Dangerous fusions: a path to cancer for arrested lymphoid progenitors

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B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is a common malignancy associated with variable chromosomal translocations, leading to fusion proteins of unknown function. To investigate how such translocations contribute to the development of BCP-ALL, Smeenk *et al* (2017) generated mouse models for Pax5 fusion proteins. The results show that a PAX5 fusion is required for BCP-ALL development by preventing B-cell differentiation and retaining cells in an arrested progenitor stage. The occurrence of further genetic aberrations eventually results in oncogenic transformation and proliferation of the arrested cells, triggering the onset of leukemia.

See also: L Smeenk *et al* (March 2017)

B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most common malignancy in children, and adults are also often diagnosed with this disease. Despite good prognosis in pediatric patient outcomes, at least 15% of patients suffer from relapse (Locatelli *et al*, 2012). Therefore, a deep understanding of the molecular mechanisms underlying the onset of this disease is needed to improve the current therapeutic approaches.

The commitment into the B-cell lineage and the subsequent cell fate decisions are regulated through different checkpoints that involve a complex transcriptional regulation. The paired box domain gene 5 (PAX5) is one of the crucial transcription factors required for maintaining B-cell identity and for further development. PAX5 is considered to be a haploinsufficient tumor suppressor in BCP-ALL, as heterozygous deletions and

loss-of-function mutations are detected in 30% of BCP-ALL (Mullighan *et al*, 2007). Moreover, chromosomal translocations of PAX5 and various partners including JAK2, ETV6, FOXP1, ZNF521, KIDINS220, and ELN have been identified in about 2.5% of BCP-ALL (Nebral *et al*, 2009). These BCP-ALL cases often show genetic aberrations affecting one allele of PAX5 while retaining the second allele functional, most likely leading to reduced PAX5 expression. Since the affected allele is involved in chromosomal translocations leading to an abnormal fusion protein, an important question is whether this fusion product itself is involved in the initiation or maintenance of the disease.

Among the interesting chimeric fusions are PAX5-ETV6 and PAX5-FOXP1, which comprise not only the DNA-binding paired box domain of PAX5 but also almost the entire coding sequence of ETV6 or FOXP1 transcription factors, as well as an oligomerization motif (Cazzaniga *et al*, 2001; Coyaudo *et al*, 2010). So far, these fusions have mainly been examined using *in vitro* systems relying on transient overexpression in cell lines. These studies suggested that a dominant-negative function of the fusion proteins interfered with wild-type PAX5, thereby affecting survival pathways (Kawamata *et al*, 2012). However, the function of these fusion proteins in the pathogenesis of ALL *in vivo* remained unclear.

The generation of animal models for Pax5 mutations may help understand their function during differentiation of B-cell progenitors and in the development of ALL. In fact, these models revealed that complete loss of Pax5 results in an early block of B-cell differentiation, while conditional Pax5

deficiency induced dedifferentiation of mature B cells into uncommitted progenitors and was associated with aggressive tumors (Cobaleda *et al*, 2007). Moreover, haploinsufficiency of Pax5 was shown to synergize with the activation of signal transducer and activator of transcription 5 (Stat5) in promoting ALL development (Heltemes-Harris *et al*, 2011). Now, the work of Smeenk *et al* (2017) provides novel knock-in mouse models for two Pax5 fusions, namely Pax5-Etv6 and Pax5-Foxp1. This is the first study to describe the mode of action of the resulting fusion proteins in B-cell development and to highlight the additional factors that promote the leukemogenesis *in vivo*.

Interestingly, neither heterozygous loss of Pax5 nor the individual expression of only the DNA-binding paired box domain of Pax5 influenced B-cell development. However, the heterozygous expression of the Pax5 fusion proteins Pax5-Etv6 and Pax5-Foxp1 led to a block in B-cell development at the transition from the pro-B to pre-B-cell stage, and a slight increase in the cell number of early B cells in comparison with control mice. This implies that the fusion proteins of Pax5 possess a distinct transcriptional activity that interferes with B-cell development. However, the developmental blockage alone was not sufficient to drive BCP-ALL, indicating that acquisition of synergistic cooperating mutations enhancing proliferation and/or survival is required for initiation or maintenance of malignant transformation. In human BCP-ALL, the generation of PAX5-ETV6 is known to co-occur with the loss of one allele of the tumor suppressor CDKN2A/B locus (Strehl *et al*, 2003). In full agreement,

Figure 1. Developmental arrest as the first step in leukemogenesis.

During normal B-cell generation, hematopoietic stem cells (HSC) give rise to progenitors that pass through steps of lineage commitment and differentiation to eventually become mature B cells. Chromosomal translocations that result in fusion proteins such as *PAX5-ETV6* block B-cell differentiation and lead to a distinct population of early B cells with limited survival capacity under normal conditions. The occurrence of secondary mutations affecting tumor suppressors or oncogenes enhances proliferation or survival and initiates BCP-ALL.

the mouse model shows that the Pax5-Etv6 fusion protein synergizes with the heterozygous loss of Cdkn2a/b in promoting BCP-ALL, which was more aggressive under the complete loss of Cdkn2a/b. On the other hand, impairment of Cdkn2a/b expression did not lead to leukemia progression in Pax5-Foxp1 knock-in mice, suggesting that the secondary events driving malignancy in human BCP-ALL are regulated differently in the case of PAX5-FOXPI fusion.

Altogether, the chromosomal alterations affecting Pax5 seem to act as first events for hematological malignancies by generating arrested lymphoid progenitors or “leukemia-initiating cells”. These cells are kept in a “ready-to-go” state until acquiring secondary events (Fig 1), which may help “arrested cells” to proliferate and survive. Identification of these events is of great importance, as it allows a better understanding of malignant transformation and leukemogenesis. Crossing the knock-in mice generated by

Smeenk *et al* (2017) with mouse strains deficient for genes important for B-cell differentiation or function could represent one approach to identify additional targets for secondary events. Moreover, introducing an experimental system for random mutagenesis into the knock-in mice carrying Pax5 fusions may help identify novel mutations for BCP-ALL development. For instance, transposon-based insertional mutagenesis has been used to screen for novel secondary mutations (van der Weyden *et al*, 2011). Identifying novel mutations or commonly altered pathways during leukemic evolution is important not only for understanding the mechanism of malignant transformation but also for the development of novel therapeutic strategies.

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