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MYELOID NEOPLASIA

Comment on Kahn et al, page 1095

CHIPing out PPM1D-mutant hematopoiesis

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Deep sequencing approaches have allowed researchers to identify a wide spectrum of somatic mutations in hematopoietic cells (HSCs) of individuals without a known hematologic disorder. Recurrent variants in protein phosphatase Mg^{2+}/Mn^{2+} 1D (PPM1D), a serine/threonine phosphatase involved in the regulation of DNA damage response (DDR), were found to be highly enriched in blood cells of cancer patients previously treated with cytotoxic agents.[1,2] In this issue of *Blood*, Kahn et al provide evidence on how *PPM1D* mutations confer a selective growth advantage in the presence of ongoing DNA damage and demonstrate that pharmacological targeting of PPM1D is able to reverse this effect.[3]

HSCs are constantly exposed to DNA damage arising from DNA replication, spontaneous reactions intrinsic to the chemical nature of DNA, or assaults by externally derived agents. Normal HSCs accumulate somatic mutations with an estimated rate of 0.13 ± 0.02 exonic mutations per year of life, ultimately resulting in a genetically heterogeneous population over time.[4] Clonal hematopoiesis (CH) arises from increased fitness of individual HSC clones and becomes manifest by a disproportional contribution to the population of mature blood cells. The presence of a clonal mutation in a gene recurrently mutated in hematologic malignancies and a variant allele frequency of ≥2% is referred to as clonal hematopoiesis of indeterminate potential (CHIP).[5] Subject to the applied technique of analysis, CH was found in 5.6% to 20% of 60- to 70-year-old individuals without known hematologic abnormalities with an increasing incidence as a function of age.[6-8] Somatic mutations were predominately detected in *DNMT3A*, *TET2*, and *ASXL1* followed in frequency by mutations in *TP53* and *PPM1D*. In contrast, in patients previously treated with cytotoxic therapy for solid or hematologic malignancies, CH was dominated by mutations in *TP53*, *PPM1D*, and other genes involved in DDR.[1,2] PPM1D (also known as WIP1 [wild-type p53-induced phosphatase 1]) acts as an important regulator of the stress response and directly suppresses the activity of several key components of the DDR pathway, including p53, ATM, CHK1/2, or p38-MAPK.[9] Amplification of PPM1D correlates with poor prognosis in a variety of solid tumor entities and confers resistance to cytotoxic agents.[9]

In this issue, Kahn et al address the question of why truncating *PPM1D* mutations are enriched in the blood of patients previously exposed to cytotoxic therapy. The authors used a sophisticated clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein-9 nuclease (Cas9) mutagenesis screen targeting the protein-coding region of *PPM1D* to demonstrate a chemotherapy resistance phenotype mediated by frameshift mutations within the C-terminal domain of the protein. The CRISPR-Cas9–induced mutational profile substantially overlaps with *PPM1D* mutations found in patients with therapy-related neoplasm or CH.[1,2] Loss of the C-terminal regulatory region increases protein stability, diminishes phosphorylation of several DDR proteins, and attenuates apoptotic cell death upon treatment with cytotoxic agents. Further, PPM1D-mutant cells outcompete their normal counterparts in the presence of chemotherapy in vitro and in vivo, recapitulating the enrichment of *PPM1D* mutations observed in patients treated with cytotoxic therapy for their primary disease.[1,2]

There is accumulating evidence that individuals with CH/CHIP have an increased risk of developing hematologic neoplasms as well as a higher all-cause mortality rate, in many cases because of ischemic cardiovascular disease.[6-8,10,11] It is tempting to speculate that early treatment of CH/CHIP might prevent hematologic neoplasms and improve overall survival. Currently, several inhibitors targeting DDR components such as ATM, ATR, and CHK1/2 are being evaluated in clinical trials. Other inhibitors will likely follow (eg, directed against PPM1D). Other inhibitors will likely follow. Therefore, we will likely have the tools to evaluate the utility of treatment soon.

Keeping in mind all the caveats related to in vitro studies, Kahn et al demonstrate that treatment of AML cells with GSK2831781, a novel PPM1D-inhibitor, had single-agent activity in PPM1D-mutant cells and specifically reversed chemotherapy resistance. Further, the authors show that GSK2831781 treatment prevents outgrowth of PPM1D-mutant cells in the presence of chemotherapy in a competitive population assay. In individuals with CH, and particularly those harboring mutations in DDR genes including *PPM1D*, previous cytotoxic therapy was associated with an increased number of somatic mutations and higher variant allele frequency.[2] Interestingly, the number of mutations and allele burden in CH correlated with an increased risk for subsequent treatment-related hematologic malignancies and all-cause mortality.[1,7,8] Although the absolute risks are still low,

patients with CH and mutations in DDR genes at the time of cytotoxic therapy might represent a high-risk population. These findings suggest several conclusions to help guide treatment decisions. (1) Patients who are supposed to receive cytotoxic therapy should be screened for CH. Patients with mutations in DDR genes could be treated, if feasible, with alternative approaches (eg, immunotherapy or inhibitors). (2) In case cytotoxic therapy is unavoidable, patients should be closely monitored for the development of treatment-related hematologic neoplasms. (3) With the increasing availability of specific inhibitors, it might be interesting to target and eliminate the clonal HSC before the onset of disease. However, each treatment strategy will likely also cause side effects in otherwise asymptomatic individuals. For example, *Ppm1d* knockout mice display defects in B- and T-cell differentiation resulting in immunodeficiency, severe neutrophilia causing inflammatory disease phenotypes, and diminished self-renewal capacity of HSC.[12] The latter is specifically relevant in elderly patients, who may rely on oligoclonal hematopoiesis to ensure proper blood production.

In summary, the work by Kahn et al provides important mechanistic insights into the function of DDR genes in clonal hematopoiesis and the first experimental evidence for successful targeting driver mutations in CHIP. As a next step, prospective clinical trials are warranted to address the questions of whether early treatment of CHIP can reduce all-cause mortality and the risk of subsequent treatment-related hematologic neoplasms.

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PLATELETS AND THROMBOPOIESIS

Comment on Tsukiji et al, page 1167

The incomparable platelet: holy alveoli!

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In this issue of *Blood*, Tsukiji et al report on the consequences of platelet–lymphatic interactions in the lung, namely the influence of platelet activation with release of platelet transforming growth factor-β (TGF-β) on lung development.[1] The small but mighty platelet continues to amaze as functions beyond canonical roles in hemostasis and thrombosis are being discovered at a rapid rate. Key roles in inflammation, autoimmunity, and metastasis have recently been elucidated. One wonders how many more tricks the platelet has up its sleeve.

As with any biologic process, there is a balance of effects on the host system. With platelets, we have to consider the good (plugging holes, antimicrobial function) with the potentially harmful (thrombotic organ injury, exaggerating inflammation). There is a growing amount of literature on the role of platelets as biologic packages that deliver key signals to tissues, including the lung. For example, in a pneumonectomy model of lung regeneration, platelets deliver stromal cell–derived factor-1 to the lung endothelium, which drives alveolar epithelial cell expansion via membrane-type metalloproteinase MMP14.[2] Platelet-rich plasma, increasingly used in sports medicine to accelerate recovery, also accelerates lung regeneration after pneumonectomy through angiogenic factors such as angiopoietin-1.[3]

There is a well-established literature field on the role of platelet–lymphatic interactions in maintaining the separation of vascular and lymphatic channels. It may seem surprising that platelets and lymphatic channels would be in contact, and indeed, the exact sites of contact during organ development are unclear. C-type lectin-like receptor-2 (CLEC-2) on platelets, originally discovered as a receptor-mediating platelet aggregation in venomous snake bites,[4] and podoplanin in the lymphatic endothelium, constitute the major receptor–ligand pairs.[5] At the junction between the thoracic duct and the subclavian vein, the CLEC-2–podoplanin interaction amazingly is in continuous operation to prevent the entry of blood into the lymphatic system.[6] Other described interactions include platelet aggregation via CLEC-2 on metastatic