Transcription Regulation in Developmental Pathways

Yoram Groner

Ditsa Levanon

Josef Lotem

Varda Negreanu

Yael Bernstein

Dalia Goldenberg

Sarit Weissmann

Shay Hantisteanu

Yaron Gruper

Omri Baure

Oren Ben-Ami

Opening Remark

We use cellular, biochemical and gene targeting approaches to investigate how differential gene expression patterns are established and maintained during mammalian development. We address this question through investigating the biology of two transcription factors Runx1 and Runx3 at the molecular level and at the *in vivo* using genetically modified mouse models (Fig. 1). The RUNX transcription factors are master regulator of linage specific gene expression in developmental pathways. RUNX1 reside on human chromosome 21 and could be involved in Down syndrome leukemia and RUNX3 reside on chromosome 1 at a region known to be involved in several human diseases. Below is a spotlight account summarizing our past two years research on Runx3.

RUNX3 Biology

We generated Runx3 knockout (KO) mice by inserting a LacZ-*neo* cassette into the gene so that the targeted allele also provided means for examining expression of the gene by LacZ staining (Fig. 1). Studies in The KO mice have delineated several cell-autonomous functions of Runx3. In neurogenesis, Runx3 is required for the development and survival of dorsal root ganglia (DRG) TrkC neurons (Fig.1). When Runx3 is mutated

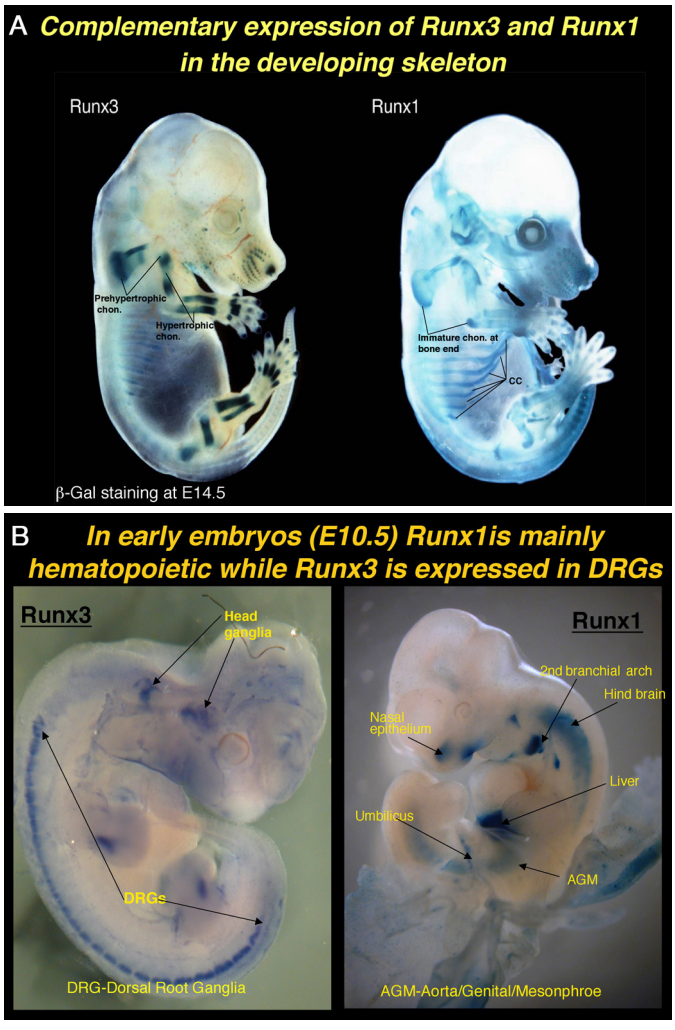

Fig. 1 *Expression patterns of Runx1 and Runx3 in developing mouse embryos.*
A- expression of β-galactosidase from Runx1 and Runx3 knock-in alleles in skeletal elements.
B- expression of Runx1 in early hematopoiesis and Runx3 in dorsal root ganglia (DRG)

Department of Molecular Genetics

972 8 934 3972

972 8 934 4108

yoram.groner@weizmann.ac.il

www.weizmann.ac.il/molgen/members/groner.html

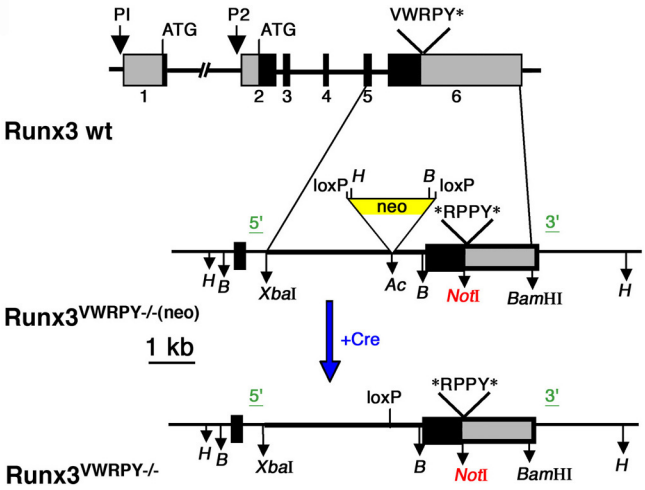

Fig. 2 *A scheme describing the disruption of the Gro/TLE binding site (VWRPY) in Runx3. The floxed neo gene was later removed giving rise to Runx3^{VWRPY-}*

these neurons do not gain their full identity and die by apoptosis due to frustration. In thymopoiesis Runx3 regulates T-cell development and is required for silencing of *CD4*. In the KO mice T cells display abnormal expression of *CD4* and impairment of CD8 T cells maturation. In a compound mutant mice that we have generated, (Runx3-/-;Runx1+/-), null for Runx3 and heterozygous for Runx1 all peripheral mature CD8 T cells also expressed *CD4*, resulting in a complete lack of single positive CD8 T cells in the spleen. Runx3 is also highly expressed in dendritic cells (DC), where it functions as a component of TGF-β signaling pathway. When Runx3 is mutated DC become insensitive to TGF-β and acquire highly active phenotype with increased potency to stimulate T cells. These hyperactive KO DC over-respond to otherwise innocuous airborne antigens and consequently induce an eosinophilic lung inflammation in the KO.

Runx3 KO mice also develop inflammation and lesions in the gastrointestinal tract including colitis and stomach hyperplasia. At 4-weeks of age, the KO mice spontaneously develop inflammatory bowel disease (IBD) characterized by leukocyte infiltration, mucosal hyperplasia, formation of lymphoid clusters and increased production of IgA. Additionally, at a considerably older age (8 months), the KO mice also develop progressive hyperplasia of the gastric mucosa associated with disturbed epithelial differentiation and cellular hyaline degeneration. Analysis of cytokines in the colonic mucosa of Runx3 KO mice revealed a mixed Th1/Th2 response. Using immunohistochemistry and RNA in situ hybridization, Runx3 expression in the gastrointestinal tract is detected in lymphoid and myeloid populations, but not in the epithelium. The data indicate that loss of leukocytic cell-autonomous function of Runx3 results in IBD and gastric lesion in the KO mice. IBD in humans is viewed as a complex genetic disorder. Several susceptibility loci were identified on different human chromosomes including the chromosomal region 1p36 where *RUNX3* resides. It is thus tempting to speculate that mutations in *RUNX3* may constitute an IBD risk factor in humans.

Regulation of gene expression by tissue specific transcription factors such as Runx3 involves not only turning on, but also turning off transcription of target genes. To investigate the mechanism of transcription shut-off by Runx3 in an *in vivo* context, we generated mice expressing a mutant Runx3 lacking the C-terminal VWRPY, a motif required for Runx3 interaction with the co-repressor Groucho/TLE (Fig. 2).

In contrast to Runx3$^{-/-}$ mice, which displayed ataxia due to death of DRG TrkC neurons, Runx3$^{VWRPY-/-}$ mice were not ataxic and had intact neurons, indicating that the ability of Runx3 to tether Groucho/TLE is not essential for neurogenesis. In the DC compartment, the mutant protein Runx3^{VWRPY-} promoted normally the development of skin Langerhans cells but failed to restrain the sponta*neo*us maturation of DC, indicating that this latter process involves Runx3-mediated repression through recruitment of Groucho/TLE. Moreover, in CD8^{+} thymocytes Runx3^{VWRPY-} up-regulated *aE*/CD103 like wild type Runx3, whereas unlike wild type it failed to repress *aE*/CD103 in CD8^{+} splenocytes. Thus, in CD8-lineage T-cells Runx3 regulates *aE*/CD103 in opposing regulatory modes and recruits Groucho/TLE to facilitate the transition from activation to repression. Runx3^{VWRPY-} also failed to mediate the epigenetic silencing of *CD4* gene in CD8^{+} T cells, but normally regulated other pan-CD8^{+} T-cell genes. The data elucidate the function of Runx3 in neurogenesis, DC and T-cell development and provide insights into the mechanism through which Runx3-regulated genes are epigenetically silenced.

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