Results: STB-HO blocked the anti-apoptotic gene BIRC5 and activated p53, p21 and the pro-apoptotic proteins Bim, Puma and p-Bad during early spontaneous differentiation. Moreover, STB-HO-pretreated differentiating hES cells did not give rise to teratomas following *in vivo*stem cell transplantation.

Conclusions: Our *in vitro* and *in vivo* results suggest a method for teratoma prevention in the context of PSC-derived cell transplantation. This novel MFP could break through the limitations of PSC therapy.

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DNA methyltransferase inhibition accelerates the immunomodulation and migration of human mesenchymal stem cells

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Introduction: DNA methyltransferase (DNMT) inhibitors regulate target gene expression through epigenetic modifications, and these compounds have primarily been studied for cancer therapy or reprogramming. However, the effect of DNMT inhibitors on the immunomodulatory capacity of human mesenchymal stem cells (hMSCs) has not been investigated.

Materials and Methods: Mixed leukocyte reaction was performed to determine the alteration in the immunomodulatory function of MSCs by the treatment of 5-azacytidine (5-aza), a DNMT inhibitor. Migration of MSCs toward inflammatory environment was detected by migration assay using transwell. Methylation array was cunducted to explore the immunomodulatory factors stimulated by 5-aza treatment. Physiological function of MSCs was finally determined using dextran sulfate sodium (DSS)-induce colitis model.

Results: In the present study, we treated hMSCs with 5-aza and confirmed that the inhibitory effects on mononuclear cell proliferation and cell migration toward activated T cells were increased. In methylation array, we observed that the promoters of immunomodulatory factors, *COX2* and *PTGES*, and migration-related factors, *CXCR2* and *CXCR4*, were hypomethylated after 5-aza treatment. In addition, we observed that the COX2-PGE_2 pathway is one of the main pathways for the enhanced immunosuppressive activity of hMSCs through 5-aza treatment. We also determined that the migration of hMSCs toward ligands for CXCR2/CXCR4 was increased after 5-aza treatment. Moreover, using an experimental colitis model, we showed that 5-aza pre-treatment could enhance the therapeutic effect of MSCs against immune-related diseases.

Conclusions: The treatment with a demethylating agent (5-aza) increased the immunomodulation and migration of hMSCs through the demethylation of target gene promoters.

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PR-domain containing protein 12 (*Prdm12*) is a downstream target of transcription factor *Zic1* during brain cell differentiation

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Introduction: Transcription factor *zic1* is an important regulator of neural plate patterning, formation of neural crest and cerebellar development where its main function is neuronal cell differentiation. Studies reported that *prdm* family is expressed in the nervous system of developing mice and zebrafish. However, the function of *prdm* in neurogenesis is still unclear. Here, we identified *prdm12* in *Xenopus* as a downstream of *zic1* transcription factor. We propose that *prdm12* is a novel and essential component of the *Xenopus* brain regulatory network downstream of *zic1*.

Materials and Methods: RT-PCR was performed by using Maxim RT-PCR Premix Kit (iNtRON). The mRNA sequence of the *Xenopus laevis prdm12* were amplified using PCR from stage 30 cDNA using a set of primers. RNA encoding *zic1*GR were synthesized in vitro with the Message Machine kit. One blastomere of 2-cell stage embryos were injected in the animal pole, with *zic1*-GR mRNA (0.5 ng), dominant negative TCF-GR, *zic1*MO (AAGTCTTCCAACAATGGGC AGCGAA), or *prdm12*MO (GCAGCACCGAGCCCATCATTAATTC). The embryos were cultured in 0.1X NAM. Embryos analyzed by *in situ* hybridization To identify the injected side, β-galactosidase mRNA was co-injected as a lineage tracer.

Results: The *prdm12* expression started from the gastrulation stage (stage 9) and continues to be present until stage 40, the last stage examined in this study. In the earlier stage, *prdm12* was detected at at the animal hemisphere and in the neuro-ectoderm. *prdm12* specifically expressed in the pre-placodal ectoderm, trigeminal ganglion, along with the dorsal spinal cord. *prdm12* expression was more prominent in the midbrain, ventral part of the forebrain and hindbrain domain, neural crest, trigeminal ganglion. In the posterior region, the *prdm12* expression was restricted to the motor neuron of the spinal cord. In a large proportion of embryos injected with *zic1*-MO

we observed a reduction of *prdm12* expression which demonstrates the position of *prdm12* downstream of *zic1* in the gene regulatory cascade leading midbrain formation.

Conclusions: *prdm12* gene is a downstream target of *zic1* and likely to modulate signaling pathways that regulate the expression of genes in the midbrain domain. In addition, *prdm12* is also regulated by *wnt* signaling. We propose that *prdm12* has a crucial role in the midbrain formation regulated by *wnt* mediated *zic1*.

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Zic1 targeted peripheral myelin protein (*pmp22*) regulates craniofacial morphogenesis

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Introduction: Peripheral myelin protein 22 (*Pmp22*), a member of the junction protein family Claudin/EMP/PMP22, contributes to the formation and maintenance of myelin sheaths in the peripheral nervous system. Deletions, duplications and mutations of *Pmp22* accounts for the majority of heritable demyelinating peripheral neuropathies, including Charcot-Marie-Tooth disease type 1A, hereditary neuropathy with liability to pressure palsies or a combination of both as a separate type called Charcot-Marie-Tooth disease type 1E. Apart from the establishment and maintenance of peripheral nerves, *Pmp22* and its family member have also been participated in a broad range of more general process including cell cycle regulation and apoptosis during development. We performed the functional analysis of *Pmp22* during *Xenopus* development. We found that *Pmp22* is a downstream of transcription factor Zic1 and it is responsible for craniofacial morphogenesis.

Materials and Methods: RNA encoding *Zic1*-GR and dnTCF-GR were synthesized in vitro with the Message Machine kit (Ambion, Austin, TX). The activity of the fusion proteins can be regulated by addition of dexamethasone to the culture medium of whole embryos. One blastomere of 2-cell stage embryos were injected in the animal pole region, with *Zic1*GR RNA (0.5 ng) and dnTCF-GR (1 ng). At early neurula stage (stage 13.5) the embryos were cultured in 0.1X NAM containing 10μm Dexamethasone (Dex; Sigma-Aldrich, St. Louis, MO) to activate both *Zic1*-GR and dnTCF-GR. Antisense morpholino oligonucleotides (*Zic1*-MO; 5'-AAGTCTTCCAACAATGGGCAGCGAA-3'; *pmp22*-MO; 5'-TCCCAGCAGTAAGAGGAGCATTTTC-3') were injected into the animal pole region of one blastomere at the 2-cell stage and embryos analyzed by *in situ* hybridization. To identify the injected side, β-galactosidase mRNA was co-injected as a lineage tracer.

Results: A *pmp22* morpholino (*pmp22*-MO) was designed to specifically interfere with translation of *pmp22* mRNA. Therefore, we observed the defective branch-arches formation during development after whole mount *in situ* hybridization. To make it confirm we performed *in situ* hybridization on sections of *pmp22*-MO injected embryos and the malformation of branch-arches was distinctly observed on the injected side of the sections. Two other branch-arches marker Sox9 and Sox11 was also down-regulated by the depletion of *pmp22*. In a large proportion of embryos injected with *Zic1*-MO we observed a reduction of *pmp22* expression in branch-arches. Using hormone inducible construct of *Zic1*, we analyzed the consequences of *pmp22*expression on the embryos. Embryos injected with 0.5 ng of *Zic1-GR* mRNA and treated with dexamethasone at the late neurula (stage 19) displayed an expansion of *pmp22 expression* in the branch-arches domain in more than 85% of injected embryos. These results further demonstrate the position of *pmp22* downstream of *Zic1* in the gene regulatory cascade leading branch-arches formation.

Conclusions: Our data suggests that pmp22 is a downstream target of transcription factor Zic1 mediated by Wnt signaling and *pmp22* has some crucial role in the maintenance of functional peripheral nerves as well as in the proliferation or differentiation of cells especially for the craniofacial morphogenesis.

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Establishment of *hPer2* transfected HepG2 cell as hepatic circadian model

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Introduction: Circadian rhythms are biological,