Transcriptional regulation by RUNX3

Chuang, LS., Ito, Y., Orlic-Milacic, M.

European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.

The contents of this document may be freely copied and distributed in any media, provided the authors, plus the institutions, are credited, as stated under the terms of Creative Commons Attribution 4.0 International (CC BY 4.0) License. For more information see our license.

13/07/2020
Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

Literature references


Reactome database release: 73

This document contains 10 pathways (see Table of Contents)
The transcription factor RUNX3 is a RUNX family member. All RUNX family members, RUNX1, RUNX2 and RUNX3, possess a highly conserved Runt domain, involved in DNA binding. For a more detailed description of the structure of RUNX proteins, please refer to the pathway 'Transcriptional regulation by RUNX1'. Similar to RUNX1 and RUNX2, RUNX3 forms a transcriptionally active heterodimer with CBFB (CBF-beta). Studies in mice have shown that RUNX3 plays a role in neurogenesis and development of T lymphocytes. RUNX3 is implicated as a tumor suppressor gene in various human malignancies.

During nervous system formation, the Cbfb:Runx3 complex is involved in development of mouse proprioceptive dorsal root ganglion neurons by regulating expression of Ntrk3 (Neurotrophic tyrosine kinase receptor type 3) and possibly other genes (Inoue et al. 2002, Kramer et al. 2006, Nakamura et al. 2008, Dykes et al. 2011, Ogihara et al. 2016). It is not yet known whether RUNX3 is involved in human neuronal development and neuronal disorders.

RUNX3 plays a major role in immune response. RUNX3 regulates development of T lymphocytes. In mouse hematopoietic stem cells, expression of Runx3 is regulated by the transcription factor TAL1 (Landry et al. 2008). RUNX3 promotes the CD8+ lineage fate in developing thymocytes. In the CD4+ thymocyte lineage in mice, the transcription factor ThPOK induces transcription of SOCS family members, which repress Runx3 expression (Luckey et al. 2014). RUNX3, along with RUNX1 and ETS1, is implicated in regulation of transcription of the CD6 gene, encoding a lymphocyte surface receptor expressed on developing and mature T cells (Arman et al. 2009). RUNX3 and ThPOK regulate intestinal CD4+ T cell immunity in a TGF-beta and retinoic acid-dependent manner, which is important for cellular defense against intestinal pathogens (Reis et al. 2013). Besides T lymphocytes, RUNX3 is a key transcription factor in the commitment of innate lymphoid cells ILC1 and ILC3 (Ebihara et al. 2015). RUNX3 regulates expression of CD11A and CD49D integrin genes, involved in immune and inflammatory responses (Dominguez-Soto et al. 2005). RUNX3 is involved in mouse TGF-beta-mediated dendritic cell function and its deficiency is linked to airway inflammation (Fainaru et al. 2004).
In addition to its developmental role, RUNX3 is implicated as a tumor suppressor. The loss of RUNX3 expression and function was first causally linked to the genesis and progression of human gastric cancer (Li et al. 2002). Expression of RUNX3 increases in human pancreatic islet of Langerhans cells but not in pancreatic adenocarcinoma cells in response to differentiation stimulus (serum withdrawal) (Levkovitz et al. 2010). Hypermethylation of the RUNX3 gene is associated with an increased risk for progression of Barrett's esophagus to esophageal adenocarcinoma (Schulmann et al. 2005). Hypermethylation-mediated silencing of the RUNX3 gene expression is also frequent in granulosa cell tumors (Dhillon et al. 2004) and has also been reported in colon cancer (Weisenberger et al. 2006), breast cancer (Lau et al. 2006, Huang et al. 2012), bladder cancer (Wolff et al. 2008) and gastric cancer (Li et al. 2002). In colorectal cancer, RUNX3 is one of the five markers in a gene panel used to classify CpG island methylator phenotype (CIMP+) (Weisenberger et al. 2006).

RUNX3 and CBFB are frequently downregulated in gastric cancer. RUNX3 cooperates with TGF-beta to maintain homeostasis in the stomach and is involved in TGF-beta-induced cell cycle arrest of stomach epithelial cells. Runx3 knockout mice exhibit decreased sensitivity to TGF-beta and develop gastric epithelial hyperplasia (Li et al. 2002, Chi et al. 2005). RUNX3-mediated inhibition of binding of TEADs:YAP1 complexes to target promoters is also implicated in gastric cancer suppression (Qiao et al. 2016).

RUNX3 is a negative regulator of NOTCH signaling and RUNX3-mediated inhibition of NOTCH activity may play a tumor suppressor role in hepatocellular carcinoma (Gao et al. 2010, Nishina et al. 2011).

In addition to RUNX3 silencing through promoter hypermethylation in breast cancer (Lau et al. 2006), Runx3+/- mice are predisposed to breast cancer development. RUNX3 downregulates estrogen receptor alpha (ESR1) protein levels in a proteasome-dependent manner (Huang et al. 2012).

Besides its tumor suppressor role, mainly manifested through its negative effect on cell proliferation, RUNX3 can promote cancer cell invasion by stimulating expression of genes involved in metastasis, such as osteopontin (SPP1) (Whittle et al. 2015).

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author/Editor</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016-12-13</td>
<td>Authored</td>
<td>Orlic-Milacic, M.</td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Reviewed</td>
<td>Ito, Y., Chuang, LS.</td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Edited</td>
<td>Orlic-Milacic, M.</td>
</tr>
</tbody>
</table>
**Regulation of RUNX3 expression and activity**

**Location:** Transcriptional regulation by RUNX3

**Stable identifier:** R-HSA-8941858

RUNX3, like other RUNX family members, is transcribed from two promoters - the proximal P2 promoter and the distal P1 promoter. The P2 promoter is positioned within a large CpG island that is frequently methylated in solid tumors, resulting in epigenetic inactivation of the RUNX3 gene (reviewed by Levanon and Groner 2004). RUNX3 transcription is affected by SMAD4 levels. RUNX3 may directly upregulate its own transcription through a positive feedback loop (Whittle et al. 2015). Under hypoxic conditions, RUNX3 transcription is downregulated. Hypoxic silencing of RUNX3 involves hypoxia-induced upregulation of the histone methyltransferase G9a and histone deacetylase HDAC1, which leads to increased dimethylation of histone H3 at lysine residue K9 (K10 when taking into account the initiator methionine) and reduced acetylation of histone H3 at the RUNX3 promoter (Lee et al. 2009).

RUNX3 protein levels are inversely related to the levels of microRNA miR-130b. Based on in silico analysis, RUNX3 is predicted to be the target of miR-130b, but binding assays and 3'UTR reporter assays have not been done to confirm this (Lai et al. 2010, Paudel et al. 2016).

Similar to RUNX1 and RUNX2, RUNX3 forms a transcriptionally active heterodimer with CBFB (CBF-beta) (Kim et al. 2013). RUNX3 activity can be regulated by changes in RUNX3 localization. SRC protein tyrosine kinase phosphorylates RUNX3 on multiple tyrosine residues, inhibiting its translocation from the cytosol to the nucleus and thus inhibiting RUNX3-mediated transcription (Goh et al. 2010). Subcellular localization of RUNX3 may be affected by PIM1-mediated phosphorylation (Kim et al. 2008).

The P1 and P2 promoters regulate RUNX3 transcription in a cell-type/differentiation dependent manner, giving rise to the p44 and p46 isoforms of RUNX3, respectively. Several splicing isoforms have also been reported. One example is the generation of a 33 kDa protein isoform (p33) by alternative splicing. The RUNX3 p33 isoform lacks the Runt domain and is unable to transactivate the regulatory regions of integrin genes. The p33 isoform is induced during maturation of monocyte-derived dendritic cells (MDCD), leading to reduced expression of genes involved in inflammatory responses, such as IL8 (interleukin-8).
E3 ubiquitin ligases MDM2 (Chi et al. 2009), SMURF1 and SMURF2 (Jin et al. 2004) are implicated in RUNX3 polyubiquitination and degradation.

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016-12-13</td>
<td>Authored</td>
<td>Orlic-Milacic, M.</td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Reviewed</td>
<td>Ito, Y., Chuang, LS.</td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Edited</td>
<td>Orlic-Milacic, M.</td>
</tr>
</tbody>
</table>
RUNX3 contributes to the upregulation of the CDKN1A (p21) gene transcription in response to TGF-beta (TGFB1) signaling. RUNX3 binds to SMAD3 and SMAD4, and cooperates with the activated SMAD3:SMAD4 complex in transactivation of CDKN1A. Runx3 knockout mice exhibit decreased sensitivity to TGF-beta and develop gastric epithelial hyperplasia (Chi et al. 2005). In response to TGF-beta signaling, the CBFB:RUNX3 complex binds to the tumor suppressor ZFHX3 (ATBF1) and, through an unknown mechanism, this complex positively regulates the CDKN1A transcription (Mabuchi et al. 2010).

In addition, RUNX3 may act as a TP53 co-factor, stimulating TP53-mediated transcription of target genes, including CDKN1A (p21) (Yamada et al. 2010).

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016-12-13</td>
<td>Authored</td>
<td>Orlic-Milacic, M.</td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Reviewed</td>
<td>Ito, Y., Chuang, LS.</td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Edited</td>
<td>Orlic-Milacic, M.</td>
</tr>
</tbody>
</table>
RUNX3 negatively regulates NOTCH signaling, which contributes to the tumor suppressor role of RUNX3 in hepatocellular carcinoma. RUNX3 binds the promoter of the JAG1 gene, encoding NOTCH ligand JAG1 and inhibits its transcription (Nishina et al. 2011). In addition, RUNX3 also binds to the NOTCH1 coactivator complex at the promoter of HES1, a NOTCH target gene, and inhibits HES1 transcription (Gao et al. 2010).

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author/Reviewer</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016-12-13</td>
<td>Authored</td>
<td>Orlic-Milacic, M.</td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Reviewed</td>
<td>Ito, Y., Chuang, LS.</td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Edited</td>
<td>Orlic-Milacic, M.</td>
</tr>
</tbody>
</table>
RUNX3 Regulates Immune Response and Cell Migration

**Location:** Transcriptional regulation by RUNX3

**Stable identifier:** R-HSA-8949275

RUNX3-mediated transcription regulates development of immune system cells. RUNX3 is necessary for the development of innate lymphoid cells (ILCs) of ILC1 and ILC3 lineages, which reside in the mucosa and are involved in response to external pathogens. RUNX3 exerts its role in the development of ILC1 and ILC3 lineages by stimulating expression of the RORC (RORgamma) gene, encoding nuclear retinoid-related orphan receptor-gamma (Ebihara et al. 2015).

RUNX3 regulates transcription of integrin genes ITGAL (CD11a) and ITGA4 (CD49d), involved in transendothelial migration of leukocytes during immune and inflammatory responses as well as co-stimulation of T cells (Domínguez-Soto et al. 2005). The RUNX3 splicing isoform p33 lacks the Runt domain and is unable to transactivate integrin genes. The p33 isoform is induced during maturation of monocyte-derived dendritic cells (MDDC), leading to reduced expression of genes involved in inflammatory responses, such as IL8 (interleukin-8) (Puig-Kröger et al. 2010).

RUNX3 positively regulates transcription of the SPP1 (osteopontin) gene, which contributes to invasiveness of pancreatic cancer cells (Whittle et al. 2015).

**Literature references**


RUNX3 regulates WNT signaling

**Location:** Transcriptional regulation by RUNX

**Stable identifier:** R-HSA-8951430

RUNX3 binds to complexes of beta-catenin (CTNNB1) and TCF/LEF family members. Binding of RUNX3 to CTNNB1:TCF/LEF complexes prevents their loading onto cyclin D1 (CCND1) and MYC gene promoters and interferes with WNT signaling-mediated activation of CCND1 and MYC1 transcription. RUNX3 therefore inhibits WNT-induced cellular proliferation (Ito et al. 2008).

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016-12-13</td>
<td>Authored</td>
<td>Orlic-Milacic, M.</td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Reviewed</td>
<td>Ito, Y., Chuang, LS.</td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Edited</td>
<td>Orlic-Milacic, M.</td>
</tr>
</tbody>
</table>
RUNX3 regulates YAP1-mediated transcription

**Location:** Transcriptional regulation by RUNX3

**Stable identifier:** R-HSA-8951671

Association of RUNX3 with the TEADs:YAP1 complex inhibits loading of the TEADs:YAP1 to the CTGF promoter, thus negatively regulating transcription of the CTGF gene which encodes the Connective tissue growth factor (Yagi et al. 1999, Zhao et al. 2008, Qiao et al. 2016).

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016-12-13</td>
<td>Authored</td>
<td>Orlic-Milacic, M.</td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Reviewed</td>
<td>Ito, Y., Chuang, LS.</td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Edited</td>
<td>Orlic-Milacic, M.</td>
</tr>
</tbody>
</table>
RUNX3 binds to Runx response elements in the distal (P1) promoter of the RUNX1 gene, repressing RUNX1 transcription (Spender et al. 2005).

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016-12-13</td>
<td>Authored</td>
<td>Orlic-Milacic, M.</td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Reviewed</td>
<td>Ito, Y., Chuang, LS.</td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Edited</td>
<td>Orlic-Milacic, M.</td>
</tr>
</tbody>
</table>
Acetylation of RUNX3 by the histone acetyl transferase p300 (EP300) and the subsequent association of acetylated RUNX3 with BRD2 correlates with upregulation of p14-ARF transcription from the CDKN2A locus. Cyclin D1 (CCND1) negatively regulates RUNX3-facilitated induction of p14-ARF by recruiting histone deacetylase HDAC4 to RUNX3, leading to RUNX3 deacetylation (Lee et al. 2013).

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016-12-13</td>
<td>Authored</td>
<td>Orlic-Milacic, M.</td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Reviewed</td>
<td>Ito, Y., Chuang, LS.</td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Edited</td>
<td>Orlic-Milacic, M.</td>
</tr>
</tbody>
</table>
RUNX3 regulates BCL2L11 (BIM) transcription

**Location:** Transcriptional regulation by RUNX3

**Stable identifier:** R-HSA-8952158

In response to TGF-beta signaling, RUNX3, in cooperation with activated SMADs and FOXO3A, induces transcription of the pro-apoptotic gene BCL2L11 (BIM) (Wildey et al. 2003, Yano et al. 2006, Yamamura et al. 2006).

**Literature references**


**Editions**

<table>
<thead>
<tr>
<th>Edition</th>
<th>Authored</th>
<th>Reviewed</th>
<th>Edited</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016-12-13</td>
<td>Orlic-Milacic, M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Ito, Y., Chuang, LS.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2017-01-31</td>
<td>Orlic-Milacic, M.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table of Contents

Introduction 1
- Transcriptional regulation by RUNX3 2
  - Regulation of RUNX3 expression and activity 4
  - RUNX3 regulates CDKN1A transcription 6
  - RUNX3 regulates NOTCH signaling 7
  - RUNX3 Regulates Immune Response and Cell Migration 8
  - RUNX3 regulates WNT signaling 10
  - RUNX3 regulates YAP1-mediated transcription 11
  - RUNX3 regulates RUNX1-mediated transcription 12
  - RUNX3 regulates p14-ARF 13
  - RUNX3 regulates BCL2L11 (BIM) transcription 14

Table of Contents 15