stem cell research&therapy

COMMENTARY

A zinc finger protein Zfp521 directs neural differentiation and beyond

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Abstract

Neural induction is largely considered a default process, whereas little is known about intrinsic factors that drive neural differentiation. Kamiya and colleagues now demonstrate that a transcription factor, Zfp521, is capable of directing embryonic stem (ES) cells into neural progenitors. They discovered that *Zfp521* transcripts were enriched in early neural lineage of ES cell differentiation. Forced expression of *Zfp521* turned ES cells into neural progenitors in culture conditions that would normally inhibit neural differentiation. *Zfp521* was expressed in mouse embryos during gastrulation. The protein was shown to associate with a co-activator p300 and directly induce expression of early neural genes. Knockdown of the Zfp521 by shRNA halted cells at the epiblast stage and suppressed neural differentiation. Zfp521 is a nuclear protein with 30 Krüppel-like zinc fingers mediating multiple protein–protein interactions, and regulates transcription in diverse tissues and organs. The protein promotes proliferation, delays differentiation and reduces apoptosis. The findings by Kamiya and colleagues that Zfp521 directs and sustains early neural differentiation now opens up a series of studies to investigate roles of Zfp521 in stem cells and brain development of mice and men.

Human embryonic stem (ES) cells offer tremendous opportunities for regenerative medicine, because they are capable of indefinite self-propagation and of differentiating into any cell types of three germ layers [1]. Significant progress has been made in understanding the maintenance of ES cells, and now induced pluripotent stem cells can be generated from most (if not all) somatic cells with the defined transcription factors c-MYC, KLF4, SOX2 and OCT4 [2] and/or small molecules [3]. A better understanding of how to derive specific cell types from stem cells will increase the rate of translation of basic laboratory work to the clinic for patient-tailored therapy.

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Neural induction in embryos and ES cells

Formation of the central nervous system has drawn the attention of developmental biologists for almost a century since Spemann and Mangold discovered that the dorsal lip of the blastopore was capable of inducing neural differentiation in *Xenopus* gastrula [4]. A list of Spemann organizers has been identified that to date includes Noggin, Gremlin, Cerberus, Twisted Gastrulation and Chordin. They are secreted molecules that directly bind and antagonize bone morphogenetic proteins (BMPs) [5,6].

BMPs bind/activate specific high-affinity type I and type II Ser/Thr kinase receptors, including ALK1 to ALK7, ActRII, ActRIIB, TβRII, BMPRI and BMPRII, and signal through the canonical SMAD pathways. BMP signaling pathways are present in ES cells and readily inhibit neural differentiation. When BMP inhibition is removed, the majority of surviving cells differentiate into neurons. Neural induction is therefore considered a default process that takes place autonomously and intrinsically [5]. Little is known, however, about the intracellular factors involved.

Kamiya and colleagues now report in *Nature*, with compelling evidence, that a zinc finger (ZF) nuclear protein, Zfp521, is a key intrinsic molecule capable of directing ES cells to neural progenitors [7]. They first knocked-in a *GFP* reporter gene into a neural precursor locus *Sox1* and cultured embryoid bodies for 3 days of default neural differentiation. They separated green (neural) and nongreen cells by flow cytometry, and identified 104 genes that were expressed highly in GFP-positive cells but were low in non-GFP cells. One of these genes, *Zfp521*, was able to turn ES cells into Sox1-GFP-positive neural progenitors by overexpression, either in the presence of BMPs or 10% FCS, which would normally inhibit neural differentiation.

Next, Zfp521 was shown to directly activate early neural genes such as *Sox1*, *Sox3* and *N-cadherin* by working together with the co-activator p300. Knockdown of

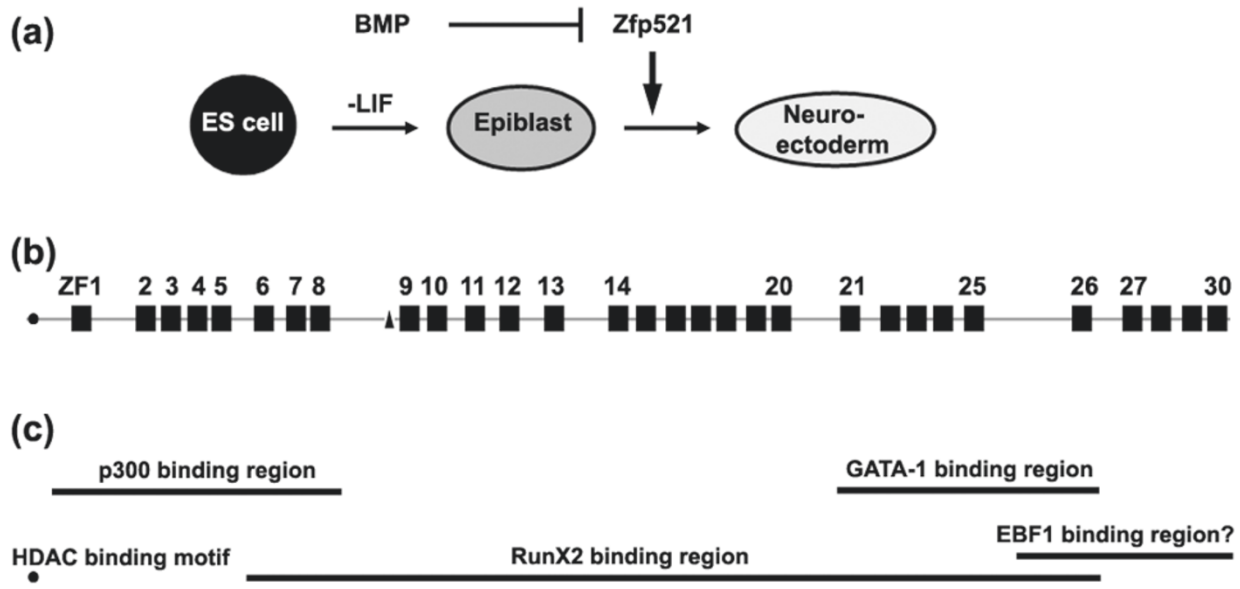

Figure 1. Zfp521 structure and function. (a) In mouse embryonic stem (ES) cells, Zfp521 is essential for directing epiblasts to neuroectoderm cells, which is inhibited by bone morphogenetic protein (BMP) signaling pathways. **(b)** Schematic presentation of the Zfp521 protein, which contains a nuclear remodeling and histone deacetylation complex (NuRD) motif (●) at the N-terminal end, 30 C_2H_2 zinc fingers (ZF1 to ZF30, ■) throughout the protein, and a putative nuclear localization signal (▲) between ZF8 and ZF9. **(c)** Zfp521 recruits histone deacetylase (HDAC1/HDAC3/HDAC4) through the NuRD motif, associates with co-activator p300 via ZF1 to ZF8, and inhibits GATA-1 and EBF1 by the C-terminal regions, and binds RunX2 through ZF6 to ZF26. LIF, leukemia inhibitory factor.

Zfp521 by shRNA had no effect on regulation of ES cell markers *Rex1* and *Nanog*, but halted cells at the epiblast stage with sustained epiblast marker gene expression including *Oct4*, *Cripto* and *Fgf5*. Meanwhile Zfp521 depletion suppressed early neural gene induction and neural differentiation. Zfp521 is therefore critical for epiblast–neuroectoderm transition (Figure 1a) [7].

Zfp521 structural domains and binding partners

Zfp521 is a nuclear protein with a putative nuclear localization signal (seven amino acids) immediately upstream of ZF9. The protein contains 30 Krüppel-like ZF motifs clustered in five to seven ZFs throughout the protein (Figure 1b), interacts with multiple partners (Figure 1c), and regulates transcription in diverse tissues and organs. In ES cells, Zfp521 associates with p300 through ZF1 to ZF8 at the N-terminus to activate early neural genes and trigger neural differentiation [7]. In the hematopoietic system, its human counterpart ZNF521 binds and inhibits GATA-1 via ZF21 to ZF26, and this maintains the stemness of hematopoietic progenitors and represses erythroid differentiation [8]. Depletion of ZNF521 by shRNA can transform K562 and HEL cells into erythroid cells synthesizing hemoglobin and glycophorin A. The C-terminus of ZNF521 is also required for interaction/inhibition of OLF1/EBF1, and the latter is important for activating B-cell-specific gene expression [9] and for the development of striatal medium spiny neurons [10]. Zfp521 also associates and attenuates RunX2 via ZF6 to ZF26 [11,12], and regulates two stages of osteoblast development during mesenchymal cell lineage commitment and during osteoblast maturation [12,13].

Additionally, Zfp521 is capable of implementing epigenetic effects through global remodeling of the nucleus. This is achieved via a 12-amino-acid motif called nuclear remodeling and histone deacetylation complex (NuRD) at its N-terminal end, which binds histone deacetylases (HDACs) [14]. The general view is that HDACs deacetylate histones and repress gene transcription. For example, ZNF521 inhibits GATA-1 through NuRD, and deletion of the motif results in reduction of the inhibitory effect in hematopoietic progenitors [8]. The NuRD motif is highly conserved among other ZF transcription factors, including ZNF423, Friend of GATA (FOG-1/ZFPM1 and FOG-2/ZFPM2), BCL11A, and SALL family members [14], and may recruit different HDACs in different cells. In chondrocytes, Zfp521 forms a complex with HDAC3/HDAC4 and inhibits RunX2 activity [11,12]. In ES cells Zfp521 antagonizes HDAC1/HDAC3, which inhibit p300-induced neural differentiation. Whether Zfp521, HDACs and p300 form one complex, however, is unclear. One scenario can be that association of p300 and Zfp521 results in either inactivation or release of HDACs from the complex.

Interestingly, an N-terminally truncated ZNF521 isoform is predicted in human (uc002kvl.2) and rat (NP_001100873), which lacks the first 220 amino acids including the NuRD motif and ZF1 to ZF5. Meanwhile, the NuRD domain is neither required for the activation of *N-cadherin* expression in ES cells [7] nor for the inhibition of *EBF1* in the hematopoietic system [9]. Information remaining to be validated includes whether the short isoform exists or is conserved during evolution, whether Zfp521 isoforms are expressed in different tissues, and whether the NuRD-truncated form is constitutively active as a result of its incapability to bind HDACs or is dominant negative as a result of missing ZF1 to ZF5.

Zfp521 expression and regulation

As a key intrinsic factor driving neural determination, we expect that *Xenopus* xZfp521 would be expressed at the early gastrula stage, such as st10.5, when neural induction is initiated by the Spemann organizer. Surprisingly, *xZfp521* mRNA was not detected at st10.5 by *in situ* hybridization [7]. In mouse embryos, *Zfp521* transcripts were not detected in blastocysts as anticipated, but were expressed weakly in E6.5 and strongly in E7.0 neuroectoderm during gastrulation. Whether Zfp521 is expressed in early postimplantation mouse embryos (E3.5 to E6.5) remains to be seen. Forced *Zfp521* expression could not drive neural differentiation in the presence of leukemia inhibitory factor or the fibroblast growth factor receptor inhibitor SU5402, suggesting that Zfp521 may be a key factor for sustaining rather than initiating neural differentiation.

Understanding how Zfp521 is regulated is equally important. In ES cells it is currently unknown whether *Zfp521* expression is purely repressed by BMP signaling, or can be induced by any other factors. What is known is that Zfp521 expression in chondrocytes can be increased by parathyroid hormone-related peptide (PTHrP) signaling or elevated adenylyl cyclase activity [11]. Knock-in of a *GFP* reporter into the *Zfp521* locus of ES cells with a similar approach may indicate whether any of the 104 genes highly expressed in Sox1-GFP cells is upstream of the *Zfp521*. Alternatively, a quantitative RT-PCR analysis of the genes in a time course of the 3-day neural induction could indicate whether any factor was induced prior to *Zfp521*. It will also be interesting to know whether WNT signaling pathways [15], downstream of the adenylyl cyclase pathway such as protein kinase A, or a well-known neural inducer such as retinoic acid may influence Zfp521 expression.

Zfp521 function

Zfp521 is expressed in a wide range of tissues including the brain, muscle, heart, kidney, spleen, lymph nodes, placenta, thymus and fetal liver [16]; in particular, in immature cells such as early mesenchymal condensations, developing bones, hematopoietic stem cells, and neural progenitors. *Zfp521* was initially called *Evi3* in mouse, which was identified as a common integration site of retrovirus in murine AKXD B-cell lymphomas [17]. The virus integrates upstream of the first coding exon, which results in upregulation of *Evi3*. The human ZNF521 was previously termed early hematopoietic zinc finger protein, and its expression in the hematopoietic system was restricted to a subpopulation of less differentiated $CD34^+$ cells. ZNF521 is abundant in leukemia, medulloblastomas and other brain tumors. iRNA-mediated ZNF521 silencing impairs the growth and clonogeneity of $MLL^+/ZNF521^+$ THP-1 cells. High levels of Zfp521 expression may therefore be associated with oncogenesis.

Zfp521 is shown to regulate a number of cellular processes. The protein promotes proliferation, delays differentiation and reduces apoptosis [13]. In cultured mesenchymal cells, Zfp521 expression is decreased by BMP2 and increased by PTHrP, which promote and antagonize osteoblast differentiation, respectively. Deleting *Zfp521* also decreases cyclin D_1 and Bcl-2 expression with increased caspase-3 activation and apoptosis. Interestingly, in stage 15 of *Xenopus* embryos developed from injection of *xZfp521* expression vector at the eight-cell stage, an altered distribution pattern of cells expressing pro-neural markers *Neurogenin* and *Estrogen Receptor* 1 is observed [7]. This observation suggests that *Zfp521* overexpression may induce ectopic expression of pro-neural genes. Alternatively, appropriate levels of *Zfp521* expression may be essential for neuronal migration. Zfp521 expression in adult brain is very limited, however, based on the Allen Brain Atlas [18] in comparison with developing neural tubes [7], whereas relatively high levels of the mRNAs for *Zfp521* and its partner *Ebf1* are detected in striatonigral neurons of the adult mouse [10] – Zfp521 may therefore be involved in neurodegenerative and psychiatric disorders.

In summary, Zfp521 plays important roles in multiple tissues. Zfp521 mediates PTHrP signaling, and regulates proliferation and differentiation of growth plate chondrocyte [11] and bone formation [13]. In embryos, Zfp521 is critical for rostral neural tube development, because Zfp521-depleted ES cells fail to contribute to the rostral neural tube, although they are found in other parts of chimeric embryos [7]. Investigation of $Zfp521^{-/-}$ mice will reveal how Zfp521 affects brain development. The excellent work carried out by Kamiya and colleagues demonstrates that Zfp521 is a key factor to sustain neural differentiation. These findings have opened up a whole series of studies to investigate how Zfp521 works together with other transcriptional factors and epigenetic factors to regulate neural differentiation in stem cells and in developing brains of mice and men.

Abbreviations
BMP, bone morphogenetic protein; ES, embryonic stem; FCS, fetal calf serum; GFP, green fluorescence protein; HDAC, histone deacetylase; iRNA, interfering RNA; NuRD, nuclear remodeling and histone deacetylation complex; PCR, polymerase chain reaction; PTHrP, parathyroid hormone-related peptide; RT, reverse transcription; shRNA, short hairpin RNA; ZF, zinc finger.

Competing interests
The authors declare that they have no competing interests.

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