Effects of the CTCF transcriptional factor in the erythroid cell differentiation and regulation of erythroid genes

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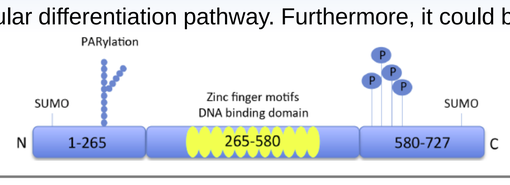
INTRODUCTION

The factor was firstly identified as a protein interacting with three repeats of the CCCTC motif within the c-myc promoter and was therefore named CTCF (CCTC-binding factor). CTCF is highly conserved from Drosophila to humans. The CTCF gene encodes an 11 zinc-finger (ZF) transcription factor, which is ubiquitously expressed and located in the nucleus. There, CTCF tethers diverse and uncommonly long DNA sequences (~50 bp) using different combinations of zinc fingers. Due to its ability of recognizing multiple target sites, CTCF is called a multivalent transcriptional factor mediating many diverse functions in gene regulation. (1, 2). Our previous data suggest a possible role of CTCF in the regulation of erythroid cell differentiation. It could be shown that CTCF was differentially expressed and post-translationally modified, comparing several human myeloid cells depending on the particular differentiation pathway. Furthermore, it could be shown that overexpression of CTCF promotes differentiation into the erythroid lineage (3). Previous results of Affymetrix microarrays comparing the transcriptome of parental K562 cells and cells overexpressing CTCF suggest that CTCF regulates erythroid specific genes. Those results indicate a feasible role of CTCF during myeloid differentiation**.**



AIMS

1. Verify a pivotal role of CTCF during differentiation.
2. Silence CTCF expression to explore its effect on K562 cell differentiation and target gene expression using lentiviral particles.
3. Characterize genes regulated by CTCF, particularly genes encoding for erythroid transcription factors.
4. Study the binding of CTCF to the regulatory regions of its putative target genes upon differentiation.
5. Elucidate the role of CTCF in human pluripotent CD34⁺ hematopoietic stem cells.

MATERIALS AND METHODS

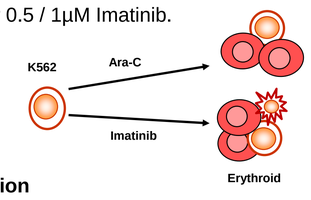
1) Cell lines

K562 is a human chronic myeloid leukemia cell line in blast crisis, which has the ability to differentiate into progenies of the erythroid, megakaryocytic and monocytic line.

CD34⁺ is a primary pluripotent cell line purified from cord blood with the ability to give rise to all cell types of the blood.

2) Treatment with Ara-C or Imatinib

K562 were seeded at 2.5x10^5 cells/mL and treated with 1µM Ara-C or 0.5 / 1µM Imatinib.

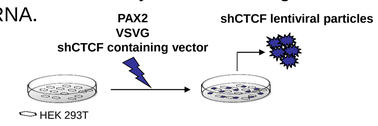


3) Evaluation of Differentiation

After treatments, differentiation was assessed by cell proliferation (cell number), cell viability (trypan blue), hemoglobinized cells (benzidine test) , RT-qPCR and westernblot.

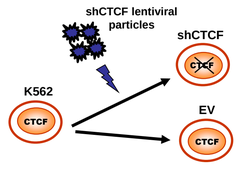
4) Production of Lentiviral Particles

- HEK293T cell were used for lentivirus production.
- Lentiviral particles deliver inducible system containing shRNA specific for CTCF mRNA.



5) Silencing of CTCF

- K562 were silenced using lentiviral particles containing shRNA specific for CTCF mRNA.
- 2 days after infection, cells were selected with 1µM/mL puromycin for 48hrs and then differentiation was induced by 1µM Ara-C and 0.5µM Imatinib.



6) Induction of Differentiation in CD34⁺

- CD34⁺ were purified from cord blood and induced with Erythropoietin (EPO).
- Erythroid differentiation was evaluated by benzidine test and westernblot.

7) ENCODE Genome Browser

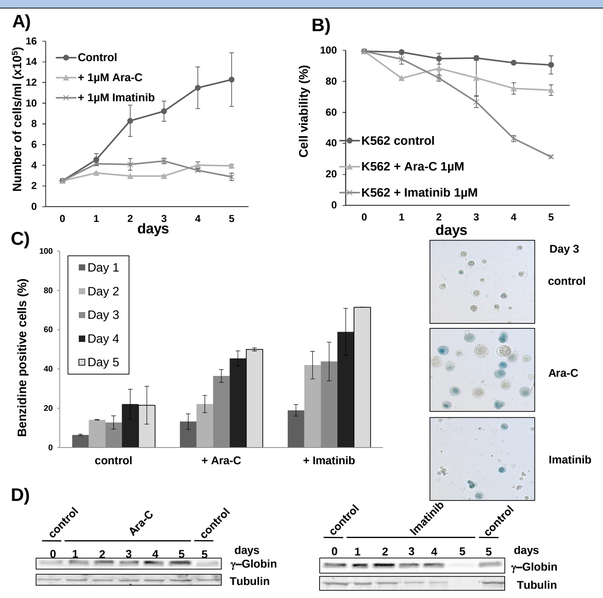
- Erythroid genes were analysed for CTCF binding sites (CTS).

8) ChIP

- 20x10^6 cells were fixed and lysed.
- ChIP was carried out with 3 CTCF antibodies.
- qPCR was performed using Primers for ETS1, MYB, LMO2 and KLF1 CTSs.

RESULTS AND DISCUSSION

1) Ara-C and Imatinib induce Erythroid Differentiation



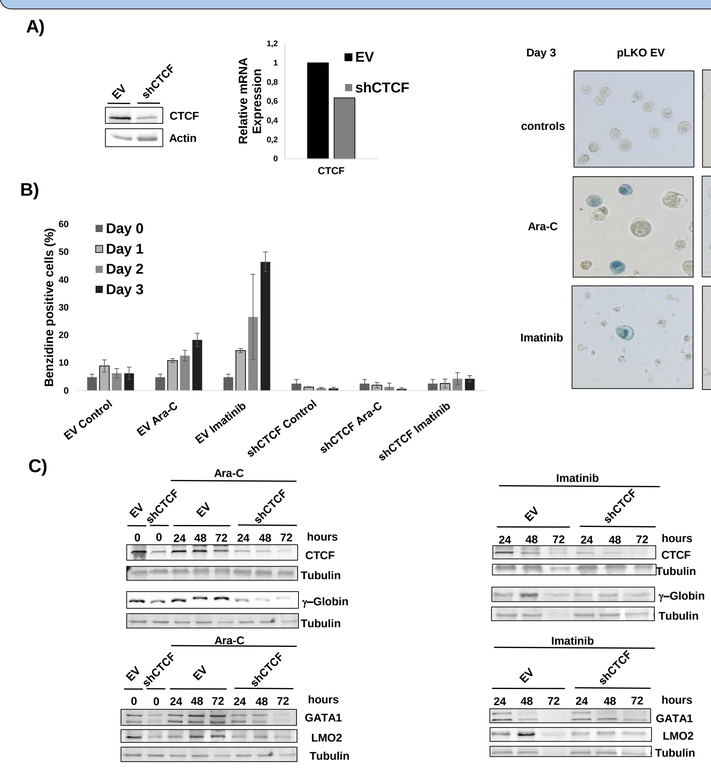
A) Ara-C and Imatinib reduce proliferation in K562 associated with differentiation

B) Ara-C reduces cell viability to 80%. Imatinib reduces cell viability to 30%.

C) Ara-C and Imatinib increase number of benzidine positive cells indicating erythroid differentiation.

D) γ-Globin expression (erythroid marker) increases upon treatment with Ara-C and Imatinib

2) Silencing of CTCF inhibits differentiation of K562 cells upon induction with Ara-C and Imatinib

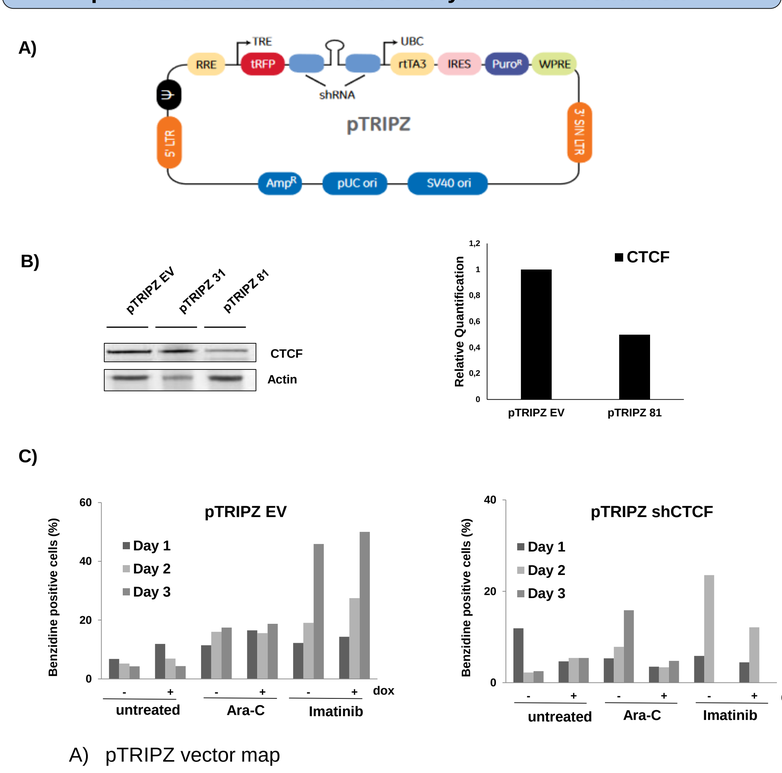


A) Silencing of CTCF using shRNA specific against CTCF mRNA

B) Silencing of CTCF reduces amount of benzidine positive cells

C) Erythroid genes are downregulated upon CTCF silencing

3) Preparation of Lentiviral Particles with inducible system pTRIPZ-shCTCF and effect on erythroid differentiation

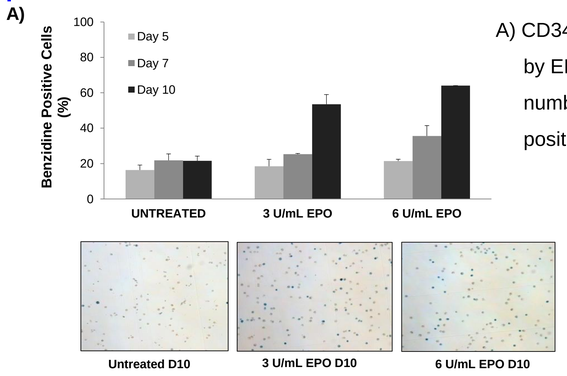


A) pTRIPZ vector map

B) Silencing of CTCF using the construct pTRIPZ shCTCF 81

C) CTCF silencing decreased number of benzidine positive cells indicating inhibition of erythroid differentiation

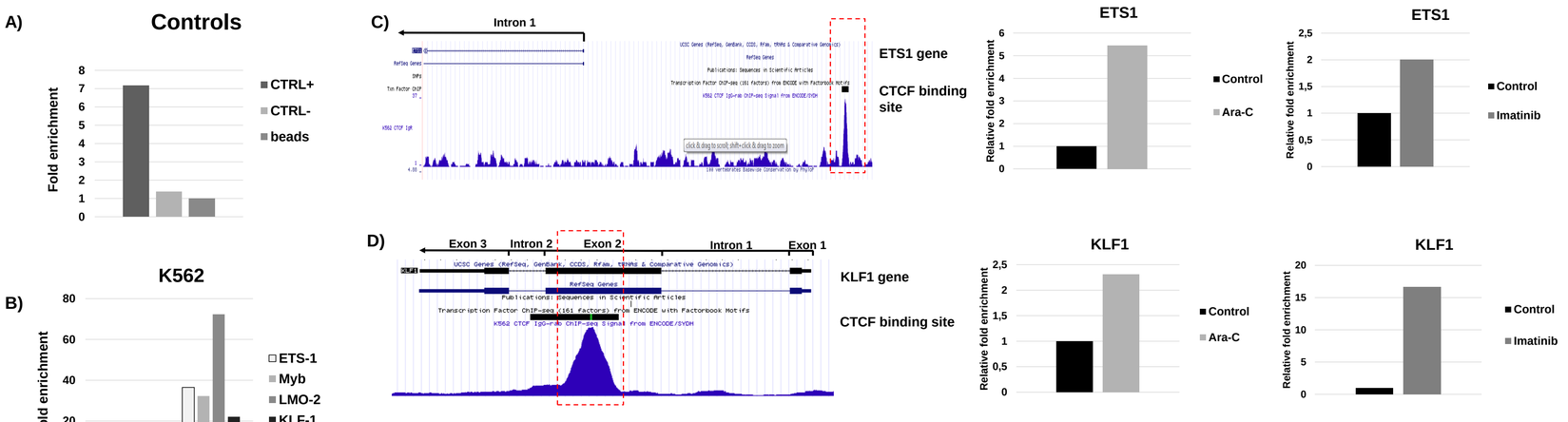
4) Erythroid differentiation of CD34⁺ cells induced by EPO



A) CD34⁺ cells induced by EPO show increased number of benzidine positive cells.

B) CD34⁺ cells induced by EPO show increased expression of γ-globin and GATA1 (erythroid markers).

5) ENCODE analysis of CTCF binding sites in erythroid genes and ChIP assays



A) Controls for ChIP. Positive Control (H42.1), negative control (H4) and without CTCF antibody (beads) are shown.

B) CTCF binding to analyzed sites of erythroid genes in K562.

C) ENCODE analysis (left) and ChIP analysis (right) of CTS upstream of ETS1. Increased occupancy was found upon induction of differentiation.

D) ENCODE analysis (left) and ChIP analysis (right) of CTS upstream of KLF1. Increased occupancy was found upon induction of differentiation.

CONCLUSIONS and FUTURE WORK

- Treatment with Ara-C and Imatinib reduce proliferation and induce differentiation along the erythroid pathway in K562 cells.
- CTCF constitutive downregulation inhibits erythroid differentiation induced by Ara-C or Imatinib.
- Inducible downregulation of CTCF confirms inhibition of erythroid differentiation.
- CD34⁺ primary cells differentiate along the erythroid pathway upon treatment with EPO.
- CTCF *in vivo* binding to erythroid genes (ETS1, KLF-1) is demonstrated and differential CTCF occupancy of erythroid genes is found upon erythroid differentiation.

- Silencing of CTCF at different time points during differentiation to obtain better insight into molecular events in K562 using the inducible system pTRIPZ.
- Silencing of CTCF using the inducible system pTRIPZ to assess its effect on CD34⁺ erythroid differentiation.
- ChIP analysis upon downregulation of CTCF to identify crucial binding sites.

REFERENCES

1) Kim, S., Yu, N.K., and Kaang, B.K. (2015). CTCF as a multifunctional protein in genome regulation and gene expression. Exp Mol Med *47*, e166.
2) Ohlsson, R., Bartkuhn, M., and Renkawitz, R. (2010). CTCF shapes chromatin by multiple mechanisms: the impact of 20 years of CTCF research on understanding the workings of chromatin. Chromosoma 119, 351-360.
3) Torrano, V., Chernukhin, I., Docquier, F., D'Arcy, V., Leon, J., Klenova, E., and Delgado, M.D. (2005). CTCF regulates growth and erythroid differentiation of human myeloid leukemia cells. J Biol Chem *280*, 28152-28161.

ACKNOWLEDGEMENTS
I would like to thank Dolores Delgado, Javier León and their group for help, support, trust and especially for their patience during my master's project.