miR-381 Regulates Neural Stem Cell Proliferation and Differentiation via Regulating Hes1 Expression

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Abstract

Neural stem cells are self-renewing, multipotent and undifferentiated precursors that retain the ability to differentiate into both glial (astrocytes and oligodendrocytes) and neuronal lineages. Neural stem cells offer cell-based therapies for neurological disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease and spinal cord injuries. However, their cellular behavior is poorly understood. MicroRNAs (miRNAs) are a class of small noncoding RNAs involved in cell development, proliferation and differentiation through regulating gene expression at post-transcriptional level. The role of miR-381 in the development of neural stem cells remains unknown. In this study, we showed that overexpression of miR-381 promoted neural stem cells proliferation. It induced the neural stem cells differentiation to neurons and inhibited their differentiation to astrocytes. Furthermore, we identified HES1 as a direct target of miR-381 in neural stem cells. Moreover, re-expression of HES1 impaired miR-381-induced promotion of neural stem cells proliferation and induce neural stem cells differentiation to neurons. In conclusion, miR-381 played important role in neural stem cells proliferation and differentiation.

Introduction

Neural stem cells are undifferentiated precursors, self-renewing cell populations that retain the ability to differentiate to both glial (astrocytes and oligodendrocytes) and neuronal lineages[1–5]. Neural stem cells are found in the adult and developing mammalian CNS (central nervous system)[6, 7]. Recent data show that neural stem cells can serve as cell replacement therapies for neurological disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease and spinal cord injuries[8–11]. Despite the great hope of using neural stem cells for clinically intervention, it is still a long distance before clinical application of neural stem cells[12–
Therefore, it is urgent to understand the molecular pathways controlling NSC proliferation and differentiation.

MicroRNAs (miRNAs) are a group of naturally occurring, conserved small non-coding, ~22-nucleotide RNA molecules that can inhibit translation or transcription by targeting protein-coding genes[15–19]. miRNAs are involved in a lot of biological functions such as cellular development, differentiation, proliferation, and apoptosis[20–23]. Deregulation of miRNAs has been found in various tumors including gastric cancer, laryngeal cancer, hepatocellular carcinoma, glioblastoma and ovarian carcinoma[16, 24–28]. Recently studies also found that miRNAs played an important role in stem cell fate determination and self-renewal by controlling the expression of stem cell regulators[3, 29–31].

In this study, we demonstrated that overexpression of miR–381 promoted neural stem cells proliferation and differentiation to neurons while it inhibited their differentiation to astrocytes. Furthermore, we identified Hairy and enhancer of split 1 (Hes1) as a direct target of miR–381 in neural stem cells.

Materials and Methods

Ethics Statement

This study was approved by the ethical board of the institute of The Second Affiliated Hospital of Harbin Medical University and complied with Declaration of Helsinki.

Cell Culture and Transfection

Rats were sacrificed by CO2 asphyxiation and neural stem cells were isolated from 13.5 days embryos of Wistar rats and cultured in growth medium with the 1% N2 (Gibco), 10 ng/ml bFGF (R&D), and 20 ng/ml human EGF (R&D) supplement. Primary neurospheres were digested using 0.25% trypsin to derive clone neurospheres. miR–381 mimic and scramble were purchased from Ambion. The transfection of miR–381 mimics and scramble (20 ng/ml), Hes–1 vector and related controls was performed using Lipofectamine 2000 (Invitrogen) following to the manufacturer’s instruction.

Immunocytochemistry

Cells were fixed with paraformaldehyde (4%) and then permeabilized by using 0.2% Triton-X. After blocking with goat serum (10%), cells were incubated with primary antibodies nestin (R&D, Minneapolis, MN, MAB1259), at 4°C overnight and then incubated the fluorescence labeled secondary antibodies. Nuclei were counter stained with DAPI (Vector labs, Burlingame, CA, H1200).

Cell Proliferation

Cell proliferation was measured by using the Counting kit 8 (CCK8) assay (Dojindo, Kumamoto, Japan) following to the manufacturer’s information. Proliferation rates were evaluated at 0, 24, 48 and 72 h after treatment. The OD (optical density) was evaluated at a wavelength of 450 nm.

qRT-PCR

Total RNA was isolated from the cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Real-time PCR was done to detect the expression of miRNA and mRNA using SYBR Green PCR Kit (QIAGEN) on 7500 Real-Time PCR System (Applied Biosystems). The PCR (polymerase chain reaction) was performed at 95°C for 8 min, then followed by 42 cycles at 95°C for 10
s, 60°C for 40 s, and 72°C for 1 s. The primer for Hes1 forward primer 5’-TGAAGGATTCGAAATATAATTCTCTGGG-3’ and reverse primer 5’-CGCTCTTCTCCATAGATAGGCTTTGATGAC-3’; β-tubulin III forward primer 5’-AAGCGGGCATGAAGAGT-3’ and reverse primer 5’-AGCCTCTTCTCCATAGATAGGCTTTGATGAC-3’; Nestin forward primer 5’-GTCATGAGCTTGAGCTTTGATGAC-3’ and reverse primer 5’-AGCCGCCCAGCAATCCAG-3’; GFAP forward primer 5’-AAGCGGGCATGAAGAGT-3’ and reverse primer 5’-CTTCCATTGGTGGTGAAGACGCCA-3’; GAPDH was forward primer 5’-ATTCCAATTGGCACCGTCAAGGCTGA-3’, reverse primer 5’-TTCTCCATGGTGGTGAAGACGCCA-3’.

Western Blot
Proteins were isolated from cells and then and separated on 12% SDS-PAGE gel. Then, it was transferred to PVDF membranes (Amersham, Buckinghamshire, UK). The membrane was blocked with milk (5%) and incubated with primary antibody as following: Nestin, β-tubulin III, GFAP, Hes1 and GAPDH (Sigma) for 2 hours. The blot was probed with HRP-conjugated secondary antibodies for 1 hour. The signal was evaluated by ECL kit (Millipore, MA, USA).

Luciferase Reporter Assay
The WT (wide type) or MT (mutant) 3’UTR of HES1 was amplified using PCR and cloned to the pGL3-luciferase reporter plasmid (Promega, Madison, WI, USA). Cell was transfected with miR-381 or scramble and luciferase reporter plasmid and the Renilla luciferase (Promega, Madison, WI, USA) using Lipofectamine 2000 (Invitrogen). Luciferase activity was performed using the Dual-Luciferase Reporter reagent (Promega) following to the manufacturer’s information.

Statistical Analysis
All data were shown as means ± SD. The difference between two groups was used Student’s t test or One-way ANOVA was performed to analyze the more than two groups.

Results
Neural stem cells could proliferate and differentiate into neurons and astrocytes Isolated cells proliferated and formed neurospheres on the second day after isolation (Fig 1A). These neurospheres also expressed the NSC-specific marker nestin (Fig 1B). Three days after withdraw of bFGF, these neurospheres differentiated into neurons and astrocytes (Fig 1C and 1D), thus confirming the identity of isolated cells as Neural stem cells.

miR-381 Promoted Neural Stem Cells Proliferation
We confirmed that miR-381 mimics could promote the expression of miR-381 in neural stem cells (Fig 2A). CCK-8 analysis demonstrated that overexpression of miR-381 increased neural stem cells proliferation (Fig 2B). In addition, miR-381 overexpression promoted the mRNA and protein expression of nestin (Fig 2C and 2D).

miR-381 Promoted Neural Stem Cells Differentiation to Neurons
miR-381 promoted neural stem cells differentiation to neurons as confirmed by immunofluorescence (Fig 3A). miR-381 overexpression promoted the mRNA and protein expression of β-tubulin III (Fig 3B and 3C).
miR–381 Inhibited Neural Stem Cells Differentiation to Astrocytes

miR–381 inhibited neural stem cells differentiation to astrocytes as confirmed by immunofluorescence (Fig 4A). miR–381 overexpression repressed the mRNA and protein expression of GFAP (Fig 4B and 4C).

Fig 2. miR–381 promoted neural stem cells proliferation. (A) The expression of miR–381 was measured by qRT-PCR. (B) CCK–8 was performed to detect the neural stem cells proliferation. (C) The mRNA expression of nestin was detected by qRT-PCR. (D) The protein expression of nestin was measured by Western blot. **p<0.01 and ***p<0.001.

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Hes1 was predicted to be target gene of miR–381 by TargetScan (Fig 5A). As shown in Fig 5B, miR-381 repressed the luciferase activity of wild type 3' UTR of Hes1 vector compared to that mutant 3' UTR of Hes1 vector (Fig 5B). Overexpression of miR–381 inhibited HES1 protein expression (Fig 5C).

Hes1 Was the Direct Target of miR–381 in Neural Stem Cells

Hes1 was predicted to be target gene of miR–381 by TargetScan (Fig 5A). As shown in Fig 5B, miR–381 repressed the luciferase activity of wild type 3' UTR of Hes1 vector compared to that mutant 3' UTR of Hes1 vector (Fig 5B). Overexpression of miR–381 inhibited HES1 protein expression (Fig 5C).

Fig 5. Hes1 was the direct target of miR–381 in neural stem cells. (A) Hes1 was predicted to be target gene of miR–381 by TargetScan. (B) Luciferase reporter assay was done to confirm the predictions in neural stem cells. (C) The protein expression of Hes1 was measured by Western blot in neural stem cells. ***p<0.001.

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**miR-381 Regulates Neural Stem Cell Proliferation and Differentiation**
miR–381 Promoted Neural Stem Cells Proliferation and Differentiation to Neurons by Targeting Hes1

We confirmed that Hes1 vector can promote the expression of Hes1 in neural stem cells (Fig 6A). Overexpression of Hes1 can impair miR-381–induced promotion of neural stem cells proliferation (Fig 6B). Ectopic expression of Hes1 inhibited miR-381–induced nestin mRNA and protein expression in neural stem cells (Fig 6C and 6D). Hes1 overexpression can repress miR-381–induced β-tubulin III mRNA and protein expression in neural stem cells (Fig 6E and 6F). Hes1 overexpression can promote miR-381–inhibited GFAP mRNA and protein expression in neural stem cells (Fig 6G and 6H).

Discussion

In this study, we demonstrated that overexpression of miR–381 promoted neural stem cells proliferation and differentiation to neurons while it inhibited the neural stem cells differentiation to astrocytes. Furthermore, we identified HES1 as a direct target of miR–381 in neural stem cells. Moreover, re-expression of HES1 impaired miR-381–induced promotion of neural stem cells proliferation and induce neural stem cells differentiation to neurons. Therefore, it is implicated that miR–381 plays important role in neural stem cells proliferation and differentiation.

Previous studies showed that miR–381 acted an important role in biological functions in both noncancerous and cancerous conditions[32–34]. For example, Lee et al[35] found that miR–381 overexpression inhibited the capacity of colony-forming of malignant mast and normal cell lines. Another study also found that miR–381 repressed the renal cancer cells.
Moreover, Zhou et al. revealed that miR–381 repressed cell migration and proliferation esophageal squamous cell carcinoma (ESCC). In addition, Hou et al. found that miR–381 expression was upregulated in arthritic cartilage and during chondrogenesis. miR–381 may contribute to absorption of the cartilage matrix by inducing MMP–13 and repressing type II collagen. However, the role of miR–381 in neural stem cells was unknown. In our study, we showed that overexpression of miR–381 promoted neural stem cells proliferation and differentiation to neurons while it inhibited the neural stem cells differentiation to astrocytes.

Hes genes are mammalian homologues of Enhancer of split and Drosophila hairy that encode bHLH (basic helix-loop-helix) transcriptional repressors. Hes1 is a downstream target of Notch signaling and it is highly expressed in the central nervous system. Previous studies demonstrated that Hes1 played an important role in the development of central nervous system. Hes1 was considered as crucial in repressing neuronal differentiation. The expression of Hes1 was essential for the maintenance of neural stem cells in the embryonic brain; however, overexpression of Hes1 repressed the differentiation and proliferation of neural stem cells. Moreover, downregulation of Hes1 induced neural stem cells differentiation into mature neurons. Furthermore, knockdown of Hes1 increased neuronal differentiation through upregulating Mash–1 (the neural differentiation factor). Tan et al. reported that miR–9 could promote the neural stem cells proliferation and differentiation to neurons by regulating Hes1 expression in developing brain. However, the underlying mechanisms of these are still unclear. Our study demonstrated that the ability of miR–381 to inhibit Hes1 expression might provide one such mechanism of post-transcriptional regulation of Hes1. In our study, we found that Hes1 as a direct target gene of miR–381 in neural stem cells. Firstly, the complementary sequence of Hes1 was predicted to be target gene of miR–381. Secondly, the data of luciferase reporter assay proved that miR–381 repressed the luciferase activity of wild type 3’UTR of HES1 vector compared to that mutant 3’UTR of HES1 vector. Thirdly, overexpression of miR–381 inhibited Hes1 protein expression in neural stem cells. The role of Hes1 was further supported by the results that promotion in neural stem cells proliferation and differentiation into neurons was attenuated by re-introduction of Hes1. These data indicate that miR-381 play an important role in the proliferation and differentiation of neural stem cells to at least partly mediated by inhibiting Hes1 expression in neural stem cells development.

In conclusion, our data demonstrated an important role of miR–381 in the regulation of proliferation, differentiation of neural stem cells. Our study also showed that miR–381 mediated the proliferation and differentiation of neural stem cells by regulating the Hes1 expression.

Author Contributions

Conceived and designed the experiments: XS C. Yan BL C. Yang XN XW JZ YW YZ. Performed the experiments: XS C. Yan BL C. Yang XN XW JZ YW YZ. Analyzed the data: XS BL C. Yan YZ. Contributed reagents/materials/analysis tools: XS BL C. Yan YZ. Wrote the paper: XS BL C. Yan YZ.

References

1. Liu S, Yin F, Zhang J, Wicha MS, Chang AE, Fan W, et al. Regulatory roles of miRNA in the human neural stem cell transformation to glioma stem cells. Journal of cellular biochemistry. 2014; 115(8):1368–80. Epub 2014/02/13. doi: 10.1002/jcb.24786 PMID: 24519663.

2. Saurat N, Andersson T, Vasistha NA, Molnar Z, Livesey FJ. Dicer is required for neural stem cell multipotency and lineage progression during cerebral cortex development. Neural development. 2013; 8:14. Epub 2013/07/31. doi: 10.1186/1749-8104-8-14 PMID: 23895693; PubMed Central PMCID: PMC3737057.
3. Garg N, Po A, Miele E, Campese AF, Begalli F, Silvano M, et al. microRNA-17-92 cluster is a direct Nanog target and controls neural stem cell through Trp53inp1. The EMBO journal. 2013; 32(21):2819–32. Epub 2013/10/01. doi: 10.1038/emboj.2013.214 PMID: 24076654; PubMed Central PMCID: PMC3817465.

4. Tan SL, Ohtsuka T, Gonzalez A, Kageyama R. MicroRNA9 regulates neural stem cell differentiation by controlling Hes1 expression dynamics in the developing brain. Genes to cells: devoted to molecular & cellular mechanisms. 2012; 17(12):952–61. Epub 2012/11/09. doi: 10.1111/j.1365-2461.2012.03416.x PMID: 22993205; PubMed Central PMCID: PMC3817465.

5. Pham JT, Gallicano GI. Specification of neural cell fate and regulation of neural stem cell proliferation by microRNAs. American journal of stem cells. 2012; 1(3):182–95. Epub 2012/01/01. PMID: 23671807; PubMed Central PMCID: PMC3636732.

6. Aranha MM, Santos DM, Sola S, Steer CJ, Rodrigues CM. miR-34a regulates mouse neural stem cell differentiation. PloS one. 2011; 6(8):e21396. Epub 2011/08/23. doi: 10.1371/journal.pone.0021396 PMID: 21857907; PubMed Central PMCID: PMC3153928.

7. Shi Y, Sun G, Zhao C, Stewart R. Neural stem cell self-renewal. Critical reviews in oncology/hematology. 2008; 65(1):43–53. Epub 2007/07/24. doi: 10.1016/j.critrevonc.2007.06.004 PMID: 17644000; PubMed Central PMCID: PMC2235812.

8. Zhu N, Lin J, Wang K, Wei M, Chen Q, Wang Y. Huperzine A protects neural stem cells against Abeta-induced apoptosis in a neural stem cells and microglia co-culture system. International journal of clinical and experimental pathology. 2011; 4(6):625–33. Epub 2011/08/12. PMID: 22113518.

9. Gonzalez C, Bonilla S, Flores AI, Cano E, Liste I. An update on human stem cell-based therapy in Parkinson’s disease. Current stem cell research & therapy. 2015. Epub 2015/06/29. PMID: 26081232.

10. Rossignol J, Fink K, Davis K, Cierco A, Crane S, Matchynski J, et al. Transplants of adult mesenchymal and neural stem cells provide neuroprotection and behavioral sparing in a transgenic rat model of Huntington’s disease. Stem Cells. 2014; 32(2):500–9. Epub 2013/08/14. doi: 10.1002/stem.1508 PMID: 23939879.

11. You Y, Che L, Lee HY, Lee HL, Yun Y, Lee M, et al. Anti-apoptotic effect of highly secreted GMCSF from neuronal cell-specific GMCSF over-expressing neural stem cells in spinal cord injury model. Spine. 2015. Epub 2015/05/29. PMID: 26018232.

12. Lopez-Ramirez MA, Nicoli S. Role of miRNAs and epigenetics in neural stem cell fate determination. Epigenetics: official journal of the DNA Methylation Society. 2014; 9(1):90–100. Epub 2013/12/18. doi: 10.4161/epi.27536 PMID: 24342893; PubMed Central PMCID: PMC3928190.

13. Luo Y, Zhu D. Combinatorial control of transgene expression by hypoxia-responsive promoter and microRNA regulation for neural stem cell-based cancer therapy. BioMed research international. 2014; 2014:751397. Epub 2014/05/28. doi: 10.1155/2014/751397 PMID: 24864258; PubMed Central PMCID: PMC4016876.

14. Rolando C, Taylor V. Neural stem cell of the hippocampus: development, physiology regulation, and dysfunction in disease. Current topics in developmental biology. 2014; 107:183–206. Epub 2014/01/21. doi: 10.1016/B978-0-12-416022-4.00007-X PMID: 24439807.

15. Yu X, Li Z, Liu J. MiRNAs in primary cutaneous lymphomas. Cell proliferation. 2015; 48(3):271–7. Epub 2015/03/05. doi: 10.1111/cpr.12179 PMID: 25736784.

16. Yu X, Li Z. The role of MicroRNAs expression in laryngeal cancer. Oncotarget. 2015. Epub 2015/06/17. PMID: 26079642.

17. Li Z, Yu X, Shen J, Law PT, Chan MT, Wu WK. MicroRNA expression and its implications for diagnosis and therapy of gallbladder cancer. Oncotarget. 2015; 6(16):13914–24. Epub 2015/06/04. PMID: 26040010.

18. Li Z, Yu X, Shen J, Chan MT, Wu WK. MicroRNA in intervertebral disc degeneration. Cell proliferation. 2015; 48(3):278–83. Epub 2015/03/05. doi: 10.1111/cpr.12180 PMID: 25736871.

19. Yang Z, Han Y, Cheng K, Zhang G, Wang X. miR-99a directly targets the mTOR signalling pathway in breast cancer side population cells. Cell proliferation. 2014; 47(6):587–95. Epub 2014/10/29. doi: 10.1111/cpr.12146 PMID: 25348507.

20. Li J, You T, Jing J. MiR-125b inhibits cell biological progression of Ewing's sarcoma by suppressing the PI3K/Akt signalling pathway. Cell proliferation. 2014; 47(2):152–60. Epub 2014/02/13. doi: 10.1111/cpr.12093 PMID: 24517182.

21. Huang J, Zhang SY, Gao YM, Liu YF, Liu YB, Zhao ZG, et al. MicroRNAs as oncogenes or tumour suppressors in oesophageal cancer: potential biomarkers and therapeutic targets. Cell proliferation. 2014; 47(4):277–86. Epub 2014/06/10. doi: 10.1111/cpr.12109 PMID: 24909356.
22. Li M, Yu M, Liu C, Zhu H, He X, Peng S, et al. miR-34c works downstream of p53 leading to dairy goat male germline stem-cell (mGSCs) apoptosis. Cell proliferation. 2013; 46(2):223–31. Epub 2013/03/21. doi: 10.1111/cpr.12013 PMID: 23510477.

23. Li Z, Yu X, Shen J, Jiang Y. MicroRNA dysregulation in uveal melanoma: a new player enters the game. Oncotarget. 2015. Epub 2015/02/17. PMID: 25682876.

24. Li Z, Yu X, Wang Y, Shen J, Wu WK, Liang J, et al. By downregulating TIAM1 expression, microRNA-329 suppresses gastric cancer invasion and growth. Oncotarget. 2015; 6(19):17559–69. Epub 2015/02/06. PMID: 25654811.

25. Li Z, Lei H, Luo M, Wang Y, Dong L, Ma Y, et al. DNA methylation downregulated mir-10b acts as a tumor suppressor in gastric cancer. Gastric cancer: official journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association. 2015; 18(1):43–54. Epub 2014/02/01. doi: 10.1007/s10120-014-0340-8 PMID: 24481854.

26. Yu L, Ding GF, He C, Sun L, Jiang Y, Zhu L. MicroRNA –424 is down-regulated in hepatocellular carcinoma and suppresses cell migration and invasion through c-Myb. PloS one. 2014; 9(3):e91661. Epub 2014/03/29. doi: 10.1371/journal.pone.0091661 PMID: 24675898; PubMed Central PMCID: PMC3968007.

27. Xie Q, Yan Y, Huang Z, Zhong X, Huang L. MicroRNA–221 targeting PI3-K/Akt signaling axis induces cell proliferation and BCNU resistance in human glioblastoma. Neuropathology: official journal of the Japanese Society of Neuropathology. 2014; 34(5):455–64. Epub 2014/05/02. doi: 10.1111/neup.12125 PMID: 24780067.

28. Dong R, Liu X, Zhang Q, Jiang Z, Li Y, Wei Y, et al. miR–145 inhibits tumor growth and metastasis by targeting metadherin in high-grade serous ovarian carcinoma. Oncotarget. 2014; 5(21):10816–29. Epub 2014/10/22. PMID: 25333261; PubMed Central PMCID: PMC4279412.

29. Zhou Y, Jiang H, Gu J, Tang Y, Shen N, Jin Y. MicroRNA–155 targets ADP-ribosylation factor-like protein 2 to induce apoptosis in human embryonic stem cell-derived neural progenitor cells. Cell death & disease. 2013; 4:e695. Epub 2013/06/29. doi: 10.1038/cddis.2013.195 PMID: 23807224; PubMed Central PMCID: PMC3702293.

30. Li X, Peng R, Huang C, Wang H, Wang J, Zhang Z, et al. MicroRNA–351 regulates TMEM 59 (DFC1) expression and mediates neural stem cell morphogenesis. RNA biology. 2012; 9(3):292–301. Epub 2012/02/18. doi: 10.4161/ma.19100 PMID: 22936717.

31. Morgado AL, Xavier JM, Dionisio PA, Ribeiro MF, Dias RB, Sebastiao AM, et al. MicroRNA-34a Modulates Neural Stem Cell Differentiation by Regulating Expression of Synaptic and Autophagic Proteins. Molecular neurobiology. 2014. Epub 2014/06/29. doi: 10.1007/s12035-014-8794-6 PMID: 24973144.

32. Tang H, Liu X, Wang Z, She X, Zeng X, Deng M, et al. Interaction of has-miR–381 and glioma suppressor LRRC4 is involved in glioma growth. Brain research. 2011; 1390:21–30. Epub 2011/03/30. doi: 10.1016/j.brainres.2011.03.034 PMID: 21435336.

33. Tang H, Wang Z, Liu Q, Liu X, Wu M, Li G. Disturbing miR–182 and -381 inhibits BRD7 transcription and glioma growth by directly targeting LRRC4. PloS one. 2014; 9(1):e84146. Epub 2014/01/10. doi: 10.1371/journal.pone.0084146 PMID: 24404152; PubMed Central PMCID: PMC3880275.

34. Hou C, Meng F, Zhang Z, Kang Y, Chen W, Huang G, et al. The Role of MicroRNA–381 in Chondrogenesis and Interleukin-1-beta Induced Chondrocyte Responses. Cellular physiology and biochemistry: international journal of experimental cellular physiology, biochemistry, and pharmacology. 2015; 36 (5):1753–66. Epub 2015/07/18. doi: 10.1159/000430148 PMID: 26184031.

35. Lee YN, Brandal S, Noel P, Wentzel E, Mendell JT, McDevitt MA, et al. KIT signaling regulates MiTF expression through miRNAs in normal and malignant mast cell proliferation. Blood. 2011; 117 (13):3629–40. Epub 2011/01/29. doi: 10.1182/blood-2010-07-293548 PMID: 21273305; PubMed Central PMCID: PMC3072881.

36. Chen B, Duan L, Yin G, Tan J, Jiang X. Simultaneously expressed miR–424 and miR–381 synergistically suppress the proliferation and survival of renal cancer cells——Cdc2 activity is up-regulated by targeting WEE1. Clinics (Sao Paulo). 2013; 68(6):825–33. Epub 2013/06/20. doi: 10.6061/clinics/2013(06)17 PMID: 23778472; PubMed Central PMCID: PMC3674285.

37. Zhou S, Ye W, Ren J, Shao Q, Qi Y, Liang J, et al. MicroRNA–381 increases radiosensitivity in esophageal squamous cell carcinoma. American journal of cancer research. 2015; 5(1):267–77. Epub 2015/01/30. PMID: 25628936; PubMed Central PMCID: PMC4300702.

38. Dailey DD, Anfinsen KP, Pfaff LE, Ehrhart EJ, Charles JB, Bonsdorff TB, et al. HES1, a target of Notch signaling, is elevated in canine osteosarcoma, but reduced in the most aggressive tumors. BMC veterinary research. 2013; 9:130. Epub 2013/07/03. doi: 10.1186/1746-6148-9-130 PMID: 23816051; PubMed Central PMCID: PMC3701487.
stem cells. Blood. 2013; 122(7):1162–73. Epub 2013/06/05. doi:10.1182/blood-2012-12-471649 PMID: 23733337.

40. Lei T, Bi Y, Gao MJ, Gao SM, Zhou LL, Zheng HL, et al. HES1 inhibits adipogenesis of porcine mesenchymal stem cells via transcriptional repression of FADD24. Domestic animal endocrinology. 2013; 45 (1):28–32. Epub 2013/04/25. doi: 10.1016/j.domaniend.2013.03.003 PMID: 23611667.

41. Zhang Z, Yan R, Zhang Q, Li J, Kang X, Wang H, et al. Hes1, a Notch signaling downstream target, regulates adult hippocampal neurogenesis following traumatic brain injury. Brain research. 2014; 1583:65–78. Epub 2014/08/02. doi: 10.1016/j.brainres.2014.07.037 PMID: 23825862.

42. Nakahara F, Kitaura J, Uchida T, Nishida C, Togami K, Inoue D, et al. Hes1 promotes blast crisis in chronic myelogenous leukemia through MMP–9 upregulation in leukemic cells. Blood. 2014; 123 (25):3932–42. Epub 2014/05/16. doi: 10.1182/blood-2013-01-476747 PMID: 24825862.

43. Li S, Liu Y, Liu Z, Wang R. Neural fate decisions mediated by combinatorial regulation of Hes1 and miR–9. Journal of biological physics. 2015. Epub 2015/07/15. doi: 10.1007/s10867-015-9391-2 PMID: 26156376.

44. Lillycrop KA, Costello PM, Teh AL, Murray RJ, Clarke-Harris R, Barton SJ, et al. Association between perinatal methylation of the neuronal differentiation regulator HES1 and later childhood neurocognitive function and behaviour. International journal of epidemiology. 2015. Epub 2015/04/25. doi: 10.1093/ije/dyv052 PMID: 25906782.

45. Kobayashi T, Iwamoto Y, Takashima K, Isonura A, Kosodo Y, Kawakami K, et al. Deubiquitinating enzymes regulate Hes1 stability and neuronal differentiation. The FEBS journal. 2015; 282(13):2475–87. Epub 2015/04/08. doi: 10.1111/febs.13290 PMID: 25846153.

46. Pfeuty B. A computational model for the coordination of neural progenitor self-renewal and differentiation through Hes1 dynamics. Development. 2015; 142(3):477–85. Epub 2015/01/22. doi: 10.1242/dev.112649 PMID: 25605780.

47. Wang W, Wang P, Li S, Yang J, Liang X, Tang Y, et al. Methylprednisolone inhibits the proliferation and affects the differentiation of rat spinal cord-derived neural progenitor cells cultured in low oxygen conditions by inhibiting HIF-1alpha and Hes1 in vitro. International journal of molecular medicine. 2014; 34 (3):788–95. Epub 2014/07/06. doi: 10.3892/ijmm.2014.1835 PMID: 24992925.

48. Indulekha CL, Divya TS, Divya MS, Sanalkumar R, Rasheed VA, Dhanesh SB, et al. Hes–1 regulates the excitatory fate of neural progenitors through modulation of Tlx3 (HOX11L2) expression. Cellular and molecular life sciences: CMLS. 2012; 69(4):611–27. Epub 2011/07/12. doi: 10.1007/s00018-011-0765-8 PMID: 21744064.

49. Keohane A, Ryan S, Maloney E, Sullivan AM, Nolan YM. Tumour necrosis factor-alpha impairs neuronal differentiation but not proliferation of hippocampal neural precursor cells: Role of Hes1. Molecular and cellular neurosciences. 2010; 43(1):127–35. Epub 2009/10/21. doi: 10.1016/j.mcn.2009.10.003 PMID: 19840854.

50. Castella P, Wagner JA, Caudy M. Regulation of hippocampal neuronal differentiation by the basic helix-loop-helix transcription factors HES–1 and MASH–1. Journal of neuroscience research. 1999; 56 (3):229–40. Epub 1999/05/21. PMID: 10336252.