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IN SILICO ANALYSIS OF PAX5 INTERACTING PROTEINS

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Abstract

The Pax5, a paired box 5 transcription factor, has been shown important for development of B-cells and brain. It also regulates expression of genes involved in receptor signalling, adhesion, migration, transcriptional control, and immune function. The Pax5 has been proposed to interact with Ets proteins, Runx1, c-Myb and Id proteins. However, the studies on Pax5 interacting proteins are very limited. *In silico* analysis revealed interaction of Pax5 with number of proteins essential for diverse biological functions including neurogenesis, neuronal and glial differentiation, inflammatory response, B-cell development in hematopoietic tissues and proteins of non-hematopoietic tissues.

Keywords: Pax5, Interacting proteins, Transcription

Introduction

The Pax5 belongs to a family of paired box transcription factors. It has been implicated in midbrain patterning, B-cell development and lymphomagenesis (Eberhard et al., 2000). The Pax5 was not detectable in multi-potent progenitors and majority of common lymphoid progenitors (Fuxa et al., 2007). It regulates differentiation and identity of B-lymphocytes (Holmes et al., 2008; Nutt and Kee, 2007; Pridans et al., 2008). The targeting of Pax5 in mice cause defect in midbrain, abolition of lymphopoiesis and incomplete V-H gene recombination in adult bone marrow (Nutt et al., 1997; Kovac et al., 2000; Hocher et al., 2001). The pre-BI precursor cells from Pax5-deficient mice can give rise to a variety of non-B cell types, including macrophages, granulocytes, osteoclasts, T cells, and natural killer cells (Nutt et al., 1999; Heavey et al., 2003; Holmes et al., 2006).

Pax5 interacts with number of genes involved in humoral immunity

The Pax5 contains paired- and partial homeodomain octapeptide motif, trans-activation and inhibitory domain. Paired domain independently binds to a distinct half-site of consensus sequence (Czerny et al., 1993; Garvie et al., 2001). The Pax5 affects activities of proteins (Figure 1) involved in receptor signalling, cell-adhesion, migration, transcriptional control, and immune function (Schebesta et al., 2007; McManus et al., 2011; Revilla-i-Domingo et al., 2012). It also forms complexes with basal transcription factor complex TFIID, the chromatin-remodelling BAF, the histone

acetyltransferase CBP and the PTIP protein (McManus et al., 2011). In B-cells, BLNK and CD19 show only Pax5-dependent expression. The promoters of both genes contain a high-affinity Pax5-binding site upstream of a cluster of transcription start sites (Kozmik et al., 1992; Nutt et al., 1999; Schebesta et al., 2002; Imoto et al., 2016).

The Pax5 exerts repressor function by interacting with groucho related gene product Grf4 through its octapeptide motif and C-terminal trans-activation domain (Eberhard et al., 2000). It interacts with other sequence specific transcription factors including Ets proteins, Runx1, c-Myb and Id proteins. The interactions between Pax5 and Runx1 or c-Myb and Ets family members affect gene expression through cooperative DNA binding (Nutt et al., 1997; Garvie et al., 2001; John et al., 2008; Maier et al., 2004; Gonda et al., 2003; Kishi et al., 2002).

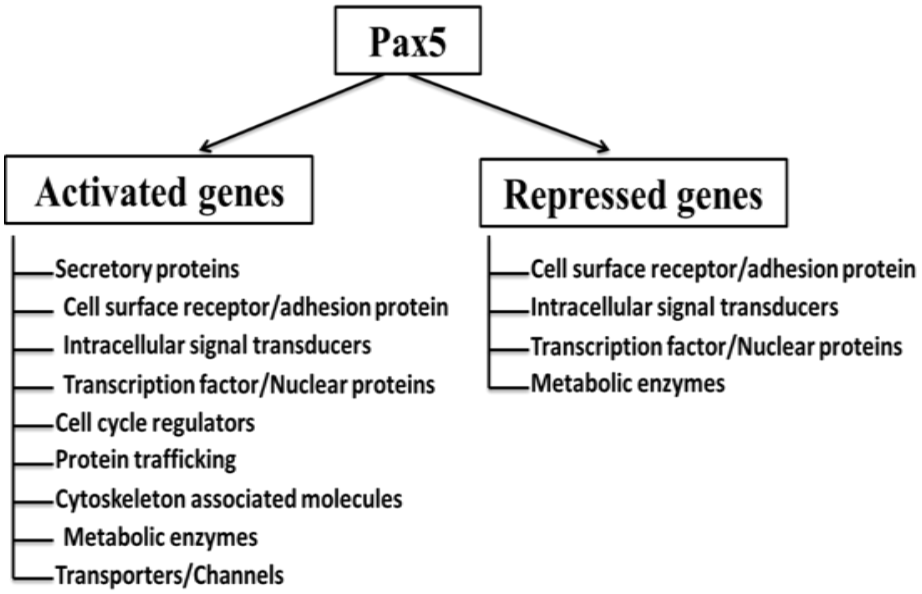

Figure 1: Representative flow chart of Pax5 activated and repressed genes functions

The binding of Pax5 to the CD79a promoter is inhibited by Id proteins (Id1-3) due to antagonizing Aicda promoter (Gonda et al., 2003; Xu et al., 2007; Holmes et al., 2008). The binding of Pax5 to enhancer and promoter region may be either direct binding of Pax5 or indirect by co-operative binding (Figure 2).

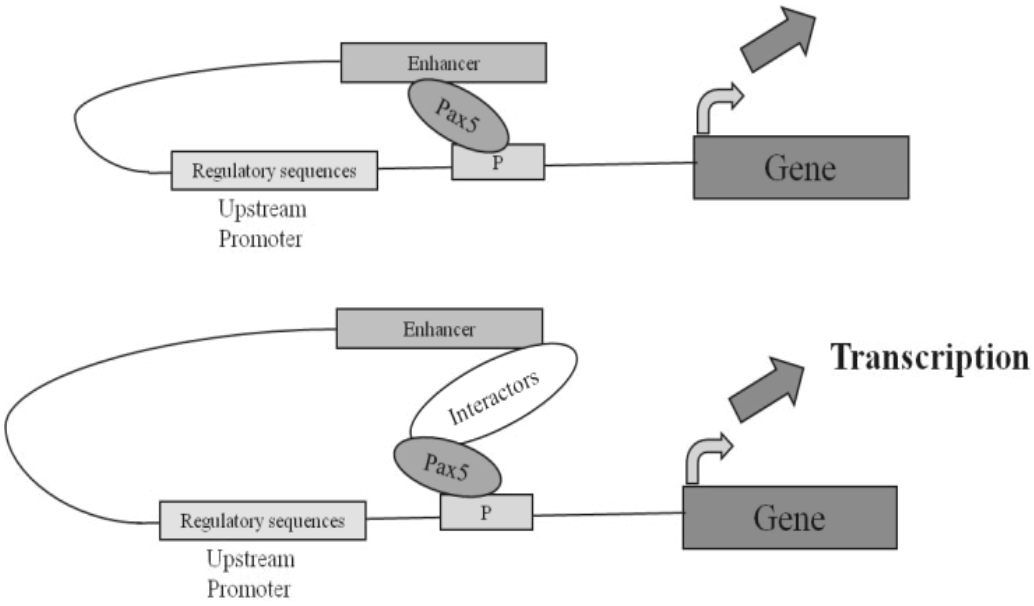

Figure 2: Diagrammatic representation of possible mechanism of action of Pax5 in transcriptional regulation of gene. The Pax5 could either binds to the regulatory sequence elements of gene or with putative interactors. The Pax5-interacting protein complex then binds to the regulatory sequences of gene and influences transcription of gene.

The *in silico* analysis reveals that Pax5 interacts with proteins of diverse biological functions including macromolecules of metabolic process, regulation of hemopoiessis, leukocyte differentiation, regulation of lymphocyte activation, B-cell activation, T-cell differentiation, NK cell differentiation, erythrocyte differentiation, mitotic cell cycle, neurogenesis, neurons differentiation and development, glial-cell differentiation, and inflammatory responses. Based on the experimental evidences, curated database, gene fusions, gene neighbourhood, co-localization, protein homology and textimining, some of the putative inetractors of Pax5 have been proposed. Among them, Myeloblastosis oncogene (Myb) plays an important role in the control of proliferation and differentiation of hematopoietic progenitor cells. Lymphoid enhancer binding factor 1 (Lef1) participates in Wnt signalling pathway. The transducin-like enhancer of split 4 (Tle4) and homologue of Drosophila E (spl) act as a transcriptional co-repressor that binds to number of transcription factor including Pax5 and inhibits transcriptional activation mediated by Pax5. The CD79a antigen (immunoglobulin-associated alpha) which is required for B-cell antigen receptor complex (BCR) helps in internalization of the complex, trafficking to late endosomes and antigen presentation. Colony stimulating factor 1 receptor (Csfr1) tyrosine-protein kinase acts as cell-surface receptor for CSF1 and IL34 to regulate survival, proliferation and differentiation of hematopoietic precursor cells. The Pax5 may also promote the release of pro-

inflammatory chemokines in response to IL34 and CSF1 important for innate immunity.

The interaction of Pax5 with catalytic component of the Rag1 (Recombination activating gene 1) mediates the DNA cleavage phase during V(D)J recombination. However, interaction with transformation related protein 53 (Trp53), Forkhead box O1 (Foxo 1), Mitogen-activated protein kinase 8 (Mapk8), serine/threonine-protein kinase, stress-activated protein kinase/c-Jun N-terminal kinase (SAP/JNK), CREB binding protein (Crebbp) shows its involvement in cell proliferation, differentiation, migration, transformation and programmed cell death in response to extracellular stimuli such as proinflammatory cytokines or physical stress. The interaction with protein tyrosine phosphatase, receptor type C (Ptprc) facilitates activation of T-cell but GATA binding protein 1 (Gata 1) transcriptional activator serves as a general switch factor for erythroid development. Pax5 promotes phosphorylation of SHC1 and AKT1, and activation of the downstream effectors MTOR, RAS, MAPK1/ERK2 and/or MAPK3/ERK1 signalling. It inhibits the activation of NF-kappa-B by TNF and IKKB. Glial cell line derived neurotrophic factor (Gdnf) enhances survival and morphological differentiation of dopaminergic neurons and increases their high-affinity dopamine uptake. Signal transducer and activator of transcription 6 (Stat6) carries out a dual function: signal transduction and activation of transcription including interleukin-4 signaling.

The *in silico* analysis of Pax5 interacting proteins indicates its association with transcriptional regulation and signalling pathways important for leukemia and other cancers, Wnt signalling, thyroid signalling, cytokine-receptor signalling, PI3K-AKT, JAK-STAT, Notch, MAPK, TGF-β,TNF, NF-κB, Ras, Apoptosis, BCR, Neurotrophin, Amyotrophic lateral sclerosis, Insulin, Toll-like receptor, and FOXO signalling. Apart from its role in biological activities of B-cell, Pax5 could be a regulator of diverse biological function including neurogenesis, inflammatory response and glial-cell differentiation in immune-privileged brain. Evaluation of Pax5 interacting proteins by suitable experimental approaches like co-immunoprecipitaion (co-IP), co-localization and chromatin-immunoprecipitation (ChIP) may decipher stable or transient interactions in hematopoietic and non-hematopoietic tissues.

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