MOLECULAR & CELLULAR ONCOLOGY
2018, VOL. 5, NO. 3, e1458014 (3 pages)
https://doi.org/10.1080/23723556.2018.1458014

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AUTHOR'S VIEWS

JAK3 mutations and HOXA9 expression are important cooperating events in T-cell acute lymphoblastic leukemia

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ABSTRACT
Sequencing data from large cohorts of T-cell acute lymphoblastic leukemia patients identified a significant association between the presence of *JAK3* mutations and ectopic *HOXA9* expression. Mouse models using a constitutive or novel inducible retroviral expression vector to express the *JAK3(M511I)* mutant and *HOXA9* led to the development of an aggressive leukemia in vivo, with shorter latency than *JAK3(M511I)* or *HOXA9* alone. This was primarily due to the co-binding of STAT5 and HOXA9 to the same genomic loci leading to increased oncogenic JAK-STAT signaling.

ARTICLE HISTORY
Received 13 March 2018
Revised 13 March 2018
Accepted 13 March 2018

KEYWORDS
signaling; transcription factor; leukemia; retroviral vector

Normal T-cell proliferation and development in the thymus requires the coordinated and sequential activation of specific growth factor signaling pathways including the PI3K/AKT (Phosphoinositide 3-kinase/AKT kinase) signaling pathway and the JAK/STAT (Janus kinase/signal transducer and activator of transcription) signaling pathway. The thymus also provides DLL4 (delta-like ligand 4) that engages NOTCH1 (Notch homolog 1, translocation-associated) to trigger the proteolytic release of its cytoplasmic domain that itself becomes a transcriptional co-activator.[1] When these signaling pathways are constitutively activated, normal T-cell development is perturbed and the accumulation of additional mutations can then eventually result in the development of T-cell acute lymphoblastic leukemia (T-ALL).[2]

Next generation sequencing efforts on large cohorts of T-ALL cases have now given us unprecedented resolution on the variety of mutations that potentially lead to this persistent signaling activation. These include activating mutations within *JAK1, JAK3* or *IL7R* (Interleukin-7 receptor) that lead to ligand independent activation of STAT5; inactivating mutations of *PTEN* (phosphatase and tensin homolog) that leads to PI3K/AKT signaling pathway activation; and a high frequency of *NOTCH1* mutations. In addition, T-ALL is also characterized by the mutually exclusive expression of transcription factors including *TAL1* (TAL bHLH transcription factor 1), *TLX1* (T cell leukemia, homeobox 1), *TLX3* (T cell leukemia, homeobox 3), *HOXA9* (Homeobox A9), *LMO2* (LIM domain only 2) and *NKX2-1* (NK2 homeobox 1), often the consequence of chromosomal rearrangements.[2] Recent analysis has shown associations between specific signaling pathway mutations and the expression of individual transcription factors.[3,4]

We hypothesized that these specific mutation combinations are likely to complement one another in driving leukemia development. To test this hypothesis, we focused on *JAK3* mutations, which are present in about 16% of T-ALL cases.[5] The analysis of two independent T-ALL cohorts revealed a strong positive association of *JAK3* mutations with *HOXA9* expression and, conversely, a strong negative association with *TAL1* expression.[6] These positive and negative associations were then modelled using a bone marrow transplant model to determine their impact on leukemia disease initiation and progression. The co-expression of *JAK3(M511I)* with *HOXA9* led to a rapid and aggressive clonal outgrowth of double positive clones with mice succumbing to leukemic disease within 40 days and was significantly shorter when compared to *JAK3(M511I)* (Median DFS = 150 days) or *HOXA9* only (median DFS = 182 days).[6] In contrast, co-expression of *JAK3(M511I)* with *TAL1* had a negative effect on leukemia development, reconciling with the negative association found in T-ALL cases and with our recent observation that TAL1 negatively regulates the IL7R/JAK/STAT signaling pathway.[7]

However, unlike the *JAK3(M511I)* driven leukemia that leads to the generation of a CD8 (cluster of differentiation 8) positive T-cell leukemia,[5] co-expression of *JAK3(M511I)* and *HOXA9* led to the development of a mixed myeloid and lymphoid leukemia likely due to the constitutive expression of both transgenes in multipotent hematopoietic progenitors.[6] Transgenic mouse lines with tissue specific promoters have been traditionally used for lineage specific expression (e.g. Lck-*TLX1*[8]) but remain time consuming and expensive. Therefore, we optimized the bone marrow transplant system by designing a novel CRE (Cre Recombinase)-inducible retroviral vector to obtain lymphoid-specific expression of the oncogenes. This novel retroviral vector included antiparallel LoxP (lox67/lox71) sites flanking the oncogenes cloned in the antisense direction. These retroviral vectors could then be used to transduce hematopoietic stem and progenitor cells isolated from the many well characterized transgenic Cre mice (e.g. CD2-Cre, Lck-Cre, CD4-Cre) for the controlled expression of the transgene at defined stages of T-cell development. Using a CD2-Cre mouse

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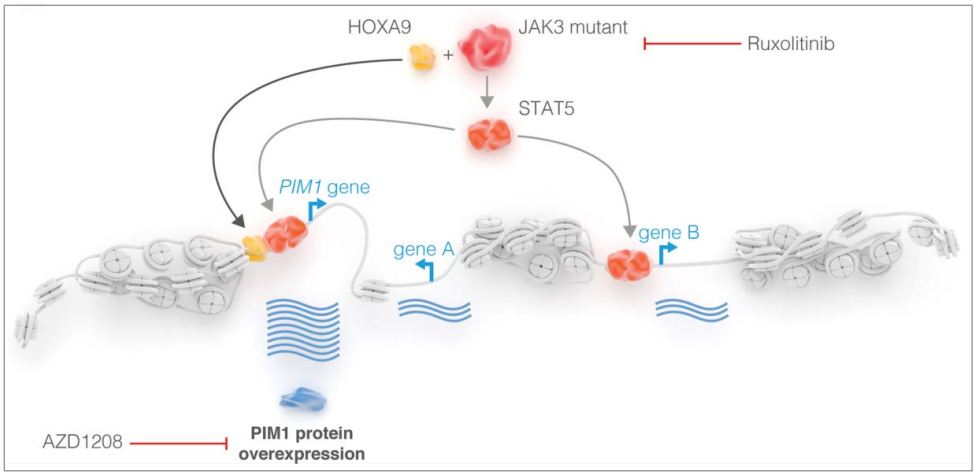

Figure 1. Mechanisms of transcriptional cooperation between STAT5 and HOXA9. Mutations in the JAK3 kinase lead to the constitutive activation of the transcription factor STAT5 that activates many genes implicated in proliferation and survival. The ectopic expression of the transcription factor HOXA9 co-binds with STAT5 to increase oncogenic JAK-STAT signaling including increasing the expression of the serine/threonine kinase PIM1. Therapeutically, this finding was exploited by using a combination of a JAK inhibitor (ruxolitinib) with a PIM1 inhibitor (AZD1208) to effectively decrease leukemia burden.

as a donor for the bone marrow transplant experiments, we were able to delay the expression of *JAK3(M511I)* and *HOXA9* to the lymphoid progenitor stage and avoid myeloid expansion.[6] Moreover, this novel inducible system has the advantage that engraftment of transduced stem cell is not compromised by expression of the transgene.

Our current study then identified that the addition of HOXA9 amplified the JAK-STAT signaling pathway and this was primarily through HOXA9 binding to the same genomic loci as STAT5.[6] Using ChIP-seq (Chromatine immunoprecipitation followed by sequencing), we detected the majority of HOXA9-STAT5 co-binding at gene promoters, which was associated with increased expression. One of these genes was *PIM1* (Pim-1 proto-oncogene), which was bound by both STAT5 and HOXA9 and also significantly upregulated in both the murine T-ALL model and human HOXA9/JAK3 mutant positive T-ALL cases. PIM1 is a serine/threonine kinase that is known to be important in a variety of hematological malignancies and for which small molecule inhibitors are under development.[9] We show that a combination of a JAK inhibitor (ruxolitinib) with a PIM1 inhibitor (AZD1208) was more effective in decreasing the leukemia burden of a patient derived T-ALL xenograft model than either agent alone. These data illustrate the potential use of PIM1 kinase inhibitors for the treatment of a subset of T-ALL cases (Figure 1).

Additional work will now be necessary to determine whether the observed cooperation between STAT5 and HOXA9 can be exploited further. For instance, it is unknown whether the combination of HOXA9 and STAT5 is dependent on alternative transcription machinery at the promoters of key regulated genes. Such dependency was recently described for the interaction between MYB (MYB proto-oncogne) and TAF12 (TATA-box binding protein associated factor 12) that when prevented led to the regression of acute myeloid leukemia in mice.[10] A greater understanding of the higher order protein complexes at STAT5-HOXA9 co-bound sites and whether this differs from STAT5 would be informative and potentially leveraged for therapeutic gain.

Disclosure of potential conflicts of interest

No potential conflicts to disclose

Funding

This work was supported by grants from KU Leuven (PF/10/016 SymBioSys), FWO-Vlaanderen, Foundation against Cancer, Swiss Bridge Foundation and an ERC-consolidator grant

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References

1. Yui MA, Rothenberg EV. Developmental gene networks: a triathlon on the course to T cell identity. Nat Rev Immunol. 2014;14:529–45. doi:10.1038/nri3702. PMID:25060579
2. Girardi T, Vicente C, Cools J, De Keersmaecker K. The genetics and molecular biology of T-ALL. Blood. 2017;129:1113–23. doi:10.1182/blood-2016-10-706465. PMID:28115373
3. Liu Y, Easton J, Shao Y, Maciaszek J, Wang Z, Wilkinson MR, McCastlain K, Edmonson M, Pounds SB, Shi L, et al. The genomic landscape of pediatric and young adult T-lineage acute lymphoblastic leukemia. Nat Genet. 2017;63:5329.
4. Vicente C, Schwab C, Broux M, Geerdens E, Degryse S, Demeyer S, Lahortiga I, Elliott A, Chilton L, La Starza R, et al. Targeted sequencing identifies association between IL7R-JAK mutations and epigenetic modulators in T-cell acute lymphoblastic leukemia. Haematologica. 2015;100:1301–10. doi:10.3324/haematol.2015.130179. PMID:26206799
5. Degryse S, de Bock CE, Cox L, Demeyer S, Gielen O, Mentens N, Jacobs K, Geerdens E, Gianfelici V, Hulselmans G, et al. JAK3 mutants transform hematopoietic cells through JAK1 activation, causing T-cell acute lymphoblastic leukemia in a mouse model. Blood. 2014;124:3092–100. doi:10.1182/blood-2014-04-566687. PMID:25193870
6. de Bock CE, Demeyer S, Degryse S, Verbeke D, Sweron B, Gielen O, Vandepoel R, Vicente C, Vanden Bempt M, Dagklis A, et al. HOXA9 cooperates with activated JAK/STAT signaling to drive leukemia development. Cancer Discov. 2018;(In press). doi: 10.1158/2159-8290.CD-17-0583. PMID:29496663

7. Bornschein S, Demeyer S, Stirparo R, Gielen O, Vicente C, Geerdens E, Ghesquière B, Aerts S, Cools J, de Bock CE. Defining the molecular basis of oncogenic cooperation between TAL1 expression and Pten deletion in T-ALL using a novel pro-T-cell model system. Leukemia. 2018;32:941–51. doi:10.1038/leu.2017.328.
8. De Keersmaecker K, Real PJ, Gatta GD, Palomero T, Sulis ML, Tosello V, Van Vlierberghe P, Barnes K, Castillo M, Sole X, et al. The TLX1 oncogene drives aneuploidy in T cell transformation. Nat Med. 2010;16:1321–7. doi:10.1038/nm.2246. PMID:20972433
9. Brault L, Gasser C, Bracher F, Huber K, Knapp S, Schwaller J. PIM serine/threonine kinases in the pathogenesis and therapy of hematologic malignancies and solid cancers. Haematologica. 2010;95:1004–15. doi:10.3324/haematol.2009.017079. PMID:20145274
10. Xu Y, Milazzo JP, Somerville TDD, Tarumoto Y, Huang Y-H, Ostrander EL, Wilkinson JE, Challen GA, Vakoc CR. A TFIID-SAGA Perturbation that Targets MYB and Suppresses Acute Myeloid Leukemia. Cancer Cell. 2018;33:13–8. doi:10.1016/j.ccell.2017.12.002. PMID:29316427