Phylogenesis and regulated expression of the RUNT domain transcription factors RUNX1 and RUNX3

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Abstract

The RUNX transcription factors are key regulators of lineage specific gene expression in developmental pathways. The mammalian RUNX genes arose early in evolution and maintained extensive structural similarities. Sequence analysis suggested that RUNX3 is the most ancient of the three mammalian genes, consistent with its role in neurogenesis of the monosynaptic reflex arc, the simplest neuronal response circuit, found in Cnidarians, the most primitive animals. All RUNX proteins bind to the same DNA motif and act as activators or repressors of transcription through recruitment of common transcriptional modulators. Nevertheless, analysis of Runx1 and Runx3 expression during embryogenesis revealed that their function is not redundant. In adults both Runx1 and Runx3 are highly expressed in the hematopoietic system. At early embryonic stages we found strong Runx3 expression in dorsal root ganglia neurons, confined to TrkC sensory neurons. In the absence of Runx3, knockout mice develop severe ataxia due to the early death of the TrkC neurons. Other phenotypic defects of Runx3 KO mice including abnormalities in thymopoiesis are also being investigated.

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Introduction

The mammalian RUNX genes comprise a small gene family of three genes that act as master regulators of gene expression in several distinct developmental pathways. The three proteins bind to the same DNA motif; hence, their pleiotropic functions are likely to result from a regulated spatial/temporal expression pattern. Molecularly, this is orchestrated through usage of transcriptional coupled translation control [1]. Interestingly, the genes contain RUNX binding sites in their promoter region, raising the possibility of positive and negative cross regulation between them.

Results and discussion

The structure of mammalian RUNX genes

Phylogenetic analysis based on protein sequence revealed that the mammalian RUNX genes arose early in evolution (Fig. 1). Interestingly, high similarities in genomic organization among the three mammalian RUNX genes were preserved through evolution (Fig. 2). Similarities are also manifested in DNA sequence of the genes and in regulation of their expression. Transcription of RUNX genes is initiated at two distantly located promoter regions
(Fig. 2) and proceeds through production of a complex repertoire of alternatively spliced transcripts, the expression of which is regulated at the level of translation [1–5].

Of the mammalian genes RUNX3 is the smallest in size and has the fewest exons [3]. Nevertheless, the RUNX3 locus harboring six exons possesses all the structural hallmarks common to RUNX family members. These hallmarks include conservation of exon/intron boundaries, the presence of two promoter regions separated by a relatively large first intron, and the preservation of a particularly large CpG island around the proximal (P2) promoter. Based on the phylogenetic analysis and on the content of MIR repetitive sequences, which are among the most ancient mammalian-wide repetitive sequences, RUNX3 emerged as the evolutionary founder of the mammalian RUNX gene family. This notion is supported by the expression pattern and biological function of Runx3, particularly its role in neurogenesis of the monosynaptic reflex arc. This neuronal circuit is the simplest information response path, found in the ancient radially symmetrical animals Cnidarians that are among the most primitive animals.

Expression of Runx1 and Runx3 during embryogenesis

In addition to similarities in genomic organization the RUNX proteins share functional features. They all bind to the same DNA motif and act as either activators or repressors of transcription through recruitment of additional transcriptional modulators [6]. This mode of operation could lead to redundancy in their biological function. To gain insight into the mechanisms that regulate expression of RUNX genes, we analyzed the expression pattern of Runx1 and Runx3 during embryogenesis [7]. Analysis revealed that Runx1 is more widely expressed than Runx3, in line with the other indications that Runx3 is the more ancient. Runx3 expression was detected only in organs that also expressed Runx1. In some compartments of the hematopoietic system expression of the two transcription factors overlapped, whereas in sensory ganglia, epidermal appendages, and developing skeletal elements, Runx3 and Runx1 expression was detected in different compartments. Overall, the expression of Runx3 was confined to mesenchymal elements whereas Runx1 was detected in both epithelial and mesenchymal tissues. Unique expression of Runx1 was detected in several types of epithelia including the gastric mucosa, the valvular region of the heart, and the CNS. The data provide new insights into the function of Runx1 and Runx3 in organogenesis and indicate that their function is not redundant.
The phenotype of Runx3-deficient mice

Runx3 KO mice were generated in our lab by inserting a LacZ-Neo cassette into exon 2, the first exon encoding the “Runt domain.” The most apparent phenotype of the homozygote Runx3 KO mice was a severe limb ataxia [8]. Of the various organs expressing Runx3 during embryogenesis, expression in the developing sensory ganglia seemed the most relevant to the ataxic phenotype and was therefore analyzed in greater detail. Analysis revealed that Runx3 expression in dorsal root ganglia starts at an early neurogenic stage and is confined to a subset of sensory neurons, the TrkC proprioceptive neurons. Further analysis revealed that in mice deficient of Runx3, the ataxia results from lack of monosynaptic connectivity between intraspinal la afferents and motoneurons because TrkC neurons in these mice do not survive long enough to extend their axons toward target cells. In addition to the sensory defect, Runx3 KO mice exhibit abnormalities in thymopoiesis.

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References