World Journal of Stem Cells

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DOI: 10.4252/wjsc.v7.i7.992

World J Stem Cells 2015 August 26; 7(7): 992-998
ISSN 1948-0210 (online)

EDITORIAL

Signaling involved in stem cell reprogramming and differentiation

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Author contributions: Tanabe S is the sole contributor to this paper.

Conflict-of-interest statement: The author declares that no conflict of interest exists.

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Received: April 21, 2015
Peer-review started: April 24, 2015
First decision: May 13, 2015
Revised: May 29, 2015
Accepted: June 18, 2015
Article in press: June 19, 2015
Published online: August 26, 2015

Abstract

Stem cell differentiation is regulated by multiple signaling events. Recent technical advances have revealed that differentiated cells can be reprogrammed into stem cells. The signals involved in stem cell programming are of major interest in stem cell research. The signaling mechanisms involved in regulating stem cell reprogramming and differentiation are the subject of intense study in the field of life sciences. In this review, the molecular interactions and signaling pathways related to stem cell differentiation are discussed.

Key words: Stem cell; Signaling; Differentiation; Gene; Genome; Reprogramming

Core tip: Signals in stem cell are regulated both genetically and epigenetically by many molecules. The programming of stem cell signaling is an important aspect of understanding stem cell phenotype transitions and functions. The differentiation process as well as intra- and inter-cellular signaling of stem cells is described in this article. The epigenetic regulation of these cells is also discussed.

Tanabe S. Signaling involved in stem cell reprogramming and differentiation. *World J Stem Cells* 2015; 7(7): 992-998 Available from: URL: http://www.wjgnet.com/1948-0210/full/v7/i7/992.htm DOI: http://dx.doi.org/10.4252/wjsc.v7.i7.992

INTRODUCTION

Stem cells are the source of all other cells, and they have the potential to differentiate into many types of cells that contribute to the various organs, such as the heart, lungs, liver, and blood. There are many kinds of stem cells: totipotent embryonic stem cells (ESCs) found in the embryo; mesenchymal stem cells (MSCs) that give rise primarily to bone marrow stroma, adipocytes, and cord blood; epithelial stem cells that reside in the intestine; induced pluripotent stem cells that are artificially reprogrammed from differentiated cells; and cancer stem cells (CSCs). In this article, signaling in stem cells and the role of signaling molecules in the differentiation and reprogramming of stem cells are discussed.

STEM CELL DIFFERENTIATION

Stem cell differentiation is tightly regulated. Among the various pathways related to cellular differentiation, epigenetic regulation is the key mechanism that controls midbrain dopaminergic neuron differentiation[1]. Urocortin, which is expressed in the developing ventral midbrain, mediates dopaminergic neuron differentiation *via* the up-regulation of acetylated histone H3 and the dopaminergic regulators *Nurr1*, *Foxa2* and *Pitx3*[1]. Dimethyl sulfoxide down-regulates the pluripotency genes *OCT4* (also known as *POU5F1*) and *NANOG* in human embryonic stem (ES) cells during definitive endoderm differentiation and controls hepatic differentiation[2].

A previous report indicated that N^6-methyladenosine (m^6A) transferase (Mettl3; methyl transferase-like 3) regulates murine naïve pluripotency[3]. mRNA methylation is a key RNA modification and an essential factor in epigenomic regulation and cell fate determination[3]. Pluripotent cells are affected by environmental factors such as culture conditions, and such factors can affect the spatial regulations of transplanted stem cells[4]. Several signaling pathways are involved in the transformation of stem cells, such as fibroblast growth factor 2 (FGF2)/Activin-A signaling and histone 3 lysine 4 trimethylation alterations[4]. Matrix-bound heparan sulfate (HS) proteoglycans are needed for the differentiation of stem cell-derived endodermal cells into airway epithelial cells[5]. The three-dimensional extracellular matrix scaffold is essential for effective functional expression of lung epithelial cells[5], and HS proteoglycans also appear to play an important role in this process. Human ES cells differentiate into osteogenic cells, which can be identified by *RUNX2* expression[6]. Monitoring the osteogenic differentiation of human ES cells is useful for the therapeutic strategies used in bone regeneration[6]. The three-dimensional poly(L-lactic acid) scaffold culture of human ES cells on a nano-fibrous matrix results in enhanced osteogenic differentiation[7]. *RUNX2* expression in nano-fibrous matrix culture is significantly higher than in control cultures[7]. Additionally, an efficient method for the osteogenic differentiation of human ES cells using primary bone-derived cells has been reported[8]. Some cellular factors from bone-derived cells may be involved in human ES cell differentiation[8]. Cell surface proteins play roles in stem cell culture differentiation[9]. The use of functionalized hyaluronic acid surfaces with either fibronectin or collagen enhances the attachment of human ES cells[9]. During the osteogenic differentiation of human ES cells, hyaluronic acid surfaces with collagen Ⅰ and collagen Ⅳ have inductive effects on differentiation compared to fibronectin alone[9]. Transcription factors are regulated dynamically during human ES cell differentiation[10]. Transcription factor binding dynamics are classified into static, dynamic, enhanced, and suppressed states[10]. Transforming growth factor (TGF)-β and WNT signals are involved in human ES cell differentiation into mesendoderm and endoderm, whereas bone morphogenetic protein (BMP), vascular endothelial growth factor, and FGF2 are key regulators of the differentiation of ES cells into mesoderm. The inhibition of TGF-β, WNT or BMP signals result in ES cell differentiation into ectoderm[10]. *RUNX2* is down-regulated in late-stage cultures of MSCs[11,12]. The osteogenic differentiation capacity is decreased in late-stage cultures of MSCs compared to early-stage cultures; thus, gene expression levels can be used as an indicator of stem cell differentiation capacity[11]. Neural differentiation is regulated by epigenetic mechanisms[13]. Deep cortical layer neuronal markers such as *BCL11B* are expressed in differentiated neurons derived from neuroepithelial and early radial glia, and superficial layer neuronal markers such as *POU3F2*/*POU3F3* and *MEF2C* are expressed in differentiated progeny derived from mid radial glia[13]. Transcription factor activity is modulated dynamically during each stage of the neural progenitor population[13]. In addition to intracellular molecular network signaling, intercellular communication is also an important factor in stem cell signaling. Human neural stem/progenitor cells (hNSCs) and peripheral blood mononuclear cells (PBMCs) interact with each other when co-cultured under allogenic conditions. hNSCs increase the production of regulatory T cells, while PBMCs promote the differentiation of hNSCs[14]. The effect of intercellular communication on stem cell differentiation is an interesting topic for investigation. Cartilaginous tissue can be generated from human induced pluripotent stem (iPS) cells[15]. In severe combined immunodeficiency (SCID) mice transplanted with human iPS cell-derived differentiated cartilaginous cells, cartilage formation without tumor formation is observed[15].

STEM CELL SIGNALING

Tools for bioinformatics

A wealth of "big data" in gene and genome information, which has been collected *via* integrative profiling and is publically available on the internet, combined with recent advances in bioinformatics, have enabled us to analyze gene and signaling information. These advances include a useful web-accessible tool called the Database for Annotation, Visualization and Integrated Discovery (DAVID) Bioinformatics Resource (http://david.abcc.ncifcrf.gov/home.jsp) and the cBioPortal for Cancer Genomics (http://www.cbioportal.org/index.do)[16,17]. The open-source tool Cytoscape is a molecular network analysis tool for integrating the networks with annotation and gene expression profiles (http://cytoscape.org/what_is_cytoscape.html).

Hedgehog pathway

Hedgehog (Hh) signaling is an important player in epithelial and mesenchymal regulation during embryonic limb and bone development and cell fate determination[18-20]. Sonic Hh signaling is involved in

vascular differentiation and regulates human CD34-positive cell function[20]. Hh signaling also has an important role in hematological CSCs[21]. Targets of the Hh signaling pathway include the Notch pathway, the epithelial-mesenchymal transition (EMT) pathway, the WNT signaling pathway, the TGF-β signaling pathway, and the regulation of cell cycle, adhesion, fate determination, and stem cell signaling[21].

Ephrin pathway

Ephrin signaling is involved in inflammatory bowel diseases[22]. Bone marrow stem cells and stem cell factors play important roles in mucosal regeneration. In the intestine of a rat model of inflammatory bowel disease, ephrin-B3 is up-regulated in bone marrow MSCs during mucosal regeneration[22]. Ephrin type-B receptor 3 (*EPHB3*), a tumor suppressor gene, is important in cancer cell metastasis, which is regulated by Notch signaling in colorectal cancers[23]. *EPHB3* down-regulation in tumorigenesis correlates with Wnt/β-catenin, Notch and mitogen-activated protein kinase (MAPK) signaling[23]. EphrinA1 expression decreases as β-catenin expression is inhibited in liver tumor cells[24]. The treatment of hepatocellular cancer with cell-permeable, gamma guanidine-based peptide nucleic acid antisense oligonucleotides resulted in the inhibition of β-catenin and Wnt target gene expression[23]. The EphrinA1 receptor has been reported to be an independent prognostic marker for different survival endpoints in clear cell renal cell carcinoma[25]. The use of Ephrin and ephrin receptor signaling as either a prognostic marker or for revealing cancer progression mechanisms is an interesting topic for future study.

WNT pathway

The WNT pathways regulate stem cell differentiation and proliferation. WNT3-WNT9B signaling is involved in the regulation of neural differentiation[26]. An increase in WNT9B is reported to control the switch between pluripotent and differentiated states *via* noncanonical Rho/JNK signaling, whereas canonical WNT3/β-catenin signaling promotes proliferation[26]. WNT5A is known to promote cancer cell invasion and proliferation[27]. WNT5A activation of the Wnt/β-catenin-independent pathway, including Src family kinases, induces the proliferation of certain cancer cell types, such as HeLa S3 cervical cancer cells and A549 lung cancer cells[27]. Interestingly, WNT5A is not involved in the proliferation of KKLS gastric cancer cells, although cancer invasion is dependent on the WNT5A pathway[27]. Wnt5A up-regulation is reported to induce EMT and metastasis by pancreatic cancer cells[28]. In this case, β-catenin-dependent canonical Wnt signaling is involved in promotion of EMT [28]. Graphene oxide is reported to target CSCs by inhibiting a number of signaling pathways including the WNT, Notch and STAT pathways[29]. Graphene oxide selectively inhibits CSC proliferation but does not affect normal fibroblast viability[29]. STAT3 signaling alters the tumor microenvironment and angiogenesis and promotes KRAS-induced lung tumorigenesis[30]. The Wnt signaling pathway is reported to be down-regulated in irradiated tumor cells[31]. Wnt signaling may be involved in tumor repopulation after radiotherapy[31]. Notch1 and Delta-like 4 signals are important factors for cell fate decisions[32]. The Notch pathway is a therapeutic target in cancer treatment[32]. Interleukin-27 (IL-27) down-regulates EMT- and stemness-related genes such as *SONIC HEDGEHOG* in adenocarcinoma and *OCT4A*, *SOX2*, *NOTCH1*, *KLF4*, *Nestin*, *SNAI1/SNAIL*, *SNAI2/SLUG* and *ZEB1* in squamous cell carcinoma[33]. *SNAI2*, an EMT-related gene, and stemness genes are known to be down-regulated by IL-27[33]. WNT signaling modulation also mediates the long-term expansion and chondrogenic differentiation of adult human MSCs[34]. WNT3A and FGF2 maintain the phenotype and multipotency of MSCs[34]. In ES cells, cyclin-dependent kinase 1 (CDK1) inhibition is reported to prevent teratoma formation[35]. CDK1 signaling and its association with other cancer signaling pathways such as the WNT pathway would be interesting topics for future study of the mechanisms of tumor formation by stem cells.

MicroRNA signaling

MicroRNAs (miRNAs) are involved in cancer cell signaling[36]. The tumor microenvironment that maintains cancer cells is regulated by microRNA-mediated gene expression[36]. Anti-miRNAs, antisense oligomers that inhibit onco-miRNAs, can be used as anti-cancer drugs[36]. The conserved, well-established promoter of the terminal differentiation *let-7a* miRNA induces mitochondrial reactive oxygen species production and up-regulates oxidative stress-responsive genes[37]. The regulation of energy metabolism in cancer cells *via* *let-7a* is a potential target of anti-cancer therapy because *let-7a* miRNA plays a tumor-suppressive role[37]. Human MSCs that support the tumor microenvironment deliver tumor regulatory miRNAs *via* extracellular vesicular trafficking[38]. The extracellular vesicles of hMSCs contain proteins and lipids on the vesicle membrane, and miRNAs and metabolites inside the vesicles[38]. The internalization of these vesicles by cancer cells may be one of the key mechanisms for cancer cell survival[38]. During the hypoxia-induced myogenic differentiation of ES cells, miRNA-26a is up-regulated and inhibits the mRNA expression of histone deacetylase 6 (*HDAC6*) and of stemness genes such as *Oct4* (also known as *Pou5f1*) and *Nanog*[39]. miRNA-26a-mediated signaling may be important for stem cell differentiation[39]. Hypoxia contributes to the development and phenotype of CSCs *via* miRNA- and cytokine-mediated microenvironmental regulation[40]. The vascular CSC niche is regulated by miRNAs, which may induce CSC generation and EMT *via* the NFkB, PI3K/Akt/mTOR, NOTCH, Wnt/β-catenin and Hedgehog signaling pathways[40].

In the murine cortex and hippocampus, subclasses of neurons and glial cells have been characterized by the expression of regulatory genes using large-scale

single-cell RNA sequencing[41]. These cells exhibit differential gene expression and epigenetic regulation and miRNAs have recently become a focus of these processes as selective cell markers[42]. Glioma cells expressing miR-302 are enriched by serum deprivation, and miR-302 may be a marker of CSCs[42]. miRNA-30c plays a role in sphere formation, self-renewal and neural differentiation in C6 glioma cells[43]. In these cells, the astrocyte marker glial fibrillary acidic protein (GFAP) is up-regulated during differentiation. However, GFAP expression decreases in miRNA-30c-overexpressing cells[43]. The fate of neural stem cells is also regulated by miRNAs[44]. The differentiation of neural stem cells into neurons is promoted by kuwanon V, a phytochemical isolated from mulberry tree (*Morus bombycis*) roots that may up-regulate the expression of miR-9, miR-29a and miR-181a[44]. Cancer stem cell self-renewal, differentiation and tumorigenesis are regulated by the Notch pathway, in which miRNAs such as the miR-34 family, miR-200 family, and miR-199-5p, miR-146a, miR-1 and miR-143 play important roles[45]. Notch signaling cross-talk with miRNAs may be involved in cancer cell proliferation, which suggests possible therapeutic approaches *via* miRNA re-expression in cancer[45]. Moreover, genome-wide analysis has revealed that long noncoding RNAs (lncRNAs) are regulated by Notch signals in acute leukemia[46]. lncRNAs are defined as transcripts of greater than 200 nucleotides that function by means other than coding for proteins, and regulate active and silent chromatin states[47]. Pluripotent states are epigenetically regulated by lncRNAs[47]. Epigenetic modifications in pluripotency are conducted *via* several steps such as histone modifications, DNA methylation and chromatin remodeling[48]. miRNA expression is also regulated in atherosclerosis[49]. Endothelial miRNA expression profiling *in silico* is one area for future study of miRNA signaling[49].

CELLULAR REPROGRAMMING

Stem cells can be reprogrammed from differentiated cells[50]. The abundant genes including *CDH1*, *RGS1*, and *NOTCH1* are related to the cell characteristics such as MSCs and gastric cancer cells[51]. A recent study revealed that neural stem cells could be reprogrammed from adult fibroblasts, senescent somatic cells, and blood cells by HMGA2/let-7[52]. These induced neural stem cells could be successfully differentiated into neurons, astrocytes, and oligodendrocytes *in vitro* and *in vivo*[52]. The clinical application of reprogrammed iPS cells has also been attempted[53]. The transplantation of reprogrammed cells efficiently overcame spinal cord injury in mice; however, the possibility of tumorigenesis is a concern during long-term therapy[53]. One of the mechanisms of tumor development in transplanted iPS cells seems to be the acquisition of mesenchymal features such as either snail up-regulation or epithelial-mesenchymal transition[53]. Careful investigation and gene expression analysis are needed before iPS cells can be utilized in clinical settings[53]. The selective targeting of human iPS cells to avoid tumorigenesis has been described using a stem cell-specific lectin probe and *Pseudomonas aeruginosa* exotoxin A fusion proteins[54]. iPS cells are reprogrammed from cancer cells that are used as a carcinogenesis model[55]. The expression of pluripotency associated genes such as either *Oct4* (also known as *Pou5f1*) or *Nanog* is regulated by the bromodomain and extraterminal domain family member BRD4[56]. BRD4 regulates transcription by binding to acetylated histones[56]. The induced differentiation of human iPS cells by interleukin-3/granulocyte colony-stimulating factor results in $CD45^+CD11b^+CD14^+CD163^+CD68^+$ monocyte/macrophage-type cell formation[57]. Mesenchymal-amoeboid transition is a suggested mechanism by which cancer cells might adopt a migration mode[58]. The cell migration mode is characterized by three qualities: adhesion, confinement, and contractility[58]. The change in cell phenotype that occurs during reprogramming may be related to migration parameters. *WNT5A* is reported to be up-regulated in MSCs compared to diffuse-type gastric cancer cells[59]. Genes related to EMT such as *WNT5A* or *NOTCH2* may have roles in maintaining mesenchymal features[59]. A genome-wide analysis of chromatin interactions in human ES cells, ES cell-derived mesendoderm, MSCs, neural progenitor cells and trophoblast-like cells revealed that 36% of active and inactive chromosomal compartment alterations occur during differentiation[60]. An integrative analysis of 111 reference human epigenomes has been performed by the NIH Roadmap Epigenomics Consortium; this analysis profiled histone modification patterns and gene regulation in several cell types and found that each identified mechanism of genetic regulation targets different tissues and cell types in human biology and disease[61].

The analysis of adipose-derived stem cell differentiation has revealed that keratinocyte progenitor cells are present in adipose tissue[62]. The reprogramming of fibroblasts into iPS cells requires WNT signaling[63]. The efficiency of cell reprogramming is regulated by WNT signaling in fibroblasts[63]. Single-cell mass cytometry has revealed that iPS cell reprogramming is related to mesenchymal-epithelial transition (MET)[64]. A time-resolved progression analysis of iPS cell reprogramming has revealed that the expression patterns are altered at each time point and that some similarities exist between Oct4-GFP, Nanog-Neo, and Nanog-GFP mouse embryonic fibroblast reprogramming systems[64]. NANOG, which regulates the pluripotency and reprogramming of iPS cells, binds to the OCT4 promoter, and its mutation enhances ES cell self-renewal[65].

In early-stage diabetic endothelial progenitor cells, the expression of anti-oxidative enzymes compensates for oxidative stress levels[66]. The relationship between oxidative stress and progenitor cell function may be an interesting future topic for future study of the reprogramming process. The expansion of human

ES cells can be successfully maintained using MSC feeder layers[67]. Considering that xenogeneic-free culture conditions are necessary for safe advances in regenerative medicine, the mechanisms that maintain pluripotent status and induce differentiation in the cellular microenvironment should be investigated.

CONCLUSION

In conclusion, stem cells have diverse internal signaling and communicate with surrounding cells. This communication among cells, such as immune cells and cancer cells, through signaling proteins and molecules leads to dramatic alterations in cell responses and pathological physiology. The intra- and inter-cellular signaling pathways in the cellular microenvironment must be elucidated to safely manipulate stem cells and program signaling cascades for therapeutic applications. Abundant data about cellular gene expression, genomics and epigenomics, which are "big-data", and database analyses are publically available *via* the internet and will contribute to our understanding of cell phenotype transition and the mechanisms that regulate various cell populations in the context of disease and therapy.

REFERENCES

1 **Huang HY**, Chiu TL, Chang HF, Hsu HR, Pang CY, Liew HK, Wang MJ. Epigenetic regulation contributes to urocortin-enhanced midbrain dopaminergic neuron differentiation. *Stem Cells* 2015; **33**: 1601-1617 [PMID: 25641682 DOI: 10.1002/stem.1949]

2 **Czysz K**, Minger S, Thomas N. DMSO efficiently down regulates pluripotency genes in human embryonic stem cells during definitive endoderm derivation and increases the proficiency of hepatic differentiation. *PLoS One* 2015; **10**: e0117689 [PMID: 25659159 DOI: 10.1371/journal.pone.0117689]

3 **Geula S**, Moshitch-Moshkovitz S, Dominissini D, Mansour AA, Kol N, Salmon-Divon M, Hershkovitz V, Peer E, Mor N, Manor YS, Ben-Haim MS, Eyal E, Yunger S, Pinto Y, Jaitin DA, Viukov S, Rais Y, Krupalnik V, Chomsky E, Zerbib M, Maza I, Rechavi Y, Massarwa R, Hanna S, Amit I, Levanon EY, Amariglio N, Stern-Ginossar N, Novershtern N, Rechavi G, Hanna JH. Stem cells. m6A mRNA methylation facilitates resolution of naïve pluripotency toward differentiation. *Science* 2015; **347**: 1002-1006 [PMID: 25569111 DOI: 10.1126/science.1261417]

4 **Wu J**, Okamura D, Li M, Suzuki K, Luo C, Ma L, He Y, Li Z, Benner C, Tamura I, Krause MN, Nery JR, Du T, Zhang Z, Hishida T, Takahashi Y, Aizawa E, Kim NY, Lajara J, Guillen P, Campistol JM, Esteban CR, Ross PJ, Saghatelian A, Ren B, Ecker JR, Izpisua Belmonte JC. An alternative pluripotent state confers interspecies chimaeric competency. *Nature* 2015; **521**: 316-321 [PMID: 25945737 DOI: 10.1038/nature14413]

5 **Shojaie S**, Ermini L, Ackerley C, Wang J, Chin S, Yeganeh B, Bilodeau M, Sambi M, Rogers I, Rossant J, Bear CE, Post M. Acellular lung scaffolds direct differentiation of endoderm to functional airway epithelial cells: requirement of matrix-bound HS proteoglycans. *Stem Cell Reports* 2015; **4**: 419-430 [PMID: 25660407 DOI: 10.1016/j.stemcr.2015.01.004]

6 **Zou L**, Kidwai FK, Kopher RA, Motl J, Kellum CA, Westendorf JJ, Kaufman DS. Use of RUNX2 expression to identify osteogenic progenitor cells derived from human embryonic stem cells. *Stem Cell Reports* 2015; **4**: 190-198 [PMID: 25680477 DOI: 10.1016/j.stemcr.2015.01.008]

7 **Smith LA**, Liu X, Hu J, Ma PX. The enhancement of human embryonic stem cell osteogenic differentiation with nano-fibrous scaffolding. *Biomaterials* 2010; **31**: 5526-5535 [PMID: 20430439 DOI: 10.1016/j.biomaterials.2010.03.065]

8 **Ahn SE**, Kim S, Park KH, Moon SH, Lee HJ, Kim GJ, Lee YJ, Park KH, Cha KY, Chung HM. Primary bone-derived cells induce osteogenic differentiation without exogenous factors in human embryonic stem cells. *Biochem Biophys Res Commun* 2006; **340**: 403-408 [PMID: 16389066 DOI: 10.1016/j.bbrc.2005.12.020]

9 **Doran MR**, Frith JE, Prowse AB, Fitzpatrick J, Wolvetang EJ, Munro TP, Gray PP, Cooper-White JJ. Defined high protein content surfaces for stem cell culture. *Biomaterials* 2010; **31**: 5137-5142 [PMID: 20378164 DOI: 10.1016/j.biomaterials.2010.03.015]

10 **Tsankov AM**, Gu H, Akopian V, Ziller MJ, Donaghey J, Amit I, Gnirke A, Meissner A. Transcription factor binding dynamics during human ES cell differentiation. *Nature* 2015; **518**: 344-349 [PMID: 25693565 DOI: 10.1038/nature14233]

11 **Tanabe S**, Sato Y, Suzuki T, Suzuki K, Nagao T, Yamaguchi T. Gene expression profiling of human mesenchymal stem cells for identification of novel markers in early- and late-stage cell culture. *J Biochem* 2008; **144**: 399-408 [PMID: 18550633 DOI: 10.1093/jb/mvn082]

12 **Wells CA**, Mosbergen R, Korn O, Choi J, Seidenman N, Matigian NA, Vitale AM, Shepherd J. Stemformatics: visualisation and sharing of stem cell gene expression. *Stem Cell Res* 2013; **10**: 387-395 [PMID: 23466562 DOI: 10.1016/j.scr.2012.12.003]

13 **Ziller MJ**, Edri R, Yaffe Y, Donaghey J, Pop R, Mallard W, Issner R, Gifford CA, Goren A, Xing J, Gu H, Cacchiarelli D, Tsankov AM, Epstein C, Rinn JL, Mikkelsen TS, Kohlbacher O, Gnirke A, Bernstein BE, Elkabetz Y, Meissner A. Dissecting neural differentiation regulatory networks through epigenetic footprinting. *Nature* 2015; **518**: 355-359 [PMID: 25533951 DOI: 10.1038/nature13990]

14 **Zhang H**, Shao B, Zhuge Q, Wang P, Zheng C, Huang W, Yang C, Wang B, Su DM, Jin K. Cross-talk between human neural stem/progenitor cells and peripheral blood mononuclear cells in an allogeneic co-culture model. *PLoS One* 2015; **10**: e0117432 [PMID: 25658950 DOI: 10.1371/journal.pone.0117432]

15 **Yamashita A**, Morioka M, Yahara Y, Okada M, Kobayashi T, Kuriyama S, Matsuda S, Tsumaki N. Generation of scaffoldless hyaline cartilaginous tissue from human iPSCs. *Stem Cell Reports* 2015; **4**: 404-418 [PMID: 25733017 DOI: 10.1016/j.stemcr.2015.01.016]

16 **Huang da W**, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 2009; **4**: 44-57 [PMID: 19131956 DOI: 10.1038/nprot.2008.211]

17 **Huang da W**, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res* 2009; **37**: 1-13 [PMID: 19033363 DOI: 10.1093/nar/gkn923]

18 **Johnson RL**, Tabin CJ. Molecular models for vertebrate limb development. *Cell* 1997; **90**: 979-990 [PMID: 9323126 DOI: 10.1016/S0092-8674(00)80364-5]

19 **St-Jacques B**, Hammerschmidt M, McMahon AP. Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. *Genes Dev* 1999; **13**: 2072-2086 [PMID: 10465785 DOI: 10.1101/gad.13.16.2072]

20 **Kanaya K**, Masaaki I, Okazaki T, Nakamura T, Horii-Komatsu M, Alev C, Akimaru H, Kawamoto A, Akashi H, Tanaka H, Asahi M, Asahara T. Sonic Hedgehog signaling regulates vascular differentiation and function in human CD34 positive cells: vasculogenic CD34(+) cells with Sonic Hedgehog. *Stem Cell Res* 2015; **14**: 165-176 [PMID: 25644021 DOI: 10.1016/j.scr.2015.01.003]

21 **Campbell V**, Copland M. Hedgehog signaling in cancer stem cells: a focus on hematological cancers. *Stem Cells Cloning* 2015; **8**: 27-38 [PMID: 25691811 DOI: 10.2147/SCCAA.S58613]

22 **Qu B**, Xin GR, Zhao LX, Xing H, Lian LY, Jiang HY, Tong JZ, Wang BB, Jin SZ. Testing stem cell therapy in a rat model of inflammatory bowel disease: role of bone marrow stem cells and stem cell factor in mucosal regeneration. *PLoS One* 2014; **9**: e107891 [PMID: 25309991 DOI: 10.1371/journal.pone.0107891]

23 **Jägle S**, Rönsch K, Timme S, Andrlová H, Bertrand M, Jäger M, Proske A, Schrempp M, Yousaf A, Michoel T, Zeiser R, Werner M, Lassmann S, Hecht A. Silencing of the EPHB3 tumor-suppressor gene in human colorectal cancer through decommissioning of a transcriptional enhancer. *Proc Natl Acad Sci USA* 2014; **111**: 4886-4891 [PMID: 24707046 DOI: 10.1073/pnas.1314523111]

24 **Delgado E**, Bahal R, Yang J, Lee JM, Ly DH, Monga SP. β-Catenin knockdown in liver tumor cells by a cell permeable gamma guanidine-based peptide nucleic acid. *Curr Cancer Drug Targets* 2013; **13**: 867-878 [PMID: 23822752 DOI: 10.2174/15680096113139990081]

25 **Toma MI**, Erdmann K, Diezel M, Meinhardt M, Zastrow S, Fuessel S, Wirth MP, Baretton GB. Lack of ephrin receptor A1 is a favorable independent prognostic factor in clear cell renal cell carcinoma. *PLoS One* 2014; **9**: e102262 [PMID: 25025847 DOI: 10.1371/journal.pone.0102262]

26 **Lee CT**, Bendriem RM, Kindberg AA, Worden LT, Williams MP, Drgon T, Mallon BS, Harvey BK, Richie CT, Hamilton RS, Chen J, Errico SL, Tsai SY, Uhl GR, Freed WJ. Functional consequences of 17q21.31/WNT3-WNT9B amplification in hPSCs with respect to neural differentiation. *Cell Rep* 2015; **10**: 616-632 [PMID: 25640183 DOI: 10.1016/j.celrep.2014.12.050]

27 **Shojima K**, Sato A, Hanaki H, Tsujimoto I, Nakamura M, Hattori K, Sato Y, Dohi K, Hirata M, Yamamoto H, Kikuchi A. Wnt5a promotes cancer cell invasion and proliferation by receptor-mediated endocytosis-dependent and -independent mechanisms, respectively. *Sci Rep* 2015; **5**: 8042 [PMID: 25622531 DOI: 10.1038/srep08042]

28 **Bo H**, Zhang S, Gao L, Chen Y, Zhang J, Chang X, Zhu M. Upregulation of Wnt5a promotes epithelial-to-mesenchymal transition and metastasis of pancreatic cancer cells. *BMC Cancer* 2013; **13**: 496 [PMID: 24156409 DOI: 10.1186/1471-2407-13-496]

29 **Fiorillo M**, Verre AF, Iliut M, Peiris-Pagés M, Ozsvari B, Gandara R, Cappello AR, Sotgia F, Vijayaraghavan A, Lisanti MP. Graphene oxide selectively targets cancer stem cells, across multiple tumor types: implications for non-toxic cancer treatment, via "differentiation-based nano-therapy". *Oncotarget* 2015; **6**: 3553-3562 [PMID: 25708684]

30 **Grabner B**, Schramek D, Mueller KM, Moll HP, Svinka J, Hoffmann T, Bauer E, Blaas L, Hruschka N, Zboray K, Stiedl P, Nivarthi H, Bogner E, Gruber W, Mohr T, Zwick RH, Kenner L, Poli V, Aberger F, Stoiber D, Egger G, Esterbauer H, Zuber J, Moriggl R, Eferl R, Győrffy B, Penninger JM, Popper H, Casanova E. Disruption of STAT3 signalling promotes KRAS-induced lung tumorigenesis. *Nat Commun* 2015; **6**: 6285 [PMID: 25734337 DOI: 10.1038/ncomms7285]

31 **Ma J**, Cheng J, Gong Y, Tian L, Huang Q. Downregulation of Wnt signaling by sonic hedgehog activation promotes repopulation of human tumor cell lines. *Dis Model Mech* 2015; **8**: 385-391 [PMID: 25713298 DOI: 10.1242/dmm.018887]

32 **Luca VC**, Jude KM, Pierce NW, Nachury MV, Fischer S, Garcia KC. Structural biology. Structural basis for Notch1 engagement of Delta-like 4. *Science* 2015; **347**: 847-853 [PMID: 25700513 DOI: 10.1126/science.1261093]

33 **Airoldi I**, Tupone MG, Esposito S, Russo MV, Barbarito G, Cipollone G, Di Carlo E. Interleukin-27 re-educates intratumoral myeloid cells and down-regulates stemness genes in non-small cell lung cancer. *Oncotarget* 2015; **6**: 3694-3708 [PMID: 25638163]

34 **Narcisi R**, Cleary MA, Brama PA, Hoogduijn MJ, Tüysüz N, ten Berge D, van Osch GJ. Long-term expansion, enhanced chondrogenic potential, and suppression of endochondral ossification of adult human MSCs via WNT signaling modulation. *Stem Cell Reports* 2015; **4**: 459-472 [PMID: 25733021 DOI: 10.1016/j.stemcr.2015.01.017]

35 **Huskey NE**, Guo T, Evason KJ, Momcilovic O, Pardo D, Creasman KJ, Judson RL, Blelloch R, Oakes SA, Hebrok M, Goga A. CDK1 inhibition targets the p53-NOXA-MCL1 axis, selectively kills embryonic stem cells, and prevents teratoma formation. *Stem Cell Reports* 2015; **4**: 374-389 [PMID: 25733019 DOI: 10.1016/j.stemcr.2015.01.019]

36 **Cheng CJ**, Bahal R, Babar IA, Pincus Z, Barrera F, Liu C, Svoronos A, Braddock DT, Glazer PM, Engelman DM, Saltzman WM, Slack FJ. MicroRNA silencing for cancer therapy targeted to the tumour microenvironment. *Nature* 2015; **518**: 107-110 [PMID: 25409146 DOI: 10.1038/nature13905]

37 **Serguienko A**, Grad I, Wennerstrøm AB, Meza-Zepeda LA, Thiede B, Stratford EW, Myklebost O, Munthe E. Metabolic reprogramming of metastatic breast cancer and melanoma by let-7a microRNA. *Oncotarget* 2015; **6**: 2451-2465 [PMID: 25669981]

38 **Vallabhaneni KC**, Penfornis P, Dhule S, Guillonneau F, Adams KV, Mo YY, Xu R, Liu Y, Watabe K, Vemuri MC, Pochampally R. Extracellular vesicles from bone marrow mesenchymal stem/stromal cells transport tumor regulatory microRNA, proteins, and metabolites. *Oncotarget* 2015; **6**: 4953-4967 [PMID: 25669974]

39 **Lee SW**, Yang J, Kim SY, Jeong HK, Lee J, Kim WJ, Lee EJ, Kim HS. MicroRNA-26a induced by hypoxia targets HDAC6 in myogenic differentiation of embryonic stem cells. *Nucleic Acids Res* 2015; **43**: 2057-2073 [PMID: 25662604 DOI: 10.1093/nar/gkv088]

40 **Collet G**, El Hafny-Rahbi B, Nadim M, Tejchman A, Klimkiewicz K, Kieda C. Hypoxia-shaped vascular niche for cancer stem cells. *Contemp Oncol* (Pozn) 2015; **19**: A39-A43 [PMID: 25691820 DOI: 10.5114/wo.2014.47130]

41 **Zeisel A**, Muñoz-Manchado AB, Codeluppi S, Lönnerberg P, La Manno G, Juréus A, Marques S, Munguba H, He L, Betsholtz C, Rolny C, Castelo-Branco G, Hjerling-Leffler J, Linnarsson S. Brain structure. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. *Science* 2015; **347**: 1138-1142 [PMID: 25700174 DOI: 10.1126/science.aaa1934]

42 **Rafiee MR**, Malekzadeh Shafaroudi A, Rohban S, Khayatzadeh H, Kalhor HR, Mowla SJ. Enrichment of A Rare Subpopulation of miR-302-Expressing Glioma Cells by Serum Deprivation. *Cell J* 2015; **16**: 494-505 [PMID: 25685740]

43 **Chao CC**, Kan D, Lu KS, Chien CL. The role of microRNA-30c in the self-renewal and differentiation of C6 glioma cells. *Stem Cell Res* 2015; **14**: 211-223 [PMID: 25698399 DOI: 10.1016/j.scr.2015.01.008]

44 **Kong SY**, Park MH, Lee M, Kim JO, Lee HR, Han BW, Svendsen CN, Sung SH, Kim HJ. Kuwanon V inhibits proliferation, promotes cell survival and increases neurogenesis of neural stem cells. *PLoS One* 2015; **10**: e0118188 [PMID: 25706719 DOI: 10.1371/journal.pone.0118188]

45 **Prokopi M**, Kousparou CA, Epenetos AA. The Secret Role of microRNAs in Cancer Stem Cell Development and Potential Therapy: A Notch-Pathway Approach. *Front Oncol* 2015; **4**: 389 [PMID: 25717438 DOI: 10.3389/fonc.2014.00389]

46 **Trimarchi T**, Bilal E, Ntziachristos P, Fabbri G, Dalla-Favera R, Tsirigos A, Aifantis I. Genome-wide mapping and characterization of Notch-regulated long noncoding RNAs in acute leukemia. *Cell* 2014; **158**: 593-606 [PMID: 25083870 DOI: 10.1016/j.cell.2014.05.049]

47 **Flynn RA**, Chang HY. Long noncoding RNAs in cell-fate programming and reprogramming. *Cell Stem Cell* 2014; **14**: 752-761 [PMID: 24905165 DOI: 10.1016/j.stem.2014.05.014]

48 **El-Badawy A**, El-Badri N. Regulators of pluripotency and their implications in regenerative medicine. *Stem Cells Cloning* 2015; **8**: 67-80 [PMID: 25960670 DOI: 10.2147/SCCAA.S80157]

49 **Kumar S**, Kim CW, Son DJ, Ni CW, Jo H. Flow-dependent regulation of genome-wide mRNA and microRNA expression in endothelial cells in vivo. *Sci Data* 2014; **1**: 140039 [PMID: 25977794 DOI: 10.1038/sdata.2014.39]

50 **Tanabe S**. Origin of cells and network information. *World J Stem Cells* 2015; **7**: 535-540 [PMID: 25914760 DOI: 10.4252/wjsc.v7.i3.535]

51 **Tanabe S**, Aoyagi K, Yokozaki H, Sasaki H. Regulated genes in mesenchymal stem cells and gastric cancer. *World J Stem Cells* 2015; **7**: 208-222 [PMID: 25621121 DOI: 10.4252/wjsc.v7.i1.208]

52 **Yu KR**, Shin JH, Kim JJ, Koog MG, Lee JY, Choi SW, Kim HS, Seo Y, Lee S, Shin TH, Jee MK, Kim DW, Jung SJ, Shin S, Han DW, Kang KS. Rapid and Efficient Direct Conversion of Human Adult Somatic Cells into Neural Stem Cells by HMGA2/let-7b.

Cell Rep 2015 Jan 15; Epub ahead of print [PMID: 25600877 DOI: 10.1016/j.celrep.2014.12.038]

53 **Nori S**, Okada Y, Nishimura S, Sasaki T, Itakura G, Kobayashi Y, Renault-Mihara F, Shimizu A, Koya I, Yoshida R, Kudoh J, Koike M, Uchiyama Y, Ikeda E, Toyama Y, Nakamura M, Okano H. Long-term safety issues of iPSC-based cell therapy in a spinal cord injury model: oncogenic transformation with epithelial-mesenchymal transition. *Stem Cell Reports* 2015; **4**: 360-373 [PMID: 25684226 DOI: 10.1016/j.stemcr.2015.01.006]

54 **Tateno H**, Onuma Y, Ito Y, Minoshima F, Saito S, Shimizu M, Aiki Y, Asashima M, Hirabayashi J. Elimination of tumorigenic human pluripotent stem cells by a recombinant lectin-toxin fusion protein. *Stem Cell Reports* 2015; **4**: 811-820 [PMID: 25866158 DOI: 10.1016/j.stemcr.2015.02.016]

55 **Curry EL**, Moad M, Robson CN, Heer R. Using induced pluripotent stem cells as a tool for modelling carcinogenesis. *World J Stem Cells* 2015; **7**: 461-469 [PMID: 25815129 DOI: 10.4252/wjsc.v7.i2.461]

56 **Wu T**, Pinto HB, Kamikawa YF, Donohoe ME. The BET family member BRD4 interacts with OCT4 and regulates pluripotency gene expression. *Stem Cell Reports* 2015; **4**: 390-403 [PMID: 25684227 DOI: 10.1016/j.stemcr.2015.01.012]

57 **Lachmann N**, Ackermann M, Frenzel E, Liebhaber S, Brennig S, Happle C, Hoffmann D, Klimenkova O, Lüttge D, Buchegger T, Kühnel MP, Schambach A, Janciauskiene S, Figueiredo C, Hansen G, Skokowa J, Moritz T. Large-scale hematopoietic differentiation of human induced pluripotent stem cells provides granulocytes or macrophages for cell replacement therapies. *Stem Cell Reports* 2015; **4**: 282-296 [PMID: 25680479 DOI: 10.1016/j.stemcr.2015.01.005]

58 **Liu YJ**, Le Berre M, Lautenschlaeger F, Maiuri P, Callan-Jones A, Heuzé M, Takaki T, Voituriez R, Piel M. Confinement and low adhesion induce fast amoeboid migration of slow mesenchymal cells. *Cell* 2015; **160**: 659-672 [PMID: 25679760 DOI: 10.1016/j.cell.2015.01.007]

59 **Tanabe S**, Aoyagi K, Yokozaki H, Sasaki H. Gene expression signatures for identifying diffuse-type gastric cancer associated with epithelial-mesenchymal transition. *Int J Oncol* 2014; **44**: 1955-1970 [PMID: 24728500 DOI: 10.3892/ijo.2014.2387]

60 **Dixon JR**, Jung I, Selvaraj S, Shen Y, Antosiewicz-Bourget JE, Lee AY, Ye Z, Kim A, Rajagopal N, Xie W, Diao Y, Liang J, Zhao H, Lobanenkov VV, Ecker JR, Thomson JA, Ren B. Chromatin architecture reorganization during stem cell differentiation. *Nature* 2015; **518**: 331-336 [PMID: 25693564 DOI: 10.1038/nature14222]

61 **Kundaje A**, Meuleman W, Ernst J, Bilenky M, Yen A, Heravi-Moussavi A, Kheradpour P, Zhang Z, Wang J, Ziller MJ, Amin V, Whitaker JW, Schultz MD, Ward LD, Sarkar A, Quon G, Sandstrom RS, Eaton ML, Wu YC, Pfenning AR, Wang X, Claussnitzer M, Liu Y, Coarfa C, Harris RA, Shoresh N, Epstein CB, Gjoneska E, Leung D, Xie W, Hawkins RD, Lister R, Hong C, Gascard P, Mungall AJ, Moore R, Chuah E, Tam A, Canfield TK, Hansen RS, Kaul R, Sabo PJ, Bansal MS, Carles A, Dixon JR, Farh KH, Feizi S, Karlic R, Kim AR, Kulkarni A, Li D, Lowdon R, Elliott G, Mercer TR, Neph SJ, Onuchic V, Polak P, Rajagopal N, Ray P, Sallari RC, Siebenthall KT, Sinnott-Armstrong NA, Stevens M, Thurman RE, Wu J, Zhang B, Zhou X, Beaudet AE, Boyer LA, De Jager PL, Farnham PJ, Fisher SJ, Haussler D, Jones SJ, Li W, Marra MA, McManus MT, Sunyaev S, Thomson JA, Tlsty TD, Tsai LH, Wang W, Waterland RA, Zhang MQ, Chadwick LH, Bernstein BE, Costello JF, Ecker JR, Hirst M, Meissner A, Milosavljevic A, Ren B, Stamatoyannopoulos JA, Wang T, Kellis M. Integrative analysis of 111 reference human epigenomes. *Nature* 2015; **518**: 317-330 [PMID: 25693563 DOI: 10.1038/nature14248]

62 **Hasegawa T**, Sakamoto A, Wada A, Fukai T, Iida H, Ikeda S. Keratinocyte progenitor cells reside in human subcutaneous adipose tissue. *PLoS One* 2015; **10**: e0118402 [PMID: 25714344 DOI: 10.1371/journal.pone.0118402]

63 **Ross J**, Busch J, Mintz E, Ng D, Stanley A, Brafman D, Sutton VR, Van den Veyver I, Willert K. A rare human syndrome provides genetic evidence that WNT signaling is required for reprogramming of fibroblasts to induced pluripotent stem cells. *Cell Rep* 2014; **9**: 1770-1780 [PMID: 25464842 DOI: 10.1016/j.celrep.2014.10.049]

64 **Zunder ER**, Lujan E, Goltsev Y, Wernig M, Nolan GP. A continuous molecular roadmap to iPSC reprogramming through progression analysis of single-cell mass cytometry. *Cell Stem Cell* 2015; **16**: 323-337 [PMID: 25748935 DOI: 10.1016/j.stem.2015.01.015]

65 **Hayashi Y**, Caboni L, Das D, Yumoto F, Clayton T, Deller MC, Nguyen P, Farr CL, Chiu HJ, Miller MD, Elsliger MA, Deacon AM, Godzik A, Lesley SA, Tomoda K, Conklin BR, Wilson IA, Yamanaka S, Fletterick RJ. Structure-based discovery of NANOG variant with enhanced properties to promote self-renewal and reprogramming of pluripotent stem cells. *Proc Natl Acad Sci USA* 2015; **112**: 4666-4671 [PMID: 25825768 DOI: 10.1073/pnas.1502855112]

66 **Sukmawati D**, Fujimura S, Jitsukawa S, Ito-Hirano R, Ishii T, Sato T, Hayashi A, Itoh S, Mizuno H, Daida H, Tanaka R. Oxidative stress tolerance of early stage diabetic endothelial progenitor cell. *Regenerative Therapy* 2015; **1**: 38-44 [DOI: 10.1016/j.reth.2014.11.001]

67 **Akutsu H**, Machida M, Kanzaki S, Sugawara T, Ohkura T, Nakamura N, Yamazaki-Inoue M, Miura T, Vemuri MC, Rao MS, Miyado K, Umezawa A. Xenogeneic-free defined conditions for derivation and expansion of human embryonic stem cells with mesenchymal stem cells. *Regenerative Therapy* 2015; **1**: 18-29 [DOI: 10.1016/j.reth.2014.12.004]

P- Reviewer: Li SC, Maioli NA, Saeki K, Teresa Valenti M
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