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PUTATIVE ROLE OF PAX5 IN IMMUNOLOGICAL SURVEILLANCE OF BRAIN

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Abstract

The *Pax5*, a member of the Paired box (Pax) gene family and B-cell Specific Activator Protein (BSAP), is expressed in brain, lymph nodes, spleen, B-lymphocytes and testes. It is also referred as redox-sensitive transcription factor because of its homology with oxido-reductases. Four alternatively spliced isoforms of *Pax5* in mouse and eleven isoforms in human have been detected responsible for B-cell development and malignancies, neuro-endrocrine tumors and astrocytomas. The Pax5 induces re-arrangement and locus contraction of immunoglobulin heavy-chain gene and conditional loss of *Pax5* results in the conversion of mature B-cells into functional T-cells by de-differentiation. In brain, the microglia serves as innate immune cells and release growth factors for healing of neurons and function as neuro-protective agent. However, in acute and chronic state of signal, microglia release cytokines like TNF-α, IFN-gamma, reactive oxygen species (ROS), nitric oxide (NO) that induce neurotoxicity and lead to neurodegeneration. The malfunctioning of microglia turns on recruitment of lymphocytes from blood at damaged sites. However, B-cell which normally resides in brain parenchyma increases in number and shows activated phenotype in pre-AIDS patients. The presence of lymphatic vessels in brain meningeal compartment lining also suggests connection of brain with peripheral lymphatic system. Therefore, it is presumed that Pax5 or its isoforms may be critical for immunological surveillance in brain by interacting with genes/proteins of neurons, microglia and astrocytes. It is likely that neuronal Pax5 may be implicated in de-differentiation of lymphocyte through ZAP-70, or microglia-mediated neuroinflammation and immunity.

Keywords: Pax5, Brain, Immunity, Microglia, Lymphocytes

Introduction

Brain is known as an immune-privileged organ separated from rest of the body through blood brain barrier (BBB). Like macrophages, unique microglia serves as innate immune cells in brain. The microglia responds to pathogens and injury. They accumulate in regions of degeneration and produce a wide variety of cytokines which maintain brain homeostasis (Cserr and Knoff, 1992; Hickey, 2001; Hickey and Kimura, 2001). They exhibit dual functions. When signal is short and moderate, microglia shows neuro-protective phenotype to clear cell debris by phagocytosis and release growth factors for healing of neurons. In acute and chronic signal, microglia release pro-inflammatory cytokines like TNF-α, IFN-gamma, reactive oxygen species (ROS),

nitric oxide (NO), all of which endanger neuronal damage. The malfunctioning of microglia results in recruitment of lymphocytes from blood to damage sites which further maintain brain homeostasis (London et al., 2013). The local microenvironment like neurons and astrocytes play an integral role in regulating microglia phenotype via interaction with both soluble and membrane bound mediators. Neurotropic factors released by neurons contribute to the down-regulated state of microglia and dampen inflammation in the central nervous system (Neumann et al., 1998). An important consequence of these interactions between neurons and microglia is that in conditions where neurons degenerate, (either acute or chronic neurodegenerative disease), the microglia gets released from the tonic inhibition provided by neurons and show activated phenotype. There is also evidence that in animal models mimicking aspects of human neurodegenerative disease, the neuropathology may be enhanced by blockade of CD200–CD200R interactions (Zhang et al., 2011) or deletion of CX3CR1 (Bhaskar et al., 2010).

The *Pax5* gene, located on chromosome 9p13, encodes Pax5 transcription factor which is critical for B-lymphoid cell commitment (Cobaleda et al., 2007a) and known as the B-cell specific activator protein (BSAP). It is expressed in spleen (Bharti et al., 2015), lymph nodes, B-cells (Nutt et al., 1999), midbrain–hindbrain boundary (Torlakovic et al., 2006) and adult testes (Adams et al., 1992). It has been detected from the pro-B to the mature lympho-plasmocytoid B-cell but not detected in the plasma cells (Adams et al., 1992; Torlakovic et al., 2002; Nutt et al., 2001; Souabni et al., 2002; Delogu et al., 2006). It activates B-cells specific gene during B-cell development but represses B-cell inappropriate genes (Souabni et al., 2002). The human *PAX5* gene is composed of nine common exons and one of two alternative initial exons, known as exons 1A and 1B (Busslinger et al., 1996). Alternative splicing generates 4 transcripts of *Pax5* in murine (Fuxa and Busslinger, 2007; Nutt et al., 1999) and 11 isoforms of *PAX5* in human. The Pax5 protein is composed of paired domain, an octapeptide motif, a partial homeodomain, and functionally conserved transactivation and inhibition domains (Dofler et al., 1996). It is highly conserved between human and mouse (Adams et al., 1992). The conditional loss of *Pax5* results in the conversion of mature B-cells into functional T-cells by dedifferentiation to uncommitted progenitors in the bone marrow (Cobaleda et al., 2007b; Cozma et al., 2007; Fuxa and Busslinger, 2007; Nutt et al., 1999). The *Pax5*, also an oncogene, is often implicated in chromosomal aberrations of different B-cell malignancies. The *Pax5* activates *CD97, CD44, Capn2, Esp8, CD19, CD21, Blnk* and repress *Flt3, CD28, Ccr2* by binding to promoter and putative enhancer present in the intronic region of genes (Adams et al., 1992; Nutt et al., 1999; Pridans et al., 2008). The isoforms of Pax5, and T-cell and B-cell associated genes also influence phenotypic traits of ascitic cells causing Dalton's lymphoma (Bharti and Mishra, 2011). The human *PAX5* variants

in normal and cancerous B-lymphocytes are due to deletion of exon 2, exons 3, exon 5, exon 7, exon 8, and exon 9 (Robichaud et al., 2004). In murine, *Pax5* variants are due to deletion of exon 2 and replacement of transactivation domain with novel sequence (Zwollo et al., 1997). In both, human and murine, full length *Pax5*a is predominant whereas expression of other transcripts varies. The full length *Pax5* expression is regulated by expression of different transcript of *Pax5* and presence of novel sequence of 42aa has significant homology with a number of oxido-reductase molecules (Lowen and Zwollo, 2001). However, in humans, novel sequence at C-terminal are encoded due to shift in reading frame and exhibit differential transactivation properties in dual luciferase assay for *Pax5* dependent transactivation of the CD19 promoter. The PST (proline-serine-threonine) region in transactivation domain of *Pax5* also varies in transcripts which further influence its transactivational properties (Robichaud et al., 2004; Palmisano et al., 2003). In brain, *Pax5* expression is reported in neurons of medulla oblongata and midbrain (Torlakovic et al., 2006) and increased oxidative stress is also reported in brain of immune-challenged mice (Bharti and Mishra, 2008).

The Pax5 serves as molecular toggle for development and de-differentiation of B-cell

During normal B-cell development in the bone marrow, productive *Igh* gene re-arrangement results in pre-BCR assembly and signalling in pre-B cells, triggering their clonal expansion (Rolink et al., 2000). These cycling pre-B-cells then exit the cells which upon productive re-arrangement of the immunoglobulin light chain gene Igl or Igk allows the surface IgM expression characteristics of immature B-cells. The Pax5 activates B-lineage specific gene expression networks while suppress alternative lineage genes cells (Rolink et al., 1999). Nutt et al. (1999) demonstrated that pro-B-cells lacking *Pax5* are incapable of *in vitro* B-cell differentiation unless *Pax5* expression are restored by retroviral transduction. The *Pax5* deficient chicken B-cell line found to exhibit slow growth, decreases surface IgM expression and cause loss of BCR signalling (Nutt et al., 1999). Cobaleda et al. (2007b) showed that conditional deletion of Pax5 in mice allowed mature B-cell from peripheral lymphoid organs to dedifferentiate *in vivo* back to early uncommitted progenitors in the bone marrow, which rescued T-lymphopoiesis in the thymus of T-cell deficient mice (Cobaleda et al., 2007b). Cozma et al. (2007) revealed that *Pax5* is required to maintain expression of several components of BCR signalling, including the immunoreceptor tyrosine-based activation motif (ITAM) - containing CD79a (Cozma et al., 2007). Thus, *Pax5* expression is necessary for the maturation of B-cell and if in mature B-cell, *Pax5* expression decreases, mature B-cell become plasma cells. The *Pax5* mediates commitment to B-cell lineage is by repressing genes and signalling pathway required for development of other lineages commitment like Notch1 (Figure 1).

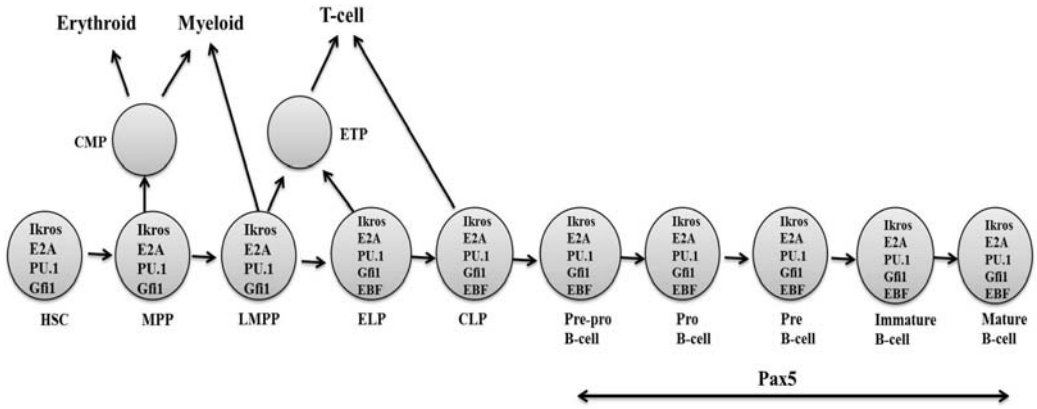

Figure1 : Schematic representation of role of Pax5 in development of B-cell lineages.

HSC=Hematopoietic stem cell, MPP=Multipotent Progenitors, LMPP=lymphoid-primed Multipotent Progenitor, ELP=Early Lymphoid Progenitors, CLP=Common Lymphoid Progenitor, CMP= Common Myeloid Progenitor, ETP= Early Thymocyte Progenitors, EBF1= Early B-cell factor1, Gfi1= Growth factor independent 1.

Pax5 regulates promoter of activation-induced cytidine-deaminase (AID)

In *Pax5*-deficient mice, B cell development is arrested at an early pro-B cell stage in the bone marrow (Urbanek et al., 1994), and pro-B cells of *Pax5*-/- mice show characteristics of uncommitted progenitors by retaining a broad lympho-myeloid developmental potential (Nutt et al., 1999). This multi-lineage potential is suppressed by restoration of *Pax5* expression, which also rescues the pro-B cell arrest of *Pax5*-/- mice. Conditional inactivation of *Pax5* leads to the total loss of identity and function of mature mouse B cells (Horcher et al., 2001). The Pax5 binds to promoter of activation-induced cytidine-deaminase (AID) (Gonda et al., 2003) that indicates active and direct role of Pax5 in the regulation of AID expression which is indispensable for somatic hypermutation (SHM), class switch recombination (CSR), and immunoglobulin gene conversion (Muramatsu et al., 2010) for diversification of rearranged Ig genes to produce different isotypes of high-affinity antibodies. Since the Blimp-1 down-regulates AID (Shaffer et al., 2002), loss of functional B-cell identity was observed due to absence or very low B Cell Receptor (BCR) and up-regulated Blimp-I which down-regulates transcription factor essential for BCR signalling and XBP-1, required for induction of Ig secretion. The microarray analysis uncovered role of *Pax5* in the control of B-cell adhesion and migration by regulating the expression of cell-surface receptors and intracellular signal transducers for remodelling of the actin cytoskeleton.

The Pax5 also regulates expression of *Tnfrsf13c, Prkcb1, T2bp, and Pea15* which are regulators of the NF-kB pathway responsible for B-cell survival by activating anti-apoptotic genes in response to stimulation of the BCR or TNF receptor family members (Schebesta et al., 2007).

Pax5 and immunological surveillance of brain

Presence of lymphocytes in brain was a major debate as brain is considered as an immune-privileged organ devoid of lymphatic vessels. Later on, it was shown that in pre-AIDS patients, number of B-cell increases which normally resides in brain parenchyma and shows an activated phenotype (Anthony et al., 2003). Recently, discovery of lymphatic vessels in brain meningeal compartment lining dural sinus, suggests connection of brain with peripheral lymphatic system. It is found to start from both eyes and track above olfactory bulb before aligning adjacent to the sinuses and drain fluids and cells from meninges/cerebrospinal fluid into the deep cervical lymph node. Meningeal lymphatic vessels are devoid of smooth muscles and are positive for T-cells and MHCII and endothelial cell marker, CD31 which are supposed to be capable of responding to the antigen (Louveau et al., 2015). In brain, the neuronal Pax5 is predominant in neurons. It would be interesting to look into neuronal Pax5 (Figure 2) either for direct relation with genes or proteins of microglia or it is indirectly influencing brain immunity by interacting with lymphocytes and macrophages residing in brain or infiltration of lymphocytes during inflammation. The investigation on role of *Zap70* in de-differentiation can also not be ignored.

During haematopoiesis, transcription factor Pax5 regulates development of B-cell lineage responsible for humoral immunity. In case of Pax5-/-, committed B-cell lineage dedifferentiates to T-cell lineage and contributes to cell-mediated immunity. In brain, Pax5 expression is predominantly found in neurons where it is proposed to modulate brain immunity through microglia derived from yolk sac macrophages which invaded neuroepithelium during embryonic development. In healthy brain, microglia activated phenotype is regulated by ligand-receptor binding interaction present on neurons and microglia, respectively. It performs regular surveillance and secretes neurotrophic factors which are important for neuronal growth and development and functioning. In case of acute or chronic infection or injury, microglia shows activated phenotype either by releasing anti-inflammatory cytokines like TGFβ, Arg1 or by releasing inflammatory cytokines like IFN-γ, TNF and oxidative stress molecules like ROS, NO which cause neuronal degradation and loss.

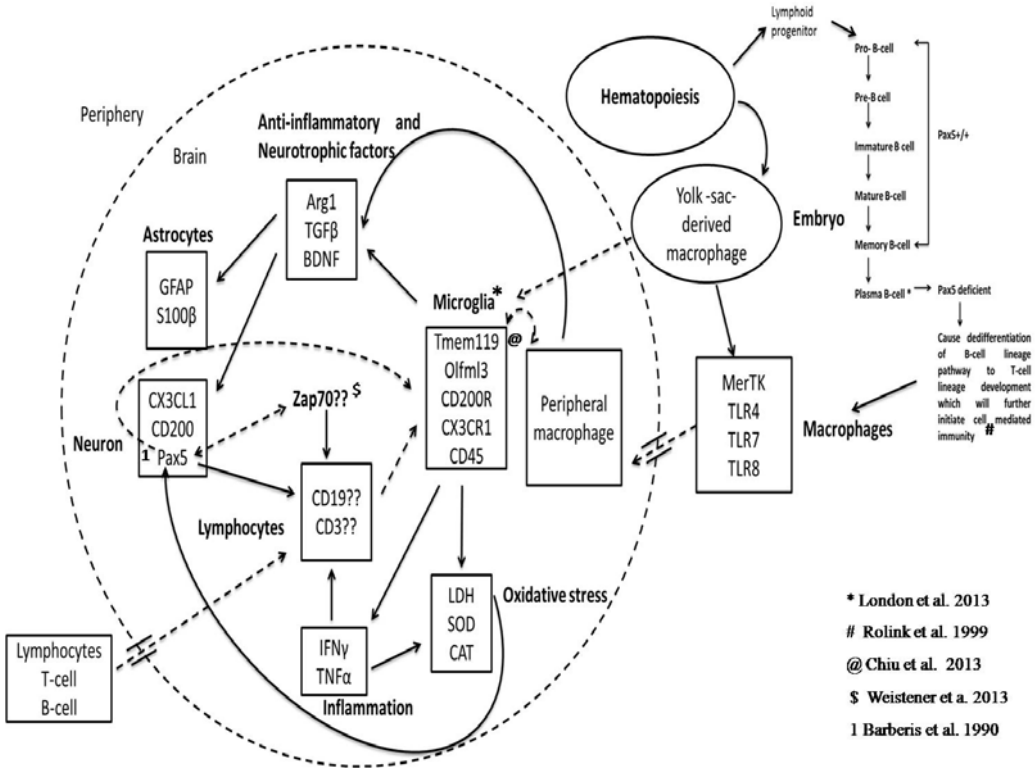

Figure 2 : Proposed role of neuronal Pax5 in brain

Conclusion:

The neuronal Pax5, expressed in immune-privileged brain, may serve as molecular toggle for immunological surveillance in brain by interacting with genes/proteins involved in neuro-inflammation, or microglia-mediated immunity. It may directly regulate re-arrangement of immunoglobulin genes of B cells normally residing in brain or may promote dedifferentiation of lymphocytes through ZAP-70. Since malfunctioning of microglia induces recruitment of lymphocytes from blood at sites of inflammation and degeneration, and B cells of brain increase in number and show activated phenotype, neuronal Pax5 is presumed may affect microglia mediated neuro-protective as well neurodegenerative functions, indirectly.

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