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Overexpression of stem cell associated *ALDH1A1*, a target of the leukemogenic transcription factor TLX1/HOX11, inhibits lymphopoiesis and promotes myelopoiesis in murine hematopoietic progenitors

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Abstract

TLX1/HOX11 is an oncogenic transcription factor in human T-cell leukemia, however, the molecular basis for its transforming activity has remained elusive. The *ALDH1A1* gene, whose product participates in retinoic acid synthesis, was previously identified as a TLX1-responsive gene. Here, we confirm regulation of *ALDH1A1* transcription by TLX1 and show that *ALDH1A1* can profoundly perturb murine hematopoiesis by promoting myeloid differentiation at the expense of lymphopoiesis. Together, these data demonstrate that *ALDH1A1* plays a key role in normal hematopoiesis, and confirm *ALDH1A1* as a TLX1 transcriptional target that may contribute to the ability of this homeoprotein to alter cell fate and induce tumor growth.
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1. Introduction

Aldehyde dehydrogenase 1a1 (*ALDH1A1*) encodes a cytosolic enzyme whose main biological role has been reported to be the irreversible oxidation of retinal to the signalling molecule retinoic acid (RA) [1,2]. Elevated levels of ALDH1A1 are a characteristic feature of hematopoietic stem cells (HSCs) [3–6], while altered expression of this RA-synthesizing enzyme has been reported in a number of solid tumors and tumor cell lines [7–9]. Despite this, possible roles for ALDH1A1 in hematopoiesis and leukemic transformation have remained largely unexplored. This needs to be addressed since dysregulation of RA synthesis, which is tightly controlled by a select group of enzymes including ALDH1A1 and ALDH1A2, may interfere with processes regulating cellular differentiation and proliferation, thereby contributing to the tumorigenic phenotype. In a separate role, ALDH1A1 is of relevance for its ability to affect the sensitivity of stem and tumor cells to chemotherapeutic agents such as cyclophosphamide [10–12].

ALDH1A1 is additionally of interest because of its status as a transcriptional target of the homeoprotein TLX1/HOX11 [13–15]. *Tlx1* has been shown to dictate the developmental fate of the spleen [16,17] and of specific neurons within the spinal cord [18], while mice lacking *Tlx1* additionally exhibit leukocytosis [16]. TLX1 is also a significant etiological factor in T-cell leukemogenesis since chromosomal translocations and other, as yet uncharacterised, molecular events involving the human 10q24 locus activate expression of the *TLX1* oncogene in up to 50% of human T-cell acute lymphoblastic leukemia (T-ALL) cases [19–23]. The

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mechanism(s) by which TLX1 induces tumor growth and controls normal development remain to be fully determined, although TLX1 has been implicated in cell cycle progression [24,25], predisposition to aneuploidy [26] and alteration of transcriptional regulator/co-regulator activity [27,28].

TLX1 is a transcription factor and several studies have linked its expression to the perturbation of gene expression programs controlling cell proliferation and apoptosis [22,25,29,30]. A plausible assumption, therefore, is that at least part of the oncogenic and/or normal activity of TLX1 is due to the direct or indirect transcriptional regulation of subordinate target genes. *Aldh1a1*, together with *Fhl1/Slim1*, which encodes a LIM-domain protein [31,32], are two such genes previously identified as being TLX1-dependent in developing mouse spleen and/or murine cell lines [13,14,33]. Since RA is a key regulator of cellular differentiation, proliferation and apoptosis in diverse situations ranging from organogenesis to hematopoiesis, *ALDH1A1* is of particular interest for its potential as a key mediator of normal and/or aberrant TLX1 function.

In this study, we demonstrate that *ALDH1A1* is also a target for transcriptional regulation by TLX1 in human hematopoietic cells. Expression of *ALDH1A1* was found in T-ALL cell lines that expressed *TLX1*, which was reminiscent of the correlation previously observed between *SCL/LMO* and *ALDH1A2* expression in T-ALL [34]. A striking feature of *TLX1* is its ability to impede hematopoietic cell differentiation [30,35–38]. We therefore employed retroviral-mediated transfer to enforce expression of *ALDH1A1* in murine bone marrow progenitors. Using this approach we found that *ALDH1A1* profoundly perturbed hematopoiesis by promoting myeloid differentiation at the expense of lymphopoiesis. These data implicate *ALDH1A1* in the regulation of hematopoiesis, and suggest one mechanism by which TLX1 may accomplish its normal and/or oncogenic functions.

2. Materials and methods

2.1. Cell culture and stable transfection

T-ALL cell lines were cultured in a humidified atmosphere at 37 °C with 5% CO_2 in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS; CSL, Melbourne, VIC, Australia), 0.03% L-glutamine, 0.03% pyruvate, 0.03% non-essential amino acids (Trace Scientific, Melbourne, VIC, Australia) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA). The erythroleukemic cell line HEL, was likewise cultured without addition of pyruvate and non-essential amino acids. The cells were grown to a density of approximately 5×10^5/ml before being harvested for RNA extraction. PER-117 and HEL cells (1×10^7) were co-transfected by electroporation with pPGKPuro (2 μg) and pEF-BOS/TLX1 (20 μg) in RPMI medium. The molar ratio of the two vectors was 1:10 (pPGKPuro: pEF-BOS/TLX1). Electroporation was performed with a Gene Pulser (Bio-Rad Laboratories, Hercules, CA, USA) at 300 V and 960 μF capacitance, followed by selection after 48 h in medium containing 0.6 μg/ml puromycin. After 3 weeks of growth in selective medium, transfected cells were cloned by repeated limiting dilution.

2.2. RT-PCR analysis

Total RNA was extracted from cell lines by NP40 lysis, phenol extraction and ethanol precipitation, and then treated with DNase I using the DNA-free kit (Ambion Inc, Austin, TX, USA). For cDNA synthesis, total RNA (5 μg) was reversed transcribed with oligo-dT using Thermoscript (Invitrogen) according to the manufacturer's instructions. One-fortieth of the cDNA (representing RNA from 1×10^4 cells) was amplified using Tth Plus DNA polymerase (Fisher Biotec, Perth, WA, Australia) in a 25 μl reaction volume according to the manufacturer's instructions. PCR cycling conditions were as previously described [33]. The primer sequences used were: 5′-CATGCCGGGCGTCAACAACCT-3′ (*TLX1* sense), 5′-TCACTCGCAGGCCGACGCCAC-3′ (*TLX1* antisense), 5′-GCCCTGGACTTCGAGCAAGAGATG-3′ (*ACTB* sense), 5′-CCTGCTTGCTGATCCACATCTGCT-3′ (*ACTB* antisense), 5′-ATTGTGTTAGCTGATGCCGACTTG-3′ or 5′-GGTTTCCATGAATATACAGAGGTC-3′ (*ALDH1A1* sense), 5′-GCGCATCTCATCTGTAACATTAGA-3′ or 5′-TGACAAGCAGACATGACATCCTAG-3′ (*ALDH1A1* antisense), 5′-ACAAAGATCCTGTGCACAGTGCAC-3′ (*FHL1* sense), 5′-AAAGCGCTTGTTGGCCAGATTCAC-3′ (*FHL1* antisense), 5′-CCAGCTCTCAGGAATACAAAGTTG-3′ (*ALDH1A2* sense), 5′-GATAGATGTACAGTGTGACAGACC-3′ (*ALDH1A2* antisense), 5′-TCAGCCAGCCGCTGGCCTCTCTCGGCAG-3′ (*SCL* sense), 5′-CTTGGCCCGCTGGGTGCCCTCCTCCTC-3′ (*SCL* antisense), 5′-CTGCAGATCCCCCCATCCCTGCTG-3′ (*LMO2* sense), 5′-ATGCTTCTGACAGGCGGCGCACTT-3′ (*LMO2* antisense), 5′-ATGGTGAAGGTCGGTGTGAACGGA-3′ (*GAPDH* sense) and 5′-GAGGTCCACCACCCTGTTGCTGTA-3′ (*GAPDH* antisense). PCR products were visualized on ethidium bromide-stained agarose gels.

2.3. Northern blotting

Total RNA samples (10 μg) from HEL cell clones were denatured with glyoxal (50 °C for 1 h), electrophoresed on 1.4% agarose gels and transferred to a nylon membrane (Magna, Osmonics Inc., Westborough, MA, USA). Human multiple tissue Northern blots (Clontech Inc., Mountain View, CA, USA) contained 2 μg poly(A)+ RNA from each tissue. ^{32}P-labelled probes were prepared by random priming (Rediprime II, GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and blots hybridised in buffer containing 3 × SSC, 10 × Denhardt's solution, 0.1% SDS and 250 mg/ml salmon sperm DNA at 65 °C for 16 h. The membranes were washed in

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