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Supplement: Inferring Epigenetic and Transcriptional Regulation during Blood Cell Development with a Mixture of Sparse Linear Models

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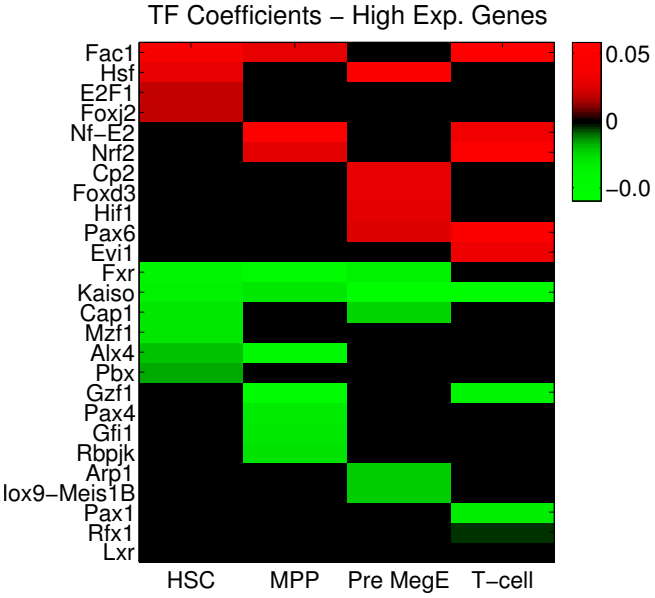

Fig. 1. Transcription factors that significantly affect the expression of high expression genes in distinct cell types (p-value < 0.05). Red arrows indicates positive coeficients and green arrows negative coefficients.

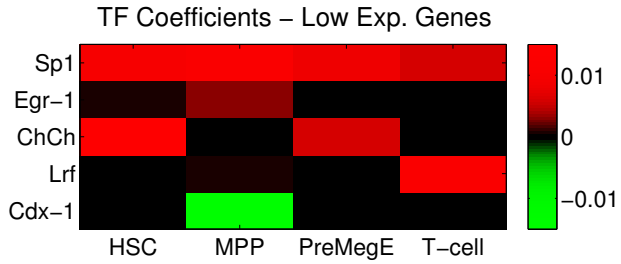

Fig. 2. Transcription factors that significantly affect the expression of low expression genes in distinct cell types (p-value < 0.05). Red arrows indicates positive coeficients and negative arrows negative coefficients.

1 TRANSCRIPTION FACTORS INFERRED FROM MIXTURE OF LINEAR MODELS

In addition to the modifications of histones, we also observe the effects of transcription factors in the four blood cell types. Out of the 600 transcription factors, 31 had a statistical significant coefficient (p-value < 0.05) in at least one condition (see Figs. 1 and 2 for the list of TFs). Out of the 31 transcription factors, 15 were related to development on hematopoietic system, 5 with chromatin structure remodelling, 8 embryonic development and only 3 had no direct link with development. This indicates an enrichment of recovering TFs related to chromatin reorganization and hematopoietic development.

Of the 31 selected transcription factors, 11 are involved in the activation process of gene expression in genes associated with high expression (Fig. 1 left). The transcription factor FAC1 — also known as Bptf—is present in the process of gene activation in HSC, MPP and T-cell. This gene is a component of the NURF complex, which is known to promote trimethylation of the H3 lysine 4 and gene activation in mammals (Wysocka *et al.*, 2006). Another protein that has been related to immune cell development is HSF (Morange, 2006), which acts as a activator of expression in HSC and PreMegE cells. This protein has been recently implicated with histone acetylation and gene activation in mammals (Fritah *et al.*, 2009). Another chromatin related protein is Kaiso, which work as a repressor in all cell types (Fig. 1 right). Kaiso is known to bind to methylated DNA and the recruitment of H3 lysine 9 methylation and gene repression (Yoon *et al.*, 2003). These examples demonstrate the recovery of a transcription factors with chromatin remodelling roles in hematopoiesis.

Some transcription factor were related to only one or two of the cell types. For example, E2F and Foxj2 were indicated to be promoters of expression in HSC cells only. The transcription factor E2F1 regulates the cell cycle of hematopoietic stem cells (Furukawa, 1998). Fork head box J2 (Foxj2) is a transcriptional activator of early embryonic development (Granadino *et al.*, 2000).

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Two factors were indicated as activators in both MPP and T cells. NRF-2 is a master regulator of the antioxidant response and has a critical role in cell identity maintenance during hematopoiesis (Merchant *et al.*, 2011). The second factor NF-E2 is interestingly associated to erytropoiesis and gene activation through the recruitment of the MLL2 complex and H3k4 methylation (Demers *et al.*, 2007). Also, Evi-1, which was related to T cell only, has been implicated in cell maintenance in HSC cells (Shimabe *et al.*, 2009).

Among the factor related to PreMegE cell, we have CP2, which has been described as a regulator of erythroid cells (Kang *et al.*, 2005). Moreover, we have a association of Foxd3 as an activator of gene expression. Note that Foxo3, which has a similar motif as Foxd3, is implicated in erytropoiesis (Bakker *et al.*, 2007). HIF-1 have been recently related to hematopoiesis (Casado *et al.*, 2010), while Pax6 is a transcription factor involved in specification dorsal/ventral axis and neurogenesis (Osumi *et al.*, 2008).

Twenty transcription factors were indicated to be involved in repression of genes with high expression. Gfi1 is involved with the proliferation of embryonic hematopoietic stem cells (HSC) (Khandanpour *et al.*, 2011) and the is important in the role in the development of myeloid, erythroid, and B cell progenitors (Orkin and Zon, 2008; Barreda and Belosevic, 2001). This protein interacts with the LSD1, a protein known to repress gene expression by demethylation of lysine 4 of H3 (Saleque *et al.*, 2007). The second complex— HOX9—works in collaboration with MEIS1. Over-expression of HOX9 can immortalize myeloid progenitors in vitro and inhibit some of the pathways of differentiation (Ghannan *et al.*, 2004). This is corroborated by our results, where the HOX9MEIS1 complex stands out in PreMegE cells of the myeloid branch in hematopoietic system.

The GZF1 transcription factors can regulate the expression of the HOX10 gene whose function is the amplification of the precursors of the hematopoietic system and megakaryocytes (Morinaga *et al.*, 2005). As we observed a negative value of correlation coefficient for this molecule in both MPP as in T cells, are likely to observe the negative regulation of GZF1 transcription factor to HOX10 gene GZF1 and thus blocking the amplification of the precursors of hematopoietic system and megakaryocytes.

Transcription factors, MZF1 and FXR, are involved in the development of myeloid cells (Barreda and Belosevic, 2001). We observed a low value of correlation coefficient for the FXR transcription factor in HSC, MPP and PreMegE cells, which might demonstrate the role of this molecule in the repression of gene expression in cells involved in the formation of myeloid branch. Pax1 is a transcriptional activator that acts on skeletal development, cell proliferation and differentiation of T cells (Wallin *et al.*, 1996). Interestingly, our results indicates its relation to gene repression in T cells only.

Many of the transcription factor with repression roles of highly expressed genes are involved in the regulation of development in embryos or tissues unrelated to hematopoiesis, such as Pbx1 (**?**), PAX4 (Sosa-Pineda, 2004), RBPJK (Miele, 2011), RFX1 (Zhao *et al.*, 2010), LXR (Cha and Repa, 2007), Cap1 (Schubert *et al.*, 2011) and Alx4 (McGonnell *et al.*, 2011). Note that two explanations can be made on the role of these transcription factors: either they are acting actively as repressors of these genes, or genes are simply not expressed in the hematopoietic tissues and there is no actual binding of these factors in such cells. Further

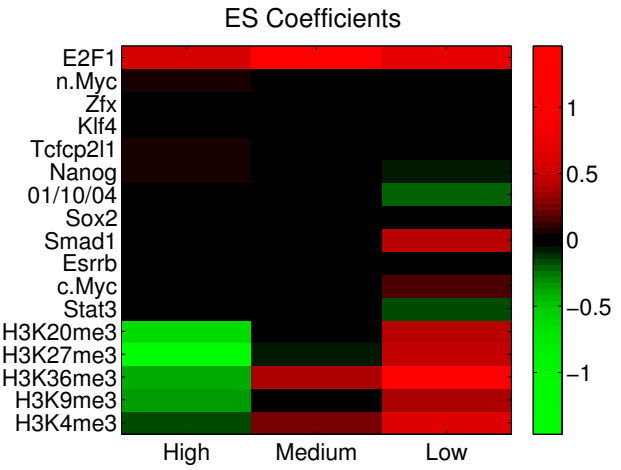

Fig. 3. Heatmaps with histone modification coeficients and TF that significantly affect the expression of distinct groups of genes (high, medium or low expression) on ES cell (p-value < 0.05). Red arrows indicate positive coeficients and negative arrows negative coeficients.

experimental data, such as chromatin imunoprecipitation essays would be necessary to validate any of these alternatives.

The regulation of expression of genes with low expression has the participation of five transcription factors: Sp1, Egr-1, Chch, LRF-1 and Cdx. Only Cdx-1—a gene related to intestin development (Beck, 2004)—have a repression role on MPP cells.

A transcription factor primarily related to activation of genes with low expression on all cells is Sp1. This gene has a known role in chromatin modelling possibly by the interaction with HDAC enzymes and promotion of gene expression (XXX epigenetics book). Another gene with a putative chromatin remodelling role is Egr-1 (Krox). It is known to interact with EP300 and Sp1, which are known to promote histone acetylation and activation of expression (Silverman *et al.*, 1998). Egr-1 was detected as a activator of gene expression in HSC and MPP cells.

We also observe the presence of LRF, which has an activity on T cells and has been implicated in the lineage decision between B and T cells by down-regulation of the Notch signals in the T cells (Maeda *et al.*, 2009). Lastly. the transcription factor Chch, which is involved in the regulation during embryo genesis (Sheng *et al.*, 2003), was indicated as an activator of expression in both HSC and PreMegE cells.

2 RESULTS FROM ES CELLS

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