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MYELOID NEOPLASIA

Comment on Papadopoulos et al, *page 1818*

Hidden conformational codes

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In this issue of *Blood*, Papadopoulos et al[1] reveal that oncogenic JAK2V617F elicits a unique pattern of active human thrombopoietin receptor (hTpoR) conformations distinct from those induced by its cognate ligand thrombopoietin (Tpo). These results point to a new therapeutic strategy of targeting JAK2V617F-specific interface with hTpoRs to eliminate pathologic JAK2V617F+ myeloproliferative neoplasms (MPNs) without impacting wild-type (WT) cells.

The TpoR is important for megakaryocyte and platelet development as well as hematopoietic stem cell (HSC) expansion.[2,3] TpoR adopts different conformations and interfaces after dimerization to modulate the activation states in response to its cognate ligand Tpo or small-molecule agonist eltrombopag.[4] JAK2 is the critical kinase for stabilizing TpoR dimers and activating downstream signaling cascade.

Activating mutations in TpoR (W515K/L and S505N), JAK2 (V617F), and calreticulin are found at high frequencies in MPNs.[5] Mechanistically, these driver mutations act through TpoR and converge on JAK-STAT signaling in all subtypes of MPNs,[6] pointing to the central role of TpoR and JAK2 in the pathogenesis of MPN. Although JAK inhibitors (JAKi) (eg, ruxolitinib) alleviate disease-associated symptoms in MPN, the allele burden of JAK2V617F is only mildly reduced in most patients, partly due to JAK2V617F+. HSCs reenter dormancy and therefore are insensitive to JAKi[7] or heterodimeric JAK1 reactivation.[8] One disadvantage of JAKi is the side effects caused by its inability to discriminate between oncogenic JAK2V617F and its WT counterpart. Thus, the exploration of novel therapeutic strategies by targeting the TpoR/JAK2 axis requires a better understanding of JAK2V617F/TpoR activation and signaling.

Despite the advance in our understanding of the TpoR/JAK2 signaling, the deeper structural basis regarding the transmembrane (TM) and juxtamembrane domain for their activation remains elusive. To overcome this barrier, Constantinescu and colleagues devised an alternative strategy to impose 7 dimeric orientations of a cytokine receptor via the replacement of the extracellular domain with an amphipathic coiled-coil domain.[9] By applying this strategy to erythropoietin receptor (EpoR), they identified 3 distinct conformations of EpoR during no, partial, and full activation, respectively.[9] In the case of mouse TpoR (mTpoR), they found that several different conformations are able to mediate its constitutive activation and induce specific MPN phenotypes.[10] These investigations indicate that a single receptor can transmit different types or strengths of downstream signaling by fine-tuning its conformation. However, the scope of the studies previously mentioned was limited to studying the receptor with JAK2 WT without considering the potential effect of oncogenic JAK2V617F. Moreover, the mechanism obtained from mTpoR cannot be assumed to be the same in hTpoRs because mTpoRs have notable amino acid differences in the cytoplasmic domain—for example, the key eltrombopag binding site in the hTpoR TM domain, H499, is absent in the mTpoR.

Papadopoulos et al further explored the activation pattern of hTpoR under physiological and pathological conditions (see figure). By applying the aforementioned strategy to hTpoR, they found that active hTpoRs adopt a distinct conformational pattern different from that of mTpoRs, attributable to the difference of 2 amino acids, G503 and H499. Next, they tested the downstream signaling spectrum of hTpoRs. Unlike EpoRs and mTpoRs, where different conformations selectively favor one signaling over another, all active hTpoR conformations appear to unbiasedly stimulate the same downstream signaling in the presence of either JAK2 WT or V617F. It is noteworthy that for a specific hTpoR conformation, JAK2V617F and WT elicit different strengths of STAT phosphorylation, and this discrepancy is only observed in hTpoR, but not mTpoR. More importantly, the authors compared

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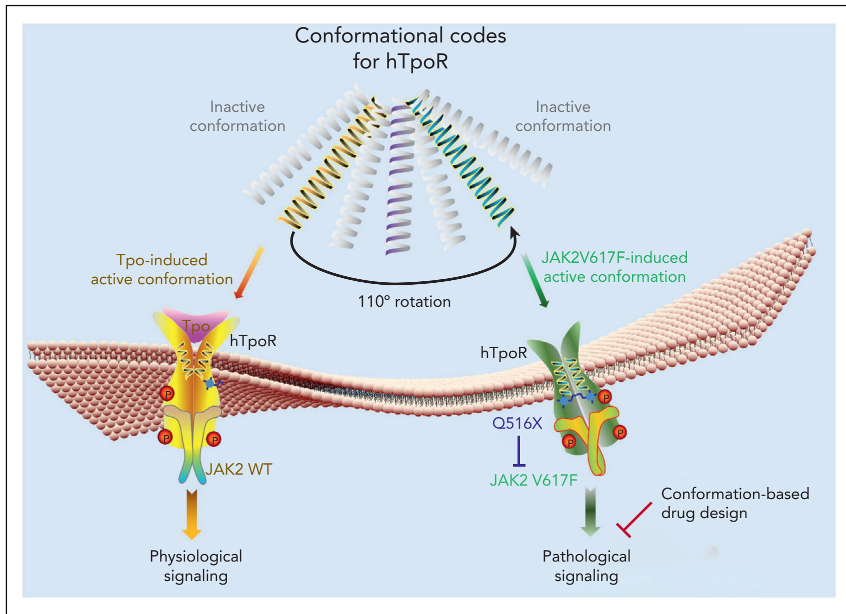

A working model describing how hTpoR responds to different activating cues and transmits downstream signaling via overlapping but distinct conformations. The active orientations and dimeric interfaces of hTpoR induced by Tpo and oncogenic JAK2V617F are strikingly distinct with a clockwise rotation of 110° from Tpo- to JAK2V617F-induced conformation. Amino acid Q516 of hTpoR is critical for JAK2V617F-induced activation with negligible effect on that induced by Tpo. Thus, targeting JAK2V617F-specific active conformation serves as a proof-of-principle strategy to develop new drugs for the treatment of JAK2V617F^{+} MPN.

the active hTpoR conformations induced by JAK2V617F or Tpo. Interestingly, the conformations of JAK2V617F-activated hTpoRs were largely different from those by Tpo. These differences may partly explain why JAK2V617F transgenic mouse models do not faithfully reflect the disease phenotypes observed in humans.

The authors next employed a live-cell cysteine-specific cross-linking assay of nonmodified hTpoR to validate their results. Indeed, they found that hTpoR adopts distinct dimeric interface upon Tpo-induced and JAK2V617F-driven dimerization, suggesting the possibility of specific inhibition of pathologic signaling via modulation of hTpoR conformations. Strikingly, disruption of JAK2V617F-induced active hTpoR conformations by mutation of key residues in full-length hTpoRs such as Q516 selectively inhibited JAK2V617F^{+}-mediated cell growth, while sparing the dimerization and activity induced by Tpo. Prompted by these encouraging data, the authors confirmed their findings in primary mouse bone marrow cells expressing full-length hTpoRs and corresponding mutants. Megakaryocyte colony forming assay and single-cell progenitor cell assay functionally recapitulated their findings. These results point to a promising therapeutic strategy by targeting specific hTpoR conformation to eliminate pathologic JAK2V617F^{+} HSCs without disturbing WT HSCs.

Elucidation of normal and pathological cytokine receptor activation remains an area of active investigation, not only for its importance in basic science but also for its therapeutic and clinical significance. This work provides a detailed characterization of the conformation-directed activation mechanism of hTpoR and a deeper understanding of the regulatory mechanism of cytokine receptor signaling. It also sheds light on our understanding of how a single receptor transmits differential downstream signaling in response to different stimuli or pathological cues. Based on this study, some interesting questions await further investigation. To what extent can the coiled-coil domain-fused truncated hTpoRs mimic physiological conformations by Tpo or JAK2V617F, and, more importantly, to what extent can the findings be validated in mouse models of MPN in vivo? Do the diverse conformations alter the hTpoR binding affinity to downstream signaling molecules or signaling modulators? Does hTpoR/JAK2V617F signal in endosome membranes adopt different conformations from that in plasma membranes? Do other membrane proteins or lipids exert specific modulatory roles on hTpoR conformations? Since JAK2V617F^{+} HSCs are heterogeneous,[7] is it possible that different JAK2V617F^{+} hematopoietic stem and progenitor cell subpopulations harbor a unique milieu of hTpoRs in the plasma membrane, for example, different expression levels of hTpoRs and local membrane composition? Furthermore, do different JAK2V617F^{+} HSC subpopulations produce distinct signaling spectra or amplitude to favor their clonal expansion and lineage-biased differentiation? The answers to these questions will help us better understand why Tpo/TpoR signaling axis functions differently in HSCs versus megakaryocytes, and why patients with JAK2V617^{+} HSCs exhibit distinct clinical phenotypes.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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TRANSPLANTATION

Comment on Patel et al, page 1831

A potential tissue-based biomarker in gut GVHD

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Patel et al used spatial transcriptomic analysis of gastrointestinal (GI) tract biopsies at the onset of graft-versus-host disease (GVHD) to determine that high expression of ubiquitin-specific protease 17 (USP17L) RNA by colonic hematopoietic cells represents a potential prognostic biomarker for GVHD.[1]

Severe acute GVHD of the lower GI tract causes high mortality and morbidity after allogeneic stem cell transplantation (SCT).[2] In particular, steroid-refractory gut GVHD portends a dismal outcome. Because of the nature of its interface with environmental microorganisms, the GI tract is immunologically active at steady state. Following allogeneic SCT, inflammatory signals in the GI tract are amplified and alloantigen presentation to donor T cells is enhanced locally, promoting donor T-cell activation and severe GVHD.[3] The mechanisms governing steroid responsiveness of gut GVHD during immunosuppressive treatment remain largely unknown. Innovative high-dimensional technology that retains spatial integrity may be useful to interrogate this question. Spatial transcriptomics is a rapidly progressing technology and merges transcriptome profiling and tissue imaging.[4] In the current study, tissue was interrogated with barcoded RNA probes, and selected areas of formalin-fixed, paraffin-embedded tissue (eg, immune cell aggregations) were analyzed to configure spatial transcriptomes.

Patel et al conducted spatial transcriptomic analysis on colonic biopsy samples from 32 patients at the time of acute GI tract GVHD diagnosis using the Nanostring GeoMx Digital Spatial Profiler,[5] one of several currently available commercial platforms. They prepared 2 serial sections from each paraffin block: one section for hematoxylin and eosin (H&E) staining and the other for the GeoMx platform. The H&E-stained sample was used for pathologic grading. The latter was stained with the large panel of RNA probes that are tagged by UV light-cleavable indexing oligonucleotides (gene barcodes) to detect >18 000 genes, and fluorophore-conjugated lineage-specific antibodies to visualize cell subsets, such as hematopoietic/immune cells (anti-CD45), epithelial cells (anti-pancytokeratin), and endothelial cells (anti-CD31). On the basis of this, they then selected lineage-enriched areas (termed areas of interest [AOIs][1]). Then, photocleavable gene barcodes bonded to RNA probes were released from an AOI by UV light projection and collected into a single well of a 96-well plate. This process was repeated for every defined AOI. UV-cleaved barcodes were quantified with next-generation sequencing, and the RNA expression data were mapped to a spatial reference (AOI location on fluorescence image).[1]

In comparison to single-cell RNA sequencing (scRNAseq) of dissociated tissues, spatial transcriptomics enables the analysis of gene expression data of cells and their neighboring microenvironment. If small but specific cell populations play a significant role only in juxtaposition to other cell lineages, this will likely be missed by scRNAseq of the same dissociated tissue. Because available discovery spatial transcriptomics has not yet reached true single-cell resolution, the ability to address cellar interactions is somewhat limited. New targeted RNA probe-based platforms, however, do now offer subcellular resolution and may well provide more informative data. In conjunction with pathologic assessment and clinical outcome information, the authors of the current study could distinguish AOI clusters in which gene signatures were associated with high-grade pathologic changes and steroid resistance. First, they generated gene expression heat maps of AOIs for each lineage (immune cells [ICs], epithelial cells, and vascular endothelial cells), and separated clear 2 clusters therein (eg, IC1 vs IC2). Because paired serial sections were assessed by pathologists, individual AOIs and their gene expression data could be associated with local pathology. Thus, the authors identified which of 2 clusters (AOIs) included higher-grade pathology. Among immune cell clusters, they found that IC1 was associated with high-grade pathology and steroid resistance, which was associated with high expression of USP17L family genes. Higher expression of USP17L family genes correlated with higher nonrelapse mortality and lower overall survival. Little is known about the USP17L gene family members, which are deubiquitinases. In particular, the current study cannot assign any causative relationship to outcome, nor were results validated in additional patient cohorts. Nevertheless, another deubiquitinase, ovarian tumor deubiquitinase 1, activates and differentiates $CD4^+$ T cells to T helper cell (Th) 1, Th17, and Th2 via its ubiquitin-cleavage function, resulting in the exacerbation of GVHD.[6] Understanding any functional role of the USP17L genes in immune cells may thus help shed light on intestinal GVHD progression and steroid resistance.

Risk-predictive biomarkers for acute GVHD that can guide therapeutic interventions are crucial to the field. The Mount Sinai Acute GVHD International Consortium algorithm probability is based on 2 serum biomarkers, suppressor of tumorigenesis 2 (ST2) and regenerating islet-derived 3α (REG3α), and is useful to predict prognosis in systemic acute GVHD.[7] A tissue-based biomarker for intestinal GVHD has not yet been developed, although RNA sequencing of biopsies has been reported in prior studies.[8] As opposed to GeoMx analysis, simple USP17L RNA in situ hybridization in isolation did not predict GVHD outcome in the current study. Thus, future clinical trials will likely require additional markers or improved staining protocols to detect USP17L RNA or protein in isolation to verify USP17L as a biomarker on intestinal biopsies.

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