Cell Research (2012) 22:948-949.

www.nature.com/cr

RESEARCH HIGHLIGHT

MLL-AF4 driven leukemogenesis: what are we missing?

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Cell Research (2012) **22**:948-949. doi:10.1038/cr.2012.16; published online 31 January 2012

Pre-leukemic MLL-AF4 fusions arise prenatally and typically lead to overt acute lymphoblastic leukemia (ALL) at or shortly after birth. In a recent study, Bueno and colleagues explored the effects of MLL-AF4 expression in human embryonic stem cells (hESCs), with a focus on early hemato-endothelial development.

Infant pro-B acute lymphoblastic leukemia (ALL) harboring MLL-AF4 fusion proteins instigated by chromosomal translocation t(4;11), represents an aggressive, high-risk type of childhood leukemia, characterized by a very brief disease latency and undisputedly originates *in utero*. Despite of recent advances and multiple important breakthroughs, MLL-AF4-driven leukemogenesis remains difficult to model and mouse models that accurately recapitulate the disease phenotype and latency are still lacking. Among several remarkable studies recently published, Montes *et al*. [1] demonstrated that enforced expression of MLL-AF4 in cord blood-derived hematopoietic stem cells (HSCs) increased the clonogenic potential of $CD34^+$ progenitors and promoted proliferation, but appeared insufficient to induce leukemia. This study undeniably questions whether MLL-AF4 fusion proteins are capable of driving leukemogenesis on their own or whether additional genetic events are required such as *RAS* mutations [2]. However, recent whole genome sequencing analysis in primary *MLL*-rearranged infant ALL samples revealed the presence of remarkably few somatic mutations [3]. Contributing to the complexity of the matter, Bursen *et al*. [4] recently showed that introducing the reciprocal fusion protein AF4-MLL (resulting from the same balanced t(4;11) translocation), but not MLL-AF4, into murine hematopoietic stem/progenitor cells induced ALL in mice without the requirement of MLL-AF4. Nonetheless, these experiments have not yet been performed in human HSCs. Moreover, MLL-AF4 and AF4-MLL knockdown experiments have shown that t(4;11)-positive cell lines display addiction to MLL-AF4 (which appeared essential for leukemic cell proliferation and survival), but not to AF4-MLL [5]. Thus, the AF4-MLL fusion protein may well be important or essential in the early transformation process and the MLL-AF4 fusion is certainly required for the maintenance of the leukemia. However, although the studies by Montes *et al*. [1] and Bursen *et al*. [4] seem to support that MLL-AF4 by itself is not sufficient to induce leukemogenesis in HSCs, others were able to induce lymphoid leukemias using MLL-AF4 knockin models in mice [2, 6]. Yet, in these latter studies both the disease phenotye and latency of the observed leukemias appeared to deviate from the highly immature pro-B cell phenotype characteristically found in humans.

While the above described contradictions make it difficult to draw solid conclusions on the oncogenic potential of the MLL-AF4 fusion protein itself, there is another important question to be asked and answered: Are these MLL-AF4-driven leukemogenesis studies targeting the right cells? In this issue of *Cell Research*, Bueno *et al*. [7] elegantly attempted to address this question by creating a human-specific cellular system to study early hemato-endothelial development in MLL-AF4-expressing human embryonic stem cells (hESCs). A recent report showed that bone marrow-derived mesenchymal stem cells from primary t(4;11)-positive pro-B infant ALL patients harbor and express the MLL-AF4 fusion gene [8]. Thus, MLL-AF4 may well arise prenatally in pre-hematopoietic mesodermal or hemangioblastic precursors sprouting from differentiating hESCs [9] rather than in more committed HSCs. From this perspective, Bueno *et al*. [7] introduced MLL-AF4 expression in hESCs and monitored the consequences. Interestingly, enforced MLL-AF4 expression in hESCs led to the accelerated emergence and elevated frequencies of hemogenic precursors. Moreover, in these hESCs, MLL-AF4 appeared to act as a global transcriptional activator, positively regulating homeobox gene expression, which is in line with what is usually (but

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not always) observed in primary t(4;11)-positive infant ALL samples [10]. Nevertheless, the MLL-AF4 fusion protein was not able to transform hESC-derived hematopoietic cells, but instead strongly impaired subsequent hematopoietic commitment in favor of an endothelial cell fate. Unfortunately, the latter brings us back to the same persistent conundrum: In order to successfully induce MLL-AF4-positive leukemia, did this system adopted by Bueno *et al*. require additional genetic hits such as the presence of the AF4-MLL fusion or *RAS* mutations? Or, despite the relevant rationale behind targeting hESC-derived pre-hematopietic precursors, did these cells not reflect the correct equivalents from which t(4;11)-positive pro-B ALL in infants originate? Perhaps MLL-AF4-positive infant ALL does arise in HSCs or early HSC progenitors, but not in those obtained from cord blood or the bone marrow. Given the strong body of evidence supporting that MLL-AF4 fusions arise during embryonic development [11, 12] when hematopoiesis still mainly takes place in the liver, the correct target cells, e.g., hematopoietic or specific lymphoid-monocytic stem cells [13], should possibly be searched for in the fetal liver.

Nonetheless, the study presented by Bueno *et al*. [7] provides unique insights into how MLL fusions regulate human embryonic hematopoietic specification and represents an intriguing experimental system to study the impact of MLL fusions from a developmental point of view. Hopefully this or similar approaches will further be exploited to unravel the riddle of MLL-AF4-driven leukemogenesis.

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