1 can serve as a robust target for the ADC. The treatment of mice with transplanted human