HoxA10 Regulates Myeloid Progenitor Proliferation by Activating Transcription of the Gene Encoding Transforming Growth Factor Beta 2.

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Abstract

HoxA10 is a homeodomain transcription factor which functions as a myeloid leukemia promoter. Correlative clinical studies found that increased expression of a group of HoxA proteins, including HoxA10, in acute myeloid leukemia (AML) was associated with poor prognosis. In murine models, overexpression of HoxA10 in the bone marrow was associated with development of a myeloproliferative disease which progressed to AML with time. These results suggested that HoxA10-overexpression dysregulated cell proliferation and/or survival, and predisposed to acquisition of additional mutations which led to differentiation block and AML. Additional investigations, we and others demonstrated that HoxA10 overexpression in murine hematopoietic stem cells (HSC) expanded the granulocyte/monocyte progenitor (GMP) population in vitro and in vivo. Despite this information about the impact of HoxA10 overexpression on myeloid leukemogenesis, the mechanisms by which HoxA10 exerts this effect are largely unknown. To investigate such mechanisms, we have been identifying HoxA10 target genes. In previous studies, we identified a number of HoxA10 target genes that encode phagocyte effector proteins. HoxA10 represses transcription of these gene in myeloid progenitors, and decreased HoxA10 repression activity contributes to phenotypic differentiation as myelopoiesis proceeds. This provided a potential mechanism for HoxA10 involvement in differentiation block, but not progenitor survival or expansion. We used a chromatin immuno-precipitation based approach to identify additional HoxA10 target genes involved in these activities. Previously, we reported that HoxA10 activated the DUSP4 gene in myeloid progenitor cells. This gene encodes Mitogen Activated Protein Kinase Phosphatase 2 (Mkp2) which inhibits Jnk-induced apoptosis in myeloid progenitor cells. This provided a mechanism for increased cell survival in HoxA10-overexpressing cells. In the current studies, we identified TGFβ2 as a HoxA10 target gene. This gene encodes Transforming Growth Factor Beta 2 (TgfB2) a member of the Tgfβ super family of cytokines. Similar to Tgfβ1 and 3, Tgfβ2 interacts with Tgfβ-receptors I and II. However, unlike these more classical family members, Tgfβ2 induces proliferation of hematopoietic stem and progenitor cells. We found that HoxA10 activated the TGFβ2 promoter via tandem cis elements in the proximal promoter. This resulted in autocrine stimulation of proliferation in HoxA10-overexpressing GMP and leukemia cells in vitro. Increased proliferation in HoxA10-overexpressing cells involved activation of the MAP kinase pathway in a Tgfβ2 dependent manner. These studies identify autocrine production of pro-proliferative cytokines as a novel mechanism for the function of Hox proteins. These findings have implications for ex vivo expansion of HSC and myeloid progenitors for tissue engineering. These result also have implications for therapeutic approaches to poor prognosis AML with increased Hox expression.

Background

Overexpression of HoxA10 is associated with AML; Increased expression of a group of Hox transcription factors is found in association with poor prognosis in human AML (HoxB3, B4, A7, A9, A10) (1). Murine models also suggest a role for Hox proteins in myeloid leukemogenesis. Overexpression of HoxB3 or B4 in murine bone marrow leads to expansion of HSC and myeloid progenitor populations, resulting in a myeloproliferative disorder. (MPD) Overexpression of HoxA10 or HoxA9 in murine bone marrow also induces MPD in vivo (2,3). This MPD progresses to AML in HoxA10 overexpressing mice, or in mice co-overexpressing HoxA9 and Meis1. However, the molecular mechanisms by which Hox proteins regulate HSC and progenitor proliferation and expansion are unknown.

Identification of HoxA10 target genes; We have investigating the role of Hox proteins in AML by identifying HoxA10 target genes. Using various high through put approaches, we identified a number of genes encoding signaling intermediates involved in cell proliferation and survival. We also identified a number of genes encoding cytokine receptors or their ligands (4). We report the identification of one such gene; the gene encoding the pro proliferative (5) cytokine, Tgfβ2.

Hypothesis of proposed studies; We hypothesize that HoxA10 overexpression in AML results in increased expression of Tgfβ2. We further hypothesize that autocrine activation of the Tgfβ-receptor by this cytokine induces proliferation in myeloid progenitor cells. Therefore, these studies identify a mechanism for progenitor expansion in HoxA10 overexpressing AML.

Results

Figure 1: **Identification of *TGFβ2* as a HoxA10 target gene in myeloid cells**

Chromatin that co-immuno-precipitated with HoxA10 from U937 myeloid leukemia cells included a Cpg island from the *TGFβ2* 5' flank. Chromatin which co-immuno-precipitated with HoxA10 from U937 cells was used to screen a CpG island microarray. A CpG island in the 5' flank of the TGFβ2 gene was identified.
HoxA10 bound to the proximal *TGFβ2* promoter region in vivo. Independent chromatin co-immuno-precipitation experiments confirmed HoxA10 binding to the *TGFβ2* 5' flank.

Figure 2: HoxA10 activates two cis elements in the *TGFβ2* promoter.

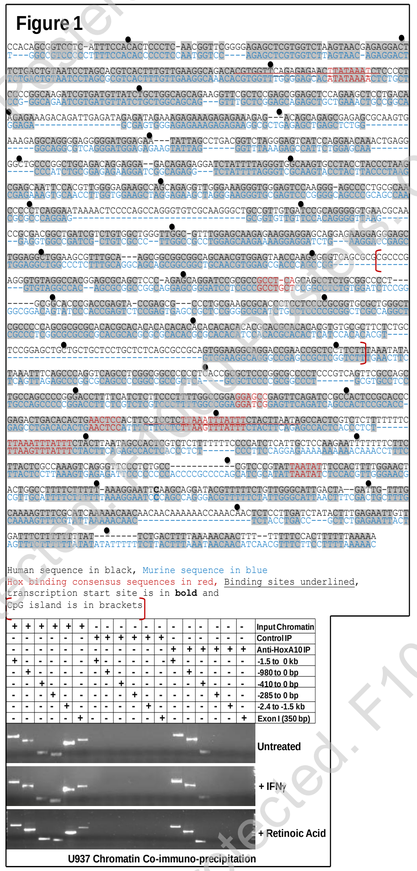
HoxA10 activates the *TGFβ2* promoter. U937 cells were co-transfected with a series of TGFβ2 promoter/reporter constructs and a vector to overexpress HoxA10. HoxA10 activated constructs with greater than 360 bp of 5' flank. Activation of constructs with 2.4 and 1.5 kb of 5' flank were activated more than constructs with less than 980 bp. Statistically significant differences are indicated by *, **, ***, #, ##, ###, &, or &&.
HoxA10 activated two *TGFβ2* cis elements. *TGFβ2* promoter analysis identified two consensus sequences for HoxA10 DNA binding at -385 to -410 bp (proximal) and -1478 to -1506 bp (distal) . Reporter constructs were generated with three copies of the proximal or distal sequences linked to a minimal promoter. U937 transfection experiments were performed in U937 cells with these constructs and a vector to overexpress HoxA10. Statistically significant differences are indicated by *, **, or ***.
HoxA10 bound the two *TGFβ2* cis elements. Chromatin co-immuno-precipitation was performed as in Figure 1, except that chromatin was sonicated to an average size of 200 bp. Probes flanking the TGFβ2 cis elements were used in real time PCR experiments.

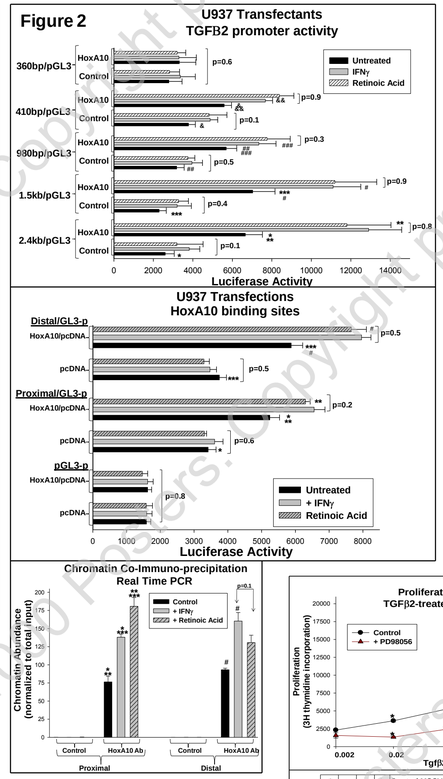
Figure 3: Tgfβ2 expression was increased in HoxA10-overexpressing cells.

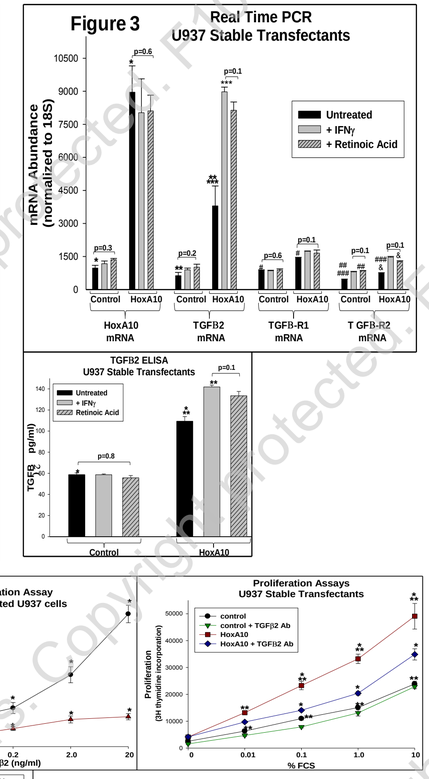
HoxA10 overexpression increased Tgfβ2 mRNA expression. U937 cells were stably transfected with a vector to overexpress HoxA10 or empty vector control. Expression of HoxA10, Tgfβ2 and Tgfβ-receptors was determined by real time PCR. Statistically significant differences are indicated by *, **, ***, #, ##, ###, & or &&.
HoxA10 overexpression increased Tgfβ2 protein expression. Media from the stable transfectants discussed above was analyzed by specific ELISA for Tgfβ2 protein secretion. Statistically significant differences are indicated by * or **.

Figure 4: HoxA10 overexpression increased myeloid cell proliferation in a Tgfβ2 and Erk dependent manner.

U937 cells exhibit Erk dependent proliferation in response to Tgfβ2. U937 cells were stimulated with a dose titration of recombinant Tgfβ2. Some cells were also treated with the specific Erk inhibitor PD98056. Proliferation was determined by incorporation of 3H thymidine. Statistically significant differences in proliferation with vs. without HoxA10 overexpression are indicated by *.
HoxA10 overexpressing U937 cells exhibit Tgfβ2 dependent cytokine hypersensitivity. U937 stable transfectants overexpressing HoxA10 or with vector control were deprived of FCS for 24 hrs followed by treatment with a dose titration of FCS. Cells were treated with a blocking antibody to Tgfβ2 vs. irrelevant antibody control. Proliferation was determined by incorporation of 3H thymidine. Statistically significant differences are indicated by * or **.
HoxA10 overexpression increases Erk activation in a Tgfβ2 dependent manner. The U937 stable transfectants described above were analyzed for Erk activation by Western blot. Blots were probed with antibodies to total Erk, phospho Erk (active), HoxA10 or GAPDH (as a loading control)







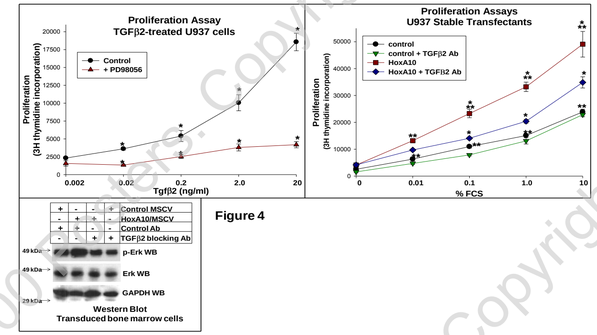


Figure 4

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Summary

1) **Increased HoxA10 expression is associated with increased Tgfβ2 expression in myeloid cells.**
2) **HoxA10 binds to and activates two cis elements in the *TGFβ2* promoter.**
3) **HoxA10 overexpression induces cytokine hypersensitivity which is at least in part due to autocrine secretion of Tgfβ2.**