TRANSCRIPTION FACTOR ZBTB20: WHAT EXPRESSION IS TELLING US OF ITS CELLULAR FUNCTION?

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*Transcription factors are key regulators of cell fate maintenance and transition. Thus, they represent key molecular targets in the fields of stem cell biology and regenerative medicine. The constant need to generate protocols with higher stem cell yield, faster and more efficient differentiation led to in-depth understanding of the function of many transcription factors in regulating developmental and adult stem cells. In this Dance Round we summarize the current understanding of the expression of a zinc finger and BTB domain-containing transcription factor ZBTB20, and comment on how its expression provides clues for its involvement in stem cell biology. **Biomed Rev 2020; 31: 1-10***

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INTRODUCTION

During normal development, stem/progenitor cells undergo multiple divisions and gradual fate restrictions eventually differentiating and leading to the generation of adult tissues. While development progresses progenitors switch between several modes of division to ensure proper balance between the dividing cell pool and the differentiated cell output (1–5). In adults, stem cells reside in specific tissue microdomains named “stem cell niches”. A fraction of adult stem cells are quiescent and these are triggered to proliferate upon activation

such as injury (6–8). Exit from quiescence is accompanied by a switch of the genetic programs in quiescent *versus* activated stem cells (9–12). Among the well-established mechanisms to achieve this change in gene expression is delivered by a family of DNA binding proteins named transcription factors (TFs) (13–15). More than 3000 TFs are known to date and their classification is not in the scope of this *Dance Round*. TFs share similar structural features: (i) one or several DNA binding domains; (ii) domain(s) for protein-protein interactions. Here we discuss one particular TF, named ZBTB20,

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also known as HOF (16). ZBTB20 has its name because it contains both a BTB (Broad-Complex, *Tramtrack* and *Bric a brac*) domain in its N-terminal region which is responsible for homo- and heteromeric protein-protein interactions, and the C-terminal region is occupied by five C2H2 Zinc finger motifs making up the DNA binding domain of this protein (16–18). Zbtb20 was identified in dendritic cells and named DPZF (*d*endritic cell-derived BTB/*P*OZ *z*inc *f*inger protein) (19). Two transcripts are generated due to alternative splicing, named HOF^L (long), and HOF^S (short) lacking 73 amino acids from the N-terminus (16). The two isoforms are considered to have redundant function.

ZBTB20 EXPRESSION IN THE FOREBRAIN

The embryonic forebrain has several subdivisions. The dorsal-most part, known as the dorsal pallium (DP), is the progenitor zone of cortical excitatory neurons and is replaced by the neocortex in adulthood (Fig. 1A2). The medial pallium (Fig. 1A2, MP) will give rise to the hippocampus and includes morphologically distinct subdomains: dentate gyrus (DG) and Ammon's horn (CA) (20). The pallial *Zbtb20* expression is initiated as early as

embryonic day (E)11.5 in the ventral forebrain and spreads to the lateral pallium (Fig. 1A2; LP). By E14.5 the *Zbtb20* expression extends through the entire pallial ventricular zone (VZ) (Fig. 1A1), where it is preserved until late embryonic stage (E18.5). In the VZ of the DP expression is concomitant with markers of apical and basal radial glia progenitors (Fig. 1A1), including Pax6, Sox2, Coup-TFI (21,22). At early postnatal stages (P0–P4), the *Zbtb20* expression in the VZ is reduced but remains strong in the pyramidal neurons of neocortical layers 2–3 (Fig. 1A3) in which it disappears by P12 (22).

The earliest studies investigating *Zbtb20* reported a strong expression in the developing hippocampus throughout development (16). *Zbtb20* expression is observed in proliferating progenitors between E12.5–E17.5, and is gradually increased in postmitotic cells starting E14.5 (Fig. 1B1) (21,23). At late embryonic stages *Zbtb20* was observed in immature migrating hippocampal CA pyramidal neurons and DG neurons (16). In the hippocampus, *Zbtb20* exhibits the strongest expression in the CA1 sector (23). At postnatal and adult stages *Zbtb20* expression persists in the upper row of CA1 pyramidal cells (Fig. 1B2, inset) (16,24). In the DG, *Zbtb20* shows a mosaic

Figure 1. A summary of TF *Zbtb20* expression in the nervous system. **A** Expression in the neocortex. **A1** A schematic summary of the *Zbtb20* expression at different stages of neocortical development. In the pallial radial glia progenitors of VZ, *Zbtb20* expression is robust as early as E14. At early postnatal stages *Zbtb20* is localized to layer 2/3 glutamatergic neurons, while in the adult neocortex the expression in neurons is down-regulated. **A2** Histological visualization of *Zbtb20* mRNA in the developing mouse cortex at E12.5. The onset of pallial expression of *Zbtb20* is evident in the LP. Strong expression in the subpallium is also seen. **A3** Immunofluorescence staining of *Zbtb20* in the mouse cortex at stage P8. Note the strong signal in neocortical layers 2/3. **B** Expression in the hippocampus. **B1** A schematic summary of the expression at different stages of hippocampal development. In the hippocampal VZ, *Zbtb20* is evident as early as E12.5. At early postnatal stages the expression is strong in the dentate gyrus and the upper row of CA1–CA3 pyramidal neurons. In the adult hippocampus the expression in both dentate gyrus and CA sectors is diminished but still present. **B2** Immunofluorescence labeling of *Zbtb20* (green) in the mouse hippocampus at P8 (all nuclei are labeled by DAPI in blue). A higher magnification inset of the CA1 sector depicts the strong *Zbtb20* signal in the upper row pyramidal neurons (an asterisk outlines the lower pyramidal row). **C** Expression in SVZ brain stem cell niche. **C1** A schematic summary of the expression in brain stem cells at different stages of SVZ development. In the embryo and around birth, *Zbtb20* is expressed in ventricular radial glia apical progenitors. At postnatal and adult stages the SVZ niche is reorganized and *Zbtb20* is present in putative stem cells and transit-amplifying progenitors (B and C cells) at a higher level, with diminished expression in neuroblasts (A cells). Ependymal expression is also evident. **C2** Immunofluorescence labeling of *Zbtb20* (green) in the mouse SVZ at P12. A strong signal is observed in both the ependymal (EL) and subependymal (SEL) layers of the SVZ. **D** Expression in cortical macroglial cells. **D1** A schematic summary of the expression at different stages of glial development. Around birth *Zbtb20* is expressed in gliogenic radial glia stem cells. After P21 its expression can be found in parenchymal glial progenitors and astrocytes, but not in oligodendrocytes. **D2** Immunofluorescence double-labeling for *Zbtb20* (red) and the mature astrocyte marker S100 β (green) in the mouse cortex at P12. A magnified view of several double positive cells is present in the insets. VZ - ventricular zone; SVZ - subventricular zone; MZ - marginal zone; MP, DP, LP - medial, dorsal and lateral pallium respectively; CP – cortical plate; CC – corpus callosum; L1–6 – Layer 1–6

expression pattern with some cells characterized by a strong expression level while others - with a low expression level. Apart from its presence in neurogenic progenitors and neurons, *Zbtb20* is also expressed in gliogenic progenitors necessary for astrocytogenesis and oligodendroglioneogenesis (Fig. 1D1) (21,25,26) as well as in the mature astrocytes of the postnatal and adult cortex (Fig. 1D2). Similarly to mice, in humans ZBTB20 is also expressed in the developing hippocampus. Expression was detected as early as 11 WPC (weeks post conception) in the dentate anlage but remained low in the neuroepithelium. At 13WPC expression has spread to all hippocampal germinal zones and the dentate migratory stream. At 20 WPC granule cells, the dentate migratory stream, and immature projection neurons of the CA sector showed a strong ZBTB20 expression (27).

EXPRESSION OF ZBTB20 IN OTHER BRAIN REGIONS

A presence of *Zbtb20* outside the forebrain is also detected but less well characterized. In the postnatal olfactory bulb, it is restricted to astrocytes and a subpopulation of glomerular interneurons. In the postnatal diencephalon, *Zbtb20* was detected in neurons of the hypothalamic suprachiasmatic nucleus (26,28). The cerebellum also shows positive for *Zbtb20* cells. The cerebellar granule cells are produced in a transient proliferative zone named external granular layer. Once generated the immature cells migrate deeper in the cerebellar cortex forming the internal granular layer (29). Immature and migrating granule cell are positive for *Zbtb20* and this expression pattern requires further investigation (16). In the peripheral nervous system, *Zbtb20* was detected in developing dorsal root ganglia, where its expression persists in postnatally in the nociceptive bipolar cells (21,30). Of note, *Zbtb20* is also expressed in the subventricular zone, a stem cell niche in the adult brain (26). The highest expression was observed in putative stem cells transient amplifying cells, and niche astrocytes, with a gradual decline of the levels in the neuroblasts (Fig. 1C) (26).

ZBTB20 EXPRESSION IN THE MUSCULOSKELETAL SYSTEM

The musculoskeletal system originates from mesodermal structures called somites (31). The early expression of *Zbtb20* (E9.5) in these structures was the first evidence for its possible involvement in the development of this system (21). At post-somite stages, *Zbtb20* was not detected in muscle fibers but was expressed in satellite cells serving as stem cells during the postnatal development. The expression of *Zbtb20* in these cells

is most likely involved in maintaining the quiescent stem cell niche in the muscle. Overexpression led to increased activation of the satellite cells and myoblast maturation (32). In the developing mouse skeleton, *Zbtb20* was not found in proliferating chondrocytes, osteoblasts, and periosteal cells. Rather, its expression was confined to the differentiating hypertrophic chondrocytes (32). Postnatally, the *Zbtb20* expression spread to the reserve cartilage zone, and a much weaker signal was observed in the proliferating zone while maintaining expression in the differentiating hypertrophic chondrocytes (33).

ZBTB20 EXPRESSION IN THE LIVER

The liver expression of *Zbtb20* is very low during development (34). Postnatally, it rises abruptly reaching a plateau by P8, when a 12-fold increase of *Zbtb20* mRNA was observed as compared to P0. The levels of *Zbtb20* persist in adulthood when *Zbtb20* is involved in repression of the transcription of the alpha-fetoprotein (Fig. 2A) (34). In line with the aforementioned results *Zbtb20* expression is reduced during the initial stages of liver regeneration after hepatectomy in adult mice and gradually reached control levels of expression when liver to body weight ratio approached normal values (34).

ZBTB20 EXPRESSION IN IMMUNE CELLS

Zbtb20 was detected in the cells of the humoral immune system. The major players in the humoral response are the B lymphocytes, which are activated upon contact with T_H2-helpers (35). Upon activation B-cells differentiate into antibody-secreting plasma cells (PCs), memory B-cells or form germinal centers (GC) where they proliferate. *Zbtb20* mRNA is present in all B-cells: developing B-cells, B-1 cells in the peritoneum, GC B-cells, and long-lived bone marrow (BM) PCs as well as splenic PCs. Protein expression increases steadily from immature GC B cell, to immature plasmablasts and to BM differentiated PCs (36). Naïve B-cells and B-cell progenitors show relatively low expression levels. (Fig. 2B) (37). *Zbtb20* expression was also shown in a subpopulation of memory T-lymphocytes (38). To our knowledge there is no evidence for *Zbtb20* expression in the granulocyte lineage and other T-lymphocytes.

ZBTB20 EXPRESSION IN OTHER ORGANS

Zbtb20 is expressed in all hormone-producing cells of the anterior pituitary (Fig. 2C) (39). However, this TF appears to be indispensable only for the development of the lactotrophs (Prolactin-producing cells) (40). Expression in the posterior

Figure 2. Schematic representation of the Zbtb20 expression outside the nervous system. *A* In liver Zbtb20 is only weakly expressed in during development with a gradual increase postnatally, eventually reaching a plateau. *B* In the immune system expression is low in centroblasts, increasing in immature B-cells, reaching its highest levels in the long-lived plasma cells. *C* Expression in the anterior pituitary is confined to all endocrine cells, but the Prolactin-secreting cells are most critically dependent on the presence of Zbtb20. *D* Expression in the testis tubules is limited to the Sertoli and Leydig cells. GS – Germinal Center GH – Gonadotropic Hormone LH – Luteinizing Hormone, FSH Follicle Stimulating Hormone, TSH Thyroid-stimulating hormone, PLR - Prolactin

lobe was also reported (Fig. 2C) (39,40). In the testis, *Zbtb20* is localized in mature Sertoli cells as well as in the testosterone-producing Leydig cells (Fig. 2D) (41). Human dental pulp stem cells upregulate both mRNA and protein levels of *Zbtb20* when induced to differentiate *in vitro* (42).

TF ZBTB20 AS A PROLIFERATION/CELL CYCLE REGULATOR

Zinc finger-BTB TFs function as cell fate determinants by

regulating the cell cycle progression in many systems (18,43). The expression pattern of *Zbtb20* (Table 1) suggests its involvement in the cell cycle regulation, and accordingly, several experimental manipulations of its levels were performed to test this. A number of loss-of-function studies revealed a decreased proliferation in the absence of *Zbtb20* (Table 2). Liver-specific *Zbtb20* deletion in mice led to a slower liver regeneration in the initial stages of recovery after partial hepatectomy (44). This effect was attributed to a slower proliferation of hepatocytes.

Table 1. Summary of available data on the expression of *Zbtb20*. VZ - ventricular zone; SVZ - subventricular zone; DG - dentate gyrus; CA – cornu ammonium; EGL/IGL – External/Internal Granule layer.

Location of expression	Developmental stage and characteristics of expression	Reference
Hippocampus	E12.5-E15.5: Apical and a few basal progenitors in VZ/SVZ E16.5: migrating and settled pyramidal CA neurons E17.5: migrating and settled DG granule cells	13, 39
	Adult: Upper row CA1 pyramidal and DG granule neurons; expression gradually decreases.	17
Neocortex	E11.5/E12.5: cortical hem and ventral pallium E14.5: entire VZ of the dorsal pallium E16.5: VZ and SVZ of the entire pallium E18.5: diminishes but remains present in the VZ	18, 19, 20
	P0-P4: pyramidal neurons in neocortical layers 2-3	19
	Postnatal and adult: expressed in glial progenitors, astrocytes and ependymal cells	40
Basal ganglia	E10.5: Progenitors of VZ and SVZ	18
Olfactory Bulb	P4: astrocytes and at low levels in some glomerular neurons	20
Spinal Ganglia	E10.5 – E14.5: developing dorsal root ganglion neurons	18, 23
	Adult: nociceptive dorsal root ganglia neurons	23
Hypothalamus	Adult: Expressed in suprachiasmatic nucleus of hypothalamus	21
Cerebellum	P5-P10: immature migrating and settled cerebellar granule cells in the EGL/IGL: expression is extinguished as they mature	13
Pituitary Gland	E14.5: embryonic pituitary gland, expression increases steadily during development.	33
	Postnatal: all endocrine anterior pituitary gland cells	
Liver	Fetal: little or no expression	27
	Postnatal: gradual increase, maximum mRNA level reached at P56	
Heart	E9.5	18
Musculoskeletal system	E10.5: somites	18
	E14.5: hypertrophic zone chondrocytes P4-P90: reserve zone and hypertrophic zone chondrocytes	26
	E16.5-8 months: during establishment and maintained in adult satellite cells; expression gradually increases	25
Dental pulp stem cells	7-14 days <i>in vitro</i> : dental pulp stem cell differentiation	36
Immune system	Various stages after antigen immunization: B cell population, highly enriched in long-lived plasma cells and memory cells	32
Testis	P7-P70: Sertoli and Leydig cells	35

Table 2. Effects of experimentally altered Zbtb20 expression on cell proliferation. CKI – Cyclin dependent Kinase Inhibitor; CDK – Cyclin Dependent Kinase

Target cells/stage (Ref.)	Experimental manipulation	Result from manipulation
Adult hepatocytes (29)	Zbtb20 loss-of-function <i>in vivo</i>	↓ Proliferation after hepatectomy ↓ Cyclin D1 and CDK4
Chondrocytes in developing cartilage/ Stage P22 (26)	Zbtb20 loss-of-function <i>in vivo</i>	↓ Proliferation ↓ Chondrocyte differentiation
Dorsal pallium radial glia/Stage E16.5 (19)	Zbtb20 loss-of-function <i>in vivo</i>	↓ Cell cycle exit ↓ Proliferation
B-cell plasma cell line (31)	Zbtb20 gain-of-function <i>in vitro</i>	↓ Proliferation ↓ CKI p15
Non-small cell lung carcinoma (37)	Zbtb20 gain-of-function <i>in vitro</i>	↑ Proliferation

This proliferation defect was concomitant with reduced levels of the cell cycle progression protein cyclin D1 and its counterpart kinase CDK4 (44). This defect was linked to a reduction of epidermal growth factor receptor (EGFR) expression in hepatocytes, and EGFR is known for its role in hepatocyte cycle progression (44). In contrast, knocking down *Zbtb20* in liver cell cultures augmented proliferation (45).

Studies in other systems also reported defects in proliferation and differentiation upon *Zbtb20* loss-of-function. A cartilage-specific *Zbtb20* inactivation showed no defect in proliferation at early stages (P4), but at later stages (P22) it produced a noticeable proliferation reduction of chondrocytes in the proliferating cartilage zone (33). Further, an enhanced chondrocyte differentiation was detected as early as P4. Because the enhanced differentiation was observed in the absence of increased apoptosis, the authors concluded that it was due to a proliferation defect (33). Whether *Zbtb20* alters cell cycle parameters was not directly tested in this setting. *Zbtb20* loss-of-function also reduced proliferation of neurogenic cortical progenitors. *Zbtb20* knockout mice showed a decreased proliferation and enhanced exit from the mitotic cell cycle at mid-neurogenesis (E15.5-E16.6) resulting in few upper layer cortical pyramidal neurons born in the mutant (22). This effect was potentially mediated by a binding of *Zbtb20* to the promoter of TF *COUP-TFI* (22), a known regulator of cell cycle progression in neocortical stem cells (46).

A few reports focus on the effect on proliferation of *Zbtb20* gain-of-function. Estrogen receptor (ER)-inducible overexpression of *Zbtb20* in plasma cell cultures lead to a much slower rate of proliferation evident by the smaller cell number in the *Zbtb20*-ER colonies. The putative cause of this

phenotype is the slower progression from G1- to S-phase and an overproduction of the inhibitor CKI p15 (36). In non-small cell lung cancer, *Zbtb20* overexpression was shown to increase proliferation (47). Upon *Zbtb20* overexpression, both *Cyclin D1* and *Cyclin E* were elevated, while the levels of the CDK inhibitors *p21* and *p27* were down-regulated (47,48). Knockdown of *Zbtb20* had an opposite effect. This was likely mediated by *FoxO1*, a gene inhibiting cell cycle progression, directly repressed by *Zbtb20* (47,48).

CONTEXT DEPENDENT REGULATION OF THE CELL CYCLE BY TF ZBTB20

Data from different systems indicate that *Zbtb20* is capable of promoting or impeding the cell cycle progression (Table 2). It is fascinating that the same gene may have opposite effects on cell cycle in a cell-dependent manner. Thus, the tissue context and the molecular partners seem critical for the effects of TF *Zbtb20* on the cell cycle (see Box). The *Zbtb20* expression in stem cell niches of the adult brain, liver and skeletal muscle places this TF as a potential gene target to manipulate organ-

Box: Questions Outstanding

- Which are the co-factors of *Zbtb20* in regulating the cell cycle?
- Are the levels of *Zbtb20* involved in maintaining stem cells quiescence?
- Are the levels of *Zbtb20* decisive in promoting or blocking cell cycle progression?
- Can *Zbtb20* induce cell cycle reentry or cell cycle progression on its own or is it a permissive factor allowing for other genes to exert their function?

specific stem cells in the context of regenerative medicine. For example, it appears that brain stem cells and proliferating hepatocytes down-regulate *Zbtb20* during their activation, while the skeletal muscle stem cells up-regulate *Zbtb20* during activation. Overexpression of *Zbtb20* in plasma cells impairs proliferation *in vitro*, while in the liver a knock-down has such effect on hepatocyte proliferation. The opposing effects on cell activation might be related to the opposing effects of *Zbtb20* on the cell cycle progression and probably depend on interaction with different molecular partners, such as COUP-TFI in cortical progenitors (19), Sox9 in chondrocytes and astrocytes (26, 40). Another important issue whether *Zbtb20* is able to affect the cell cycle directly or rather it acts a permissive factor allowing for other genes to exert their function. These and other outstanding questions need to be answered in order to get a better understanding regarding the role of *Zbtb20* in progenitor cell biology.

CONFLICT OF INTERESTS

There authors declare no conflict of interests exists.

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