MOLECULAR & CELLULAR ONCOLOGY
2021, VOL. 8, NO. 2, e1871172 (3 pages)
https://doi.org/10.1080/23723556.2020.1871172

Taylor & Francis
Taylor & Francis Group

AUTHOR'S VIEWS

A JAK of all trades: how global phosphoproteomics reveal the Achilles heel of MPNs

Tina M. Schnoeder[a], Florian Perner[b], and Florian H. Heidel[a]

[a]Innere Medizin C, Universitätsmedizin Greifswald, Greifswald, Germany; [b]Department of Pediatric Oncology, Dana-Farber Cancer Institute & Harvard Medical School, Boston, MA, USA

ABSTRACT

While Janus-kinase (JAK)-inhibitors effectively reduce the inflammatory phenotype of myeloproliferative neoplasms (MPN), they do not affect disease burden or presence of the mutated clone to a major extent. Here, we show how Janus-kinase 2 (*JAK2*)-mutated cells persist through maintenance of the mitogen-activated protein kinase Interacting Serine/Threonine Kinase 1 (MKNK1) – Extracellular Signal-regulated Kinase (ERK)-axis by hijacking the splicing machinery through post-translational modifications.

ARTICLE HISTORY
Received 22 December 2020
Revised 24 December 2020
Accepted 28 December 2020

KEYWORDS
JAK2; MPN; clonal persistence

Author's views

Myeloproliferative neoplasms (MPN) are clonal disorders of aging hematopoietic stem cells and early myeloid progenitors. Mutation of Janus-kinase 2 (*JAK2*) is the predominant genetic event in Philadelphia-chromosome negative MPNs.[1] Janus-kinase 1/2 inhibitors are well tolerated and highly effective in reducing pro-inflammatory cytokine production and inflammation-related clinical symptoms. However, reduction of disease burden is rarely seen,[2] which has restricted their use to rather symptomatic approaches.[1] This finding is unique as the use of targeted therapies such as tyrosine kinase inhibitors frequently leads to regression of the mutated clone. The persistence of *JAK2*-mutated cells under treatment with JAK-inhibitors raises questions about the role of JAK2 as a 'driver' mutation and suggests the alteration of downstream signaling pathways to protect the malignant clone.

Pathways that protect the integrity of the malignant clone from stress-induced apoptosis but are otherwise dispensable for normal (steady state) hematopoiesis have been described in many cancers. Moreover, as a result of an oncogenic mutation, cancer cells may also develop secondary dependency on proteins or signaling molecules that are themselves not oncogenes. Pharmacologic perturbation of these molecules may result in oncogene-specific 'synthetic lethal' interactions.[3] Synthetic lethal interactions can basically involve genes within the same pathway, or distinct pathways that get functionally connected particularly in cancer cells carrying the specific oncogene. Activating mutations of JAK2-kinase (e.g., JAK2V617F) lead to constitutive activation of the kinase and of bona fide downstream signaling nodes such as Phosphoinositide 3-kinase (PI3-kinase), signal transducer and activator of transcription 3/5 (STAT3/5) or mitogen-activated protein kinase kinase (MEK)-extracellular signal-regulated kinase (ERK). Mutation of *JAK2* may result in the recruitment of aberrant binding partners or downstream signaling nodes to facilitate persistent signal transduction. Also, constitutive activation may lead to enhanced recruitment of physiologic binding partners.

In order to assess for the global signaling landscape downstream of mutated JAK2-kinase, we used in-depth phosphoproteomic analysis.[4] This data provides a first unbiased and global view on relevant downstream effectors of mutated JAK2. This global view on JAK2-regulated proteins identified *bona fide* JAK2 targets such as STAT5, STAT3, and Proto-oncogene serine/threonine-protein kinase (PIM), kinase motifs of glycogen synthase kinase-3 (GSK3), and cyclin-dependent kinases (CDKs). Among the most highly enriched cellular processes in *JAK2*-mutated cells, gene ontology analysis revealed 'mRNA splicing and processing.' This novel finding is of major interest as mutations affecting members of the spliceosome machinery have been identified in advanced phases of MPN[5] and specific mutations such as *Serine And Arginine Rich Splicing Factor 2* (*SRSF2*) or *U2 Small Nuclear RNA Auxiliary Factor 1* (*U2AF1*) are prognostically relevant and therefore incorporated in advanced scoring systems. Recently, seminal publications have shown how modulation of splicing catalysis can facilitate therapeutic targeting of myeloid malignancies harboring mutations of spliceosome factors.[6] In murine models of myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML), they provide evidence that *SRSF2* mutations, which occur always heterozygous in human MDS or AML, rely on the wildtype allele for survival. Pharmacologic perturbation of the spliceosome machinery revealed that *SRSF2*-mutated cells appear more sensitive than non-mutated cells. Our findings add another layer of complexity, highlighting the hijacking of the spliceosome machinery through post-translational modification by an oncogenic kinase rather than mutation of the splicing factor itself. We have identified phosphorylation of splicing factor Y-box Protein 1 (YBX1) by mutated JAK2 as a critical downstream event that is essentially required for mRNA splicing of the ERK-signaling component Mitogen-activated Protein Kinase Interacting Serine/Threonine Kinase 1 (MKNK1). MKNK1 in turn is required for maintenance of the *JAK2*-mutated cells during JAK-inhibitor treatment and mediates a rebound of ERK-phosphorylation despite JAK-inhibition (Figure 1). This has

CONTACT Florian H. Heidel florian.heidel@uni-greifswald.de Innere Medizin C, Universitätsmedizin Greifswald, Greifswald 17475, Germany.

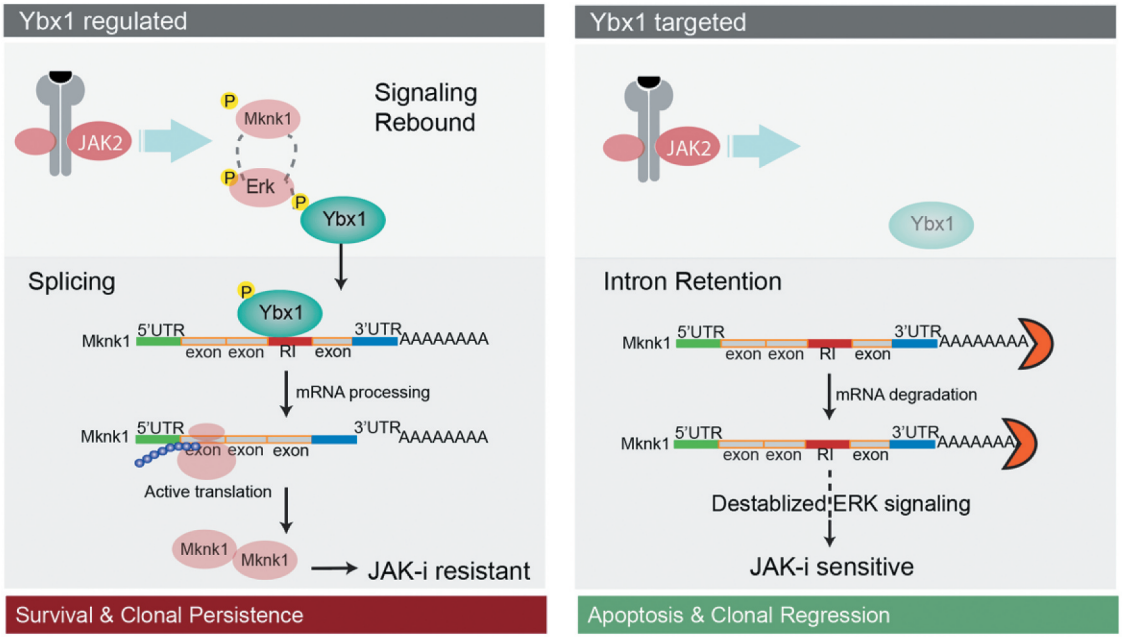

Figure 1. Mechanisms of rebounding mitogen-activated protein kinase interacting serine/threonine kinase 1 (MKNK1) extracellular signal-regulated kinase (ERK) signaling and persistence of Janus-kinase 2 (JAK2)-mutated MPN cells.

exposed a major regulatory cell-intrinsic mechanism of disease persistence.

Recent studies by other groups have highlighted the role of ERK-signaling in *JAK2*-mutated cells stimulated by cell-extrinsic factors, such as Platelet-derived Growth Factor Receptor (PDGFR) signaling, *in vivo* that eventually maintained ERK activation.[7] While this study focused on bona fide signaling pathways demonstrated by Western Blotting, the results indicate that also extrinsic stimuli may contribute to the maintenance of *JAK2*-mutated cells.

While direct and specific inhibitors of YBX1 are not available for clinical use so far, identification of relevant protein domains using genetic screens are clearly warranted. Both studies identifying a role of ERK signaling in the persistence of *JAK2*-mutated MPN clones have confirmed the efficacy of dual JAK2-ERK targeting in primary murine and human cells. These findings establish a novel therapeutic principle for *JAK2*-mutated neoplasms and a strong experimental rationale for incorporating the JAK2-YBX1 mediated regulation of MKNK1-dependent ERK signaling into therapies directed at the elimination of the persistent *JAK2*-mutated clone.

Other strategies investigated to target the persistence of *JAK*-mutated MPN clones include immunotherapeutic approaches[8] and Mouse Double Minute 2 Homologue (MDM2)-inhibition,[9] among others.

Most notably, several of these preclinical findings are currently validated in early (phase 1b/2) clinical trials, testing MDM2-inhibition, MEK-ERK targeting, and checkpoint inhibitor treatment in combination with JAK1/2-inhibitor therapy.[10] These pick-the-winner concepts may help to identify combinations with disease-modifying potential to reduce disease burden and induce durable remissions.

Disclosure of potential conflicts of interest

F.H.H. has served as an advisory board member for and received research funding from Novartis, Celgene and CTI.

Funding

This work was supported by grants of the German Research Council (DFG) to F.P. [PE-3217/1-1] and F.H.H. [HE6233/4-1 and 4-2].

ORCID

Florian H. Heidel http://orcid.org/0000-0003-2438-1955

References

1. Perner F, Perner C, Ernst T, Heidel FH. Roles of JAK2 in aging, inflammation, hematopoiesis and malignant transformation. Cells. 2019;8:8. doi:10.3390/cells8080854.
2. Deininger M, Radich J, Burn TC, Huber R, Paranagama D, Verstovsek S. The effect of long-term ruxolitinib treatment on JAK2p.V617F allele burden in patients with myelofibrosis. Blood. 2015;126(13):1551–1554. doi:10.1182/blood-2015-03-635235.
3. Kaelin WG Jr. The concept of synthetic lethality in the context of anticancer therapy. Nat Rev Cancer. 2005;5(9):689–698. doi:10.1038/nrc1691.
4. Jayavelu AK, Schnoder TM, Perner F, Herzog C, Meiler A, Krishnamoorthy G, Huber N, Mohr J, Edelmann-Stephan B, Austin R, et al. Splicing factor YBX1 mediates persistence of JAK2-mutated neoplasms. Nature. 2020;588(7836):157–163. doi:10.1038/s41586-020-2968-3.
5. Grinfeld J, Nangalia J, Baxter EJ, Wedge DC, Angelopoulos N, Cantrill R, Godfrey AL, Papaemmanuil E, Gundem G, MacLean C, et al. Classification and personalized prognosis in myeloproliferative neoplasms. N Engl J Med. 2018;379(15):1416–1430. doi:10.1056/NEJMoa1716614.

6. Lee SC, Dvinge H, Kim E, Cho H, Micol JB, Chung YR, Durham BH, Yoshimi A, Kim YJ, Thomas M, et al. Modulation of splicing catalysis for therapeutic targeting of leukemia with mutations in genes encoding spliceosomal proteins. Nat Med. 2016;22(6):672–678. doi:10.1038/nm.4097.
7. Stivala S, Codilupi T, Brkic S, Baerenwaldt A, Ghosh N, Hao-Shen H, Dirnhofer S, Dettmer MS, Simillion C, Kaufmann BA, et al. Targeting compensatory MEK/ERK activation increases JAK inhibitor efficacy in myeloproliferative neoplasms. J Clin Invest. 2019;130:1596–1611. doi:10.1172/JCI98785.
8. Prestipino A, Emhardt AJ, Aumann K, O'Sullivan D, Gorantla SP, Duquesne S, Melchinger W, Braun L, Vuckovic S, Boerries M, et al. Oncogenic JAK2(V617F) causes PD-L1 expression, mediating immune escape in myeloproliferative neoplasms. Sci Transl Med. 2018;10:429. doi:10.1126/scitranslmed.aam7729.
9. Mascarenhas J, Lu M, Kosiorek H, Virtgaym E, Xia L, Sandy L, Mesa R, Petersen B, Farnoud N, Najfeld V, et al. Oral idasanutlin in patients with polycythemia vera. Blood. 2019;134(6):525–533. doi:10.1182/blood.2018893545.
10. Perkins AC, Burbury K, Lehmann T, Ross DM, Reiter A, Gupta V, Harrison C, Kiladijan JJ, Vannucchi A, Wodergem M, et al. Adore: a randomized, open-label, phase 1/2 open-platform study evaluating safety and efficacy of novel ruxolitinib combinations in patients with myelofibrosis. Blood (62nd ASH Annu Meet Abstr). 2020;136.