IL-4 and TAL1 in T-cell acute lymphoblastic leukemia: studies on the participation of microenvironmental cues and cell-autonomous alterations in leukemogenesis

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Abstract

Acute lymphoblastic leukemia (ALL) is the most frequent cancer found in children and results from the clonal expansion of transformed lymphoid precursors. Approximately 15% of pediatric ALL patients present with a T-cell phenotype (T-ALL). Despite the recent improvements in the treatment of T-ALL, there are still a high number of relapses and the intensive chemotherapeutic regiments used are associated with long-term severe complications. In order to develop new therapeutic strategies that can further increase efficacy while reducing side effects, one needs to better understand the pathobiology of T-ALL. In particular, it is necessary to understand how microenvironmental and cell-autonomous mechanisms influence the initiation and the progression of leukemia. The present thesis has the preocupation of exploring the mechanisms by which both an extracellular cue (IL-4) and a cell-intrinsic transcription factor (TAL1) may partake in leukemia development and maintenance.

Interleukin-4 (IL-4) is a γ-common chain cytokine produced within the bone marrow microenvironment that is known to promote the *in vitro* proliferation of T-ALL cells. In Chapter 2, we present evidence that IL-4 induces primary T-ALL cell cycle progression from G0/G1 into S and G2/M, by up-regulating cyclin D2, E and A and down-regulating the cyclin-dependent kinase inhibitor $p27^{kip1}$. Transfection of T-ALL cells with the VP22-$p27^{kip1}$ fusion protein, which is able to translocate into the cytoplasm and nucleus of target cells, abrogates IL-4-mediated proliferation. This indicates that $p27^{kip1}$ downregulation is mandatory for cell cycle progression of T-ALL cells stimulated with IL-4. Furthermore, IL-4 stimulates mTOR activation, as

determined by increased phosphorylation of its downstream targets $p70^{S6K}$, S6 and 4E-BP1. Inhibition of mTOR signaling with rapamycin prevents IL-4-induced T-ALL cell growth, cell cycle progression and proliferation. Our results identify mTOR as a critical regulator of IL-4-mediated effects in T-ALL cells and support the rationale for using mTOR pharmacological inhibitors in T-ALL therapy (Cardoso et al. *Leukemia* 2009).

The basic helix-loop-helix transcription factor TAL1 is aberrantly expressed in up to 65% of T-ALL patients. LMO2, a Lim-only domain protein, is often co-expressed ectopically with TAL1 in this malignancy. These genes appear to have leukemogenic potential, since both TAL1 and LMO2 transgenic mice develop leukemias of T-cell phenotype. However, it is still unclear whether TAL1 is effectively leukemogenic in humans, or whether merely participates as a secondary event in the transformation process in T-ALL. To address this question, we transduced hematopoietic progenitors with *TAL1* and/or *LMO2* and co-cultured them with OP9-Dll1 stromal cells, which have the capacity to induce T-cell differentiation in vitro. We found that *TAL1* and *LMO2* genes deregulate human T-cell differentiation in stromal cell co-cultures. Interestingly, the coordinated expression of both *TAL1* and *LMO2* led to a relative increase in $CD3^{+}CD4^{+}CD8^{+}$ T-cell precursors with increased cell size. This observation is particularly interesting given that TAL1-expressing patients normally display a similar phenotype. These preliminary results show that *TAL1* and *LMO2* can disrupt normal human T-cell development, therefore likely predisposing thymocytes to malignant transformation (Chapter 3).

In our effort to characterize the mechanisms by which TAL1 might promote T-cell leukemogenesis, we developed a TAL1 inducible system, by fusing TAL1 with the hormone binding domain (HBD) of the estrogen receptor (ER), which we expressed in a TAL1-negative T-cell line. Upon 4-Hydroxi-Tamoxifen (4OHT) treatment, ER-TAL1 fusion protein is able to translocate into the nucleus and consequently trigger its transcriptional program. Gene expression profiling of 4OHT-treated HPB-ALL cells stably transduced with the ER-TAL1 fusion revealed a total of 26 genes up- or down-regulated by TAL1 activation, in at least two independent experiments. We selected seven of those genes on the basis of their function/potential interest in cancer and confirmed the differential expression of three (CASZ1, DMGDH and OR5M3) by qRT-PCR. Accordingly, transfection of another TAL1-negative T-ALL cell line, P12, with TAL1, also led to increased expression of the validated TAL1 target genes. The possible

involvement of CASZ1 in TAL1-mediated anti-apoptotic and proliferative effects in T-ALL cells was subsequently investigated. Knock-down of TAL1 with siRNA in the TAL1-positive T-ALL cell line Jurkat decreased the expression of CASZ1, correlating with loss of cell viability. Moreover, CASZ1 knockdown in Jurkat cells led to functional effects similar to those of TAL1 knockdown, namely a decrease in survival and proliferation. Overall, these studies allowed the identification of three novel TAL1 downstream targets, likely with functional relevance for TAL1-mediated leukemogenic potential (Chapter 4).

TAL1 binds to repressive chromatin complexes, namely involving HDAC1, and incubation with HDAC inhibitors (HDACis) promotes apoptosis of leukemia cells derived from TAL1 transgenic mice. In Chapter 5, we evaluated the impact of HDACis on TAL1 from a somewhat different perspective, namely by analyzing their impact on TAL1 expression rather than transcriptional activity. We found that incubation of T-ALL cells with HDACis strikingly down-regulates TAL1 protein expression. This is due to decreased *TAL1* gene transcription in cells with an intact *TAL1* locus, and to impaired *TAL1* mRNA translation in cells that harbor the TAL1^{d} deletion. Importantly, HDACi-induced apoptosis of T-ALL cells is significantly reversed by TAL1 forced over-expression. Our results indicate that the HDACi-mediated apoptotic program in T-ALL cells is partially dependent on the down-regulation of TAL1 expression, and suggest that integration of HDACis into T-ALL treatment protocol may be of potential therapeutic benefit (Cardoso et al, *Leukemia* 2011, *advance online publication*).

Taken together, the results described in this thesis highlight the importance that microenviromental factors, such as IL-4, might have in the progression of T-ALL (for instance, by activating mTOR and promoting cell cycle progression), and hint on the importance that cell-autonomous factors, such as TAL1 and LMO2, may have in predisposing T-cells for malignant transformation and promoting survival of T-ALL cells. Importantly, our results further demonstrate that both extracellular cues and intracellular molecular lesions can constitute targets for therapeutic intervention in T-ALL.