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Splicing the innate immune signalling in leukaemia

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Abstract

Components of the spliceosome are frequently mutated in haematopoietic malignancies. Identification of mis-spliced genes promoting transformation will uncover novel targeted therapies. Now, a long isoform of IRAK4 is shown to be upregulated in a subset of acute myeloid leukaemia patients, conferring susceptibility for IRAK4 inhibition therapy.

Recent transcriptomic mapping of a number of cancer types revealed that physiological gene splicing patterns are frequently altered in malignant cells¹. Consistent with this notion, recent studies have identified frequent mutations in genes—components of the basic RNA splicing machinery—that are particularly prevalent in haematopoietic malignancies². Indeed, a significant number of people afflicted with myeloid neoplasms and malignancies carry such mutations, which alter spliceosome machinery function. These include somatic, non-overlapping mutations on SF3B1, SRSF2, U2AF1 and ZRSR2, all essential components of the core spliceosome. These findings have led to the discovery of novel mechanisms for malignant transformation, revealing unknown cancer dependencies and introducing potential new therapeutic interventions directly targeting the spliceosome³. Despite these studies, our knowledge of the contribution of altered splicing genes to cancer pathogenesis is still limited. Indeed, it is currently not known whether there are additional mechanisms of regulation for RNA splicing in cancer that do not involve the acquisition of somatic mutations targeting the spliceosome⁴. Also, despite a number of elegant studies using animal modelling and pharmacologic targeting, there is only scarce mechanistic data on the roles of aberrantly spliced genes and the proteins that they encode in leukaemia.

In this issue of *Nature Cell Biology*, Smith et al., have addressed this last question, focusing on oncogenic signalling dependencies created by aberrant RNA processing in both myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML)⁵. This elegant study proposes a subtype of MDS and AML that accumulates novel mRNA isoform changes in innate immune pathway genes. Particularly, the authors identify a long isoform of the interleukin-1 receptor associated kinase 4 (IRAK4-L) overexpressed in a subset of myeloid neoplasms. Indeed, human AML primary cells and cell lines express IRAK4-L, encoding a longer protein isoform, whereas normal stem cells and differentiated myeloid cells predominantly express the shorter IRAK4-S version of the protein. The authors further

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Competing interests

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demonstrate that specific inhibition of IRAK4L abrogates leukaemia growth in vitro and in vivo, suggesting IRAK4 targeting as a future therapy option in MDS and AML.

MDS is a condition characterized by peripheral blood cytopenia and abnormal cellular maturation accompanied by an increased risk of progression to AML⁶. AML is an aggressive haematologic malignancy characterized by a high rate of relapse, absence of targeted therapies and complex subclassification⁷. Given the recurrent and frequent mutations in splicing factor genes in both neoplasms, the authors interrogated whether aberrant alternative splicing defines molecular subsets of AML by analysing the differential RNA isoform variants of 160 AML samples using The Cancer Genome Atlas (TCGA). Based on the pattern of expression of those genes showing mutually exclusive isoforms, and therefore alternative expression programs, the authors identified three subgroups of AML that correlate with distinct clinical outcomes. Remarkably, the poor-prognosis group showed enrichment of isoforms of genes implicated in innate immune response and activation of the NF- κ B pathway. The authors go on to connect this innate and inflammatory signalling activation to the AML-specific overexpression of IRAK4-L. IRAK4 is a kinase that transduces signalling downstream of toll-like receptors (TLRs) and interleukin 1 receptor (IL1R), interacting with key additional signalling components including MYD88, forming active myddosomes leading to activation of pro-inflammatory gene expression programs⁸.

Briefly, upon TLR receptor stimulation by their ligands, the adaptor protein MYD88 recruits IRAK4 through its death domain, initiating a cascade that results in TRAF6-mediated activation of NF- κ B and MAPK signalling. These signalling events trigger inflammatory response pathways. In agreement with the notion that IRAK4-L participates actively in these events, the authors performed a series of transcriptional and biochemical experiments demonstrating that AML samples expressing IRAK4-L show stronger induction of NF- κ B transcription factor activity and inflammatory response genes. On the other hand, in non-transformed cells expressing IRAK4-S, MAPK pathways are predominantly activated, as suggested by the phosphorylation of p38 and JNK. Moreover, the authors validated the oncogenic relevance of IRAK4-L isoforms in myeloid malignancies, as genetic suppression of IRAK4-L expression resulted in a lower number of transformed colonies in vitro and increased expression of monocytic pathway genes, indicating induced differentiation of AML cells and a lower tumour burden in in vivo experiments. Importantly, all these functions depend on the IRAK4-L kinase activity, as a selective IRAK4 inhibitor suppressed tumorigenic activity both in vitro and in vivo, including xenograft studies. It will be exciting to test this inhibitor, a compound currently approved for human clinical use, in a wide variety of MDS/AML animal models and primary xenografts, especially as the authors show that primary human CD34⁺ stem and progenitor cells are not affected by IRAK4 activity inhibition. Obviously, such experiments have potential caveats, including the fact that the human innate immune system is constantly stimulated by a constellation of TLR agonists, and IRAK4-targeting compounds could affect the patient's ability to keep bacterial and viral infections in check.

Finally, Starczynowski and colleagues⁵ sought to determine the genetic alterations that lead to aberrant IRAK4-L splicing. Analysing the TCGA data set, they found that mutations in the U2 small nuclear RNA auxiliary factor 1 (U2AF1) tightly correlate with the inclusion of

the IRAK-4 exon 4. Further experiments demonstrated that overexpression of mutant U2AF1-S34F resulted in increased reads of IRAK4 exon 3–4 junctions and induction of NF- κ B pathway gene signatures. Moreover, the authors explored whether inhibition of IRAK4 directly abrogates tumourigenesis of U2AF1-mutated leukaemia. To this end, they generated myeloid leukaemia lines that overexpress wild-type U2AF1 or the mutant form (U2AF1-S34F) and confirmed that IRAK4 inhibitors preferentially decrease NF- κ B signalling and result in toxicity in mutant cells, but not wild-type cells. Importantly, similar results were obtained using primary patient-derived cells carrying U2AF1 mutations, in which inhibition of IRAK4 enhanced differentiation, although such effects were not seen in CD34⁺ stem and progenitor cells. Supporting these findings, patient xenografts carrying U2AF1 mutations treated with IRAK4 inhibitors showed decreased tumour burden, indicating that U2AF1 mutant AML cells are preferentially sensitive to IRAK4 inhibition.

Overall, this is an exciting piece of work (Fig. 1) that focuses on how differential splicing of a single gene (IRAK4 in this case) results in novel oncogenic dependencies in leukaemia. Most importantly, these studies introduce us to a bold new world of interactions between the innate immune system, pathogens/TLR stimuli and the mechanism of transformation in leukaemia. Although previous reports have suggested that specific innate signalling mediators are highly expressed in myeloid malignancies⁹, it remains unclear how the activation of innate immune pathways is involved in promoting or maintaining leukaemogenesis. One should not forget that the key TLR signalling transducer MYD88 was initially cloned as a myeloid differentiation factor¹⁰, underscoring the intimate connection between these processes. Moreover, recent data have shown that haematopoietic stem cells (HSC) and progenitor cells sense and respond to systemic infection^{11,12}. It is thus tempting to propose that the very same pathways that activate the innate immune response also regulate HSC proliferation and differentiation and that fine tuning of the innate system through IRAK4 inhibitors, TLR agonists and antagonists could be used as differentiation therapy for selected types of hematological neoplasms.

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Fig. 1 |. Differential splicing of IRAK4 leads to a specific long isoform that can be therapeutically targeted.

a, Normal CD34⁺ stem and progenitor cells preferentially express a short isoform of IRAK4 (IRAK4-S), which promotes stronger activation of MAPK signalling than the NF-κB pathway. **b**, Mutations in U2AF1 lead to predominant expression of a long isoform of IRAK4 (IRAK4-L). The N-terminal domain retained in IRAK4-L interacts with the death domain of MYD88, facilitating the assembly of the myddosome complex, which mediates IRAK4 autotransphosphorylation and NF-κB maximal activation. Treatment with IRAK4 inhibitors decreases NF-κB signalling and promotes the differentiation of AML cells in vitro, significantly reducing the tumour burden in in vivo experiments. Ex, exon.