Title

Runx1 Regulates c-Myc Expression And The Expansion Of Hematopoietic Precursors In A C-terminally Dependent Manner

Author

Paejonette Jacobs, *University of Massachusetts - Amherst*

Date of Award

5-2012

Document Type

Campus Access

Degree Name

Doctor of Philosophy (PhD)

Degree Program

Molecular and Cellular Biology

First Advisor

Janice C. Telfer

Second Advisor

Juan Anguita

Third Advisor

Surita R. Bhatia

Subject Categories

Immunology and Infectious Disease | Molecular Biology

Abstract

Runx1 regulates the expression of several important target genes and plays critical roles in the process of hematopoiesis. Runx1 by itself is a poor regulator of transcription and instead nucleates transcription complexes through its C-terminus to transactivate or repress the expression of target genes. We generated a C-terminally deleted Runx1 construct (Runx1.d190), which lacks important co-factor sites, to further investigate the function of Runx1 in development. A potential role for Runx1 in regulating the expression of another potent transcriptional regulator, c-Myc, has been suggested by published studies, which show that Runx1 and c-Myc collaborate in oncogenesis. In these studies, we show that endogenous Runx1 binds to three Runx consensus sites upstream of the *c-Myc* transcriptional start site in Jurkat T cells and murine primary splenocytes. Retroviral transduction of Jurkat T cells with Runx1.d190 results in the increased transcription of *c-Myc* as determined by microarray analysis. In order to monitor c-Myc expression in response to early-acting and transient Runx1.d190, we generated a cell membrane-permeable TAT-Runx1.d190 fusion protein. Treatment of murine primary splenocytes with TAT-Runx1.d190 protein results in a transient increase in the transcription of *c-Myc* and a corresponding increase in c-Myc protein levels. This effect is dependent on the ability of Runx1.d190 to bind to DNA. These data demonstrate that Runx1 directly regulates c-Myc expression in a C-terminally and DNA-binding dependent manner. In these studies, we also investigate the effects of the truncation of Runx1 C-terminus on hematopoietic stem cells (HSCs). We found that treatment of bone marrow cells enriched for HSCs with TAT-Runx1.d190 results in a 12.5 fold increase in hematopoietic precursors compared to untreated precursors *in vitro* as determined by Colony Forming Cell assays. We also show that hematopoietic precursors treated with TAT-Runx1.d190 are able to able to differentiate normally both *in vitro* and *in vivo* and thus represent functional hematopoietic precursors. Our findings show that we are able to transiently expand hematopoietic precursors*ex vivo* by treating the cells with a Runx1 construct that lacks the C-terminus. Collectively, this work demonstrates that Runx1 regulates the expression of c-Myc and the expansion of hematopoietic precursors in a C-terminally dependent manner.

Recommended Citation