Change in Expression Patterns of Micro RNAs in the Primary Culture of Parotid Acinar Cells

Hiroaki Ogawa,1 Megumi Yokoyama,2, 3 Osamu Katsumata-Kato,2, 3 and Junko Fujita-Yoshigaki2, 3

1Nihon University Graduated School of Dentistry at Matsudo, Cellular Physiology, Matsudo, Chiba 271–8587, Japan
2Department of Physiology, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba 271–8587, Japan
3Research Institute of Oral Science, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba 271–8587, Japan

Introduction

Decrease in saliva is a serious problem in clinical dentistry since saliva maintains healthy environment of the oral cavity. Hyposcretion of saliva and consequent dry mouth lead to severe dental caries, periodontal disease, and mucosal infections (1). We have established a system for primary culture of parotid acinar cells in order to investigate the mechanism of dysfunction of salivary glands (2). Salivary acinar cells gradually lose their function during the primary culture. For example, synthesis of amylase, a major saliva protein, and generation of new secretory granules were declined (3). In addition, the expression levels of aquaporin-5, which is essential for water secretion, decreased during the culture (4). We found that the process of cell isolation from the glands triggered the stress signal mediated by Src and p38 MAP kinases (4, 5).

On the other hand, the cells not only lost function as acinar cells, but also began to express genes that were not originally expressed. For example, duct markers such as claudin-4 and markers of epithelial-mesenchymal transition such as matrix metallopeptidase-9 and fibronectin began to be detected (3, 4). In addition, stem/progenitor cell markers such as nestin and cytokeratin-14 were expressed in the primary culture (4, 6). There is a possibility that the changes occurred in the primary culture are not only loss of function, but transition to undifferentiated state that can re-differentiate to acinar cells. The regulatory mechanism for
the transition of cell characters remains poorly understood.

Micro RNAs (miRNAs) are small non-coding RNAs consisting of 20–25 nucleotides. They are not used as templates for protein synthesis and regulate the expression of other genes by degradation of mRNAs or suppression of translation. They are involved in the development, differentiation, cell proliferation and apoptosis. In the salivary glands, it was reported that miRNAs secreted from the mesenchyme reacted with the epithelium and modulated branching morphogenesis (7). In addition, different miRNAs are detected in the saliva from patients with a parotid gland neoplasm compared with the healthy control (8). Therefore, miRNAs may regulate development and function of salivary glands.

In this study, we examined changes in expression pattern of miRNAs in the primary culture of parotid acinar cells by microarray analysis. Pathway analysis suggested that changes in miRNA expression in parotid acinar cells were related to acquisition of characteristics of stem/progenitor cells.

Materials and Methods

Primary culture of parotid acinar cells

Parotid glands were taken from male Sprague-Dawley rats under anesthesia with 3% sevoflurane. Acinar cells were isolated by digestion with collagenase and hyaluronidase as previously reported (9). Cells were cultured in Waymouth’s medium containing 10% rat serum. The low molecular weight inhibitor for Src family kinases PP1 (4-amino-1-tert-butyl-3-(1’-naphthyl) pyrazolo[3,4-d] pyrimidine) was added to the isolating buffer and medium at a final concentration of 10 µM. Dimethylsulfoxide as a vehicle was added to the control buffer and medium (final concentration of 0.05%). All animal experiments were carried out in accordance with institutional and national guidelines for the care and use of experimental animals and were approved by the Experimental Animal Committee of the NIHl University School of Dentistry at Matsudo (API5MD018).

Comparison of the expression patterns of the total miRNAs by microarray analysis

Total RNAs were purified from the cells just after isolation (Day 0) and after 1-day culture (Day 1) with miRNeasy mini kit (Qiagen, Hilden, Germany). We used each one sample of Day 0 and Day 1 to microarray analysis. The quality of RNAs was confirmed by Agilent 2100 (Agilent Technology, Palo Alto, CA, USA). The expression patterns of miRNA were analyzed by GeneChip® miRNA 4.0 Array (Affymetrix, Santa Clara, CA, USA), and the signal intensities were normalized with robust multi-array average (RMA) method by Affymetrix Expression Console Software (Affymetrix). The ratio of the normalized values of Day 1 to those of Day 0 was calculated, and expression-altered miRNAs were identified GeneSpring software (Agilent Technologies). The values of weighted average difference between Day 0 and Day 1 were calculated as previously reported (10). The threshold value of up-regulated or down-regulated miRNA was a fold change greater than 2.0. The analysis protocol was shown in Fig. 1.

Prediction of target genes for miRNAs

The expression-altered miRNAs were further filtered based on their expression values. After identification of altered miRNAs from the microarray data, the predicted targets for the miRNAs were identified using TargetScan 7.2 (http://www.targetscan.org), miRDB (http://mirdb.org), miRSearch 3.0 (https://www.exiqon.com/mirsearch) and DIANA-microT-CDS 5.0 (http://www.microrna.gr/micr oT-CDS) software tools. We selected genes that were commonly predicted by two or more prediction tools.

Pathway analysis

The Kyoto encyclopedia of genes and genomes (KEGG) database provides searchable pathways for molecular interactions and cellular processes. DIANA-mirPath 3.0 (http://snp.515788.vm.okeanos.grnet.gr) was used to search KEGG pathways related to the set of expression-altered miRNAs. The set of target gene candidates for the chosen miRNAs was analyzed by DAVID functional annotation tool 6.8 (https://david.ncifcrf.gov).

Real time RT-PCR of miRNA and mRNA

The expression level of miR-3473 was determined by miRCURY LNA micro RNA PCR kit (Takara). U6 snRNA was used as a reference. The expression level of Sox10 was determined with One Step TB Green PrimeScript PLUS kit (Takara Bio, Kusatsu, Japan). Rps18 was used as a reference. The sequence of primer pair for Sox10 was forward: 5’-TCA CTA CAA GAG TGC CCA C-3’ and reverse: 5’-CAC ATT GCC GAA GTC GAT G-3’. The predicted size of PCR fragment was 215 bp. The primer pair for Rps18 was purchased from Takara Bio.
Antibodies and immunoblot analysis

Rabbit monoclonal anti-Sox10 antibody was purchased from Abcam (Cambridge, UK). Cells were lysed with 20 mM HEPES/NaOH (pH 7.4) containing 0.1% Triton X-100 and 1 × Complete Protease Inhibitor Cocktail (Roche, Basel, Switzerland). The proteins were separated by SDS-PAGE and transferred to Hybond-LFP membranes (GE healthcare, Chicago, IL, USA). The membranes were blocked at room temperature for 1 h in Blocking reagent (GE Healthcare), and probed with anti-Sox10 antibody. Immunoreactivity was determined using ECL-Plex (GE Healthcare) and images were acquired using Typhoon Trio (GE Healthcare). Intensities of immunoreactivities are quantified using ImageQuantTL software (GE Healthcare).

Results

Expression profiles of miRNAs in parotid acinar cells

Among the 728 mature miRNAs on the array, 377 miRNAs were absent both just after isolation (Day 0) and after 1-days culture (Day 1), and were excluded from further analysis. The expression levels of 18 mature miRNAs were up-regulated and 52 mature miRNAs were down-regulated from Day 0 to Day 1 (Table 1). To analyze the role of these miRNAs, KEGG pathway enrichment analysis was performed. Candidates of target genes were predicted by microT-CDS 5.0 and pathways were searched by mirPath 3.0. We excluded two miRNAs from the analysis because the two were not assigned by miRBase (ver. 21). From 68 miRNAs, 43 pathways were identified (Table 2).
Table 1. Microarray analysis of miRNAs whose expression was changed during the culture

| miR   | Fold Change | miR   | Fold Change |
|-------|-------------|-------|-------------|
| rno-miR-6216 | 2.3 | rno-miR-375-3p | 0.38 |
| rno-miR-17-5p | 2.0 | rno-miR-3473 | 0.30 |
| rno-miR-146b-5p | 3.4 | rno-miR-429 | 0.48 |
| rno-miR-31a-3p | 3.1 | rno-miR-30b-5p | 0.47 |
| rno-miR-1224 | 2.3 | rno-miR-15b-5p | 0.40 |
| rno-miR-18a-5p | 2.5 | rno-miR-7C-5p | 0.44 |
| rno-miR-21-5p | 2.9 | rno-miR-25-3p | 0.44 |
| rno-miR-92b-5p | 2.7 | rno-miR-352 | 0.46 |
| rno-miR-874-3p | 2.6 | rno-miR-6215 | 0.15 |
| rno-miR-503-5p | 4.0 | rno-miR-1949 | 0.33 |
| rno-miR-31a-3p | 3.6 | rno-miR-298-3p | 0.48 |
| rno-miR-326-5p | 2.8 | rno-miR-30s-3p | 0.40 |
| rno-miR-92a-1-5p | 2.1 | rno-miR-672-3p | 0.41 |
| rno-miR-7b-3p | 2.5 | rno-miR-181c-5p | 0.48 |
| rno-miR-150-3p | 2.1 | rno-miR-26b-5p | 0.43 |
| rno-miR-207 | 2.1 | rno-miR-140B-3p | 0.20 |
| rno-miR-327 | 2.5 | rno-miR-29B-2-5p | 0.32 |
| rno-miR-365-5p | 4.5 | rno-miR-30b-3p | 0.43 |
| rno-miR-7c-1-3p | 3.3 | rno-miR-382-5p | 0.42 |
| rno-miR-466b-5p | 0.33 | rno-miR-664-1-5p | 0.43 |
| rno-miR-944 | 0.29 | rno-miR-19a-3p | 0.45 |
| rno-miR-134-5p | 0.48 | rno-miR-204-5p | 0.38 |
| rno-miR-128-3p | 0.41 | rno-miR-1843-5p | 0.26 |
| rno-miR-674-3p | 0.44 | rno-miR-487b-5p | 0.28 |
| rno-miR-872-3p | 0.50 | rno-miR-298-3p | 0.25 |
| rno-miR-550 | 0.42 | rno-miR-101B-1p | 0.46 |
| rno-miR-24-1-5p | 0.14 | rno-miR-34c-5p | 0.48 |
| rno-miR-138-3p | 0.47 | rno-miR-195-3p | 0.40 |
| rno-miR-130B-1p | 0.42 | rno-miR-326-3p | 0.42 |
| rno-miR-368-5p | 0.44 | rno-miR-30m-3p | 0.39 |
| rno-miR-204-5p | 0.39 | rno-miR-29c-5p | 0.25 |
| rno-miR-162-5p | 0.25 | rno-miR-329-3p | 0.48 |
| rno-miR-322-3p | 0.30 | rno-miR-297 | 0.22 |
| rno-miR-576b-1p | 0.37 | rno-miR-493-3p | 0.44 |
| rno-miR-335-3p | 0.33 | rno-miR-338-3p | 0.33 |
| rno-miR-221-5p | 0.38 | rno-miR-299a-5p | 0.46 |

The values of Fold change were shown as ratio (Day 1/Day 0) of expression values. Up-regulated and down-regulated miRNAs were sorted in order of normalized expression values at Day 1 and Day 0, respectively.

We have already performed the comprehensive analysis on the expression patterns of miRNAs of parotid acinar cells in the primary culture (11). Thus, we compared the expression patterns of miRNAs and mRNAs. Since the expression levels of miRNAs were various, we selected seven miRNAs (miR-375-3p, miR-3473, miR-429, miR-30b-5p, miR-15b-5p, let-7i-3p and miR-25-3p) whose normalized expression values were more than 1000 at Day 0 from the 52 down-regulated miRNAs (Fig. 2). As target genes for the seven miRNAs, 1088 genes were predicted. Among them, we selected 202 genes whose miRNAs were up-regulated more than two-fold after 1- or 2-day culture. From the 202 genes, 21 pathways were identified (Table 3). Among them, 13 pathways were common to the result of direct KEGG pathway analysis from expression-altered miRNA (compare Tables 2 and 3). The down-regulation of miRNAs in the culture may be involved in the 13 pathways.

Change in the expression level of rno-miR-3473 in parotid acinar cells during the culture

Among the seven down-regulated miRNAs that were highly expressed, the fold change of miR-3473 was the largest (Table 1 and Fig. 2). We also calculated values of weighted average difference in order to rank differentially expressed miRNAs (10). The weighted average difference of miR-3473 was the largest among all rat miRNAs in the array. We confirmed the change in expression of miR-3473 by real time RT-PCR. The expression level of miR-3473 was significantly decreased during the culture (Fig. 3). In contrast, in the presence of a Src kinase inhibitor PP1, its expression was gradually declined but the difference was not significant.

Change in the expression level of Sox10 mRNA in parotid acinar cells during the culture

We searched target gene candidates for miR-3473 by TargetScan 7.2. Among them, we focused on Sox10. Sox10 is known as a transcription factor that regulates stem/progenitor cell states in mammary epithelial cells. We have already reported that stem/progenitor cell marker nestin began to be expressed and increased during the culture (6). The expression of nestin has been reported to be regulated by Sox10 (12, 13). Thus, we examined the expression of Sox10 in parotid acinar cells by real time RT-PCR. As a result, the expression level of Sox10 mRNA was increased and was sustained for 3 days (Fig. 4A). Although Sox10 in
Table 2. KEGG pathways that were identified from 68 expression-altered miRNAs

| KEGG pathway                                | p-value | Number of genes | Number of miRNAs |
|----------------------------------------------|---------|-----------------|------------------|
| Metabolism                                  |         |                 |                  |
| N-Glycan biosynthesis                        | 0.002   | 17              | 25               |
| Valine, leucine and isoleucine biosynthesis  | 0.031   | 2               | 3                |
| Mucin type O-Glycan biosynthesis             | 0.004   | 11              | 15               |
| 2-Oxocarboxylic acid metabolism              | 0.050   | 7               | 9                |
| Genetic Information Processing               |         |                 |                  |
| Protein processing in endoplasmic reticulum  | < 0.001 | 62              | 46               |
| Ubiquitin mediated proteolysis               | 0.002   | 53              | 54               |
| Environmental Information Processing         |         |                 |                  |
| MAPK signaling pathway                       | < 0.001 | 100             | 63               |
| Ras signaling pathway                        | < 0.001 | 78              | 54               |
| Wnt signaling pathway                        | < 0.001 | 59              | 45               |
| TNF signaling pathway                        | 0.007   | 41              | 46               |
| Hippo signaling pathway                      | 0.011   | 56              | 45               |
| Rap1 signaling pathway                       | 0.013   | 70              | 56               |
| FoxO signaling pathway                       | 0.022   | 47              | 44               |
| TGF-beta signaling pathway                   | 0.031   | 33              | 34               |
| PI3K-Akt signaling pathway                   | 0.040   | 102             | 57               |
| Cellular Process                             |         |                 |                  |
| Gap junction                                 | 0.001   | 34              | 36               |
| Signaling pathways regulating pluripotency of stem cells | 0.001   | 51              | 43               |
| Endocytosis                                  | 0.001   | 78              | 49               |
| Focal adhesion                               | 0.028   | 70              | 57               |
| Organismal Systems                           |         |                 |                  |
| Endocrine and other factor-regulated calcium reabsorption | < 0.001 | 19              | 30               |
| Neurotrophin signaling pathway               | < 0.001 | 51              | 43               |
| Circadian rhythm                             | < 0.001 | 16              | 28               |
| Synaptic vesicle cycle                       | 0.001   | 25              | 35               |
| Estrogen signaling pathway                   | 0.001   | 36              | 39               |
| Axon guidance                                | 0.010   | 49              | 47               |
| Adrenergic signaling in cardiomyocytes       | 0.011   | 50              | 50               |
| Dorsal-ventral axis formation                | 0.026   | 13              | 25               |
| Melanogenesis                                | 0.036   | 37              | 38               |
| Oxytocin signaling pathway                   | 0.049   | 53              | 53               |
| Vasopressin-regulated water reabsorption     | 0.049   | 19              | 31               |
| Human Diseases                               |         |                 |                  |
| Prion diseases                               | < 0.001 | 12              | 22               |
| MicroRNAs in cancer                          | < 0.001 | 66              | 46               |
| Proteoglycans in cancer                      | < 0.001 | 83              | 52               |
| Transcriptional misregulation in cancer      | < 0.001 | 64              | 50               |
| Pathways in cancer                           | < 0.001 | 130             | 54               |
| Glioma                                       | < 0.001 | 28              | 34               |
| Renal cell carcinoma                         | < 0.001 | 29              | 37               |
| Chronic myeloid leukemia                     | < 0.001 | 33              | 37               |
| Hepatitis B                                  | 0.001   | 48              | 50               |
| Prostate cancer                              | 0.004   | 37              | 45               |
| Cocaine addiction                            | 0.007   | 19              | 30               |
| Pancreatic cancer                            | 0.036   | 26              | 33               |
| Acute myeloid leukemia                       | 0.044   | 22              | 32               |
Table 3. KEGG pathways that were identified from combination analysis of the 7 down-regulated miRNAs and mRNA expression microarray

| KEGG pathway                                      | p-value | Number of genes |
|---------------------------------------------------|---------|-----------------|
| Metabolism                                        |         |                 |
| Amino sugar and nucleotide sugar metabolism       | 0.027   | 4               |
| Genetic Information Processing                     |         |                 |
| Ubiquitin mediated proteolysis                    | 0.003   | 8               |
| Environmental Information Processing               |         |                 |
| PI3K-Akt signaling pathway                        | < 0.001 | 14              |
| HIF-1 signaling pathway                           | < 0.001 | 8               |
| Hippo signaling pathway                           | 0.001   | 9               |
| Wnt signaling pathway                             | 0.011   | 7               |
| VEGF signaling pathway                            | 0.045   | 4               |
| Cellular Process                                  |         |                 |
| Oocyte meiosis                                    | < 0.001 | 9               |
| Signaling pathways regulating pluripotency of stem cells | 0.041   | 6               |
| Organismal Systems                                |         |                 |
| Melanogenesis                                     | < 0.001 | 8               |
| Estrogen signaling pathway                        | 0.010   | 6               |
| Neurotrophin signaling pathway                    | 0.028   | 6               |
| GnRH signaling pathway                            | 0.036   | 5               |
| Insulin signaling pathway                         | 0.041   | 6               |
| Human Diseases                                    |         |                 |
| MicroRNAs in cancer                               | < 0.001 | 9               |
| Renal cell carcinoma                              | 0.002   | 6               |
| Pathways in cancer                                | 0.007   | 13              |
| Choline metabolism in cancer                      | 0.012   | 6               |
| Viral carcinogenesis                              | 0.040   | 8               |
| Hepatitis B                                       | 0.040   | 6               |
| HTLV-I infection                                  | 0.042   | 9               |

the PP1-treated cells was increased at Day 1 as well as in the control, it decreased later. The expression level of Sox10 mRNA after 3-day culture with PP1 became almost the same level as before culture. The Sox10 protein in the control cells also increased during the culture and addition of PP1 suppressed its expression (Fig. 4B).

Discussion

First, we performed KEGG pathway analysis on 68 expression-altered miRNAs. Next, we performed pathway analysis on the genes extracted by combination of miRNA and mRNA expression array analyses. As a result, the pathways that are involved in the stemness were commonly identified (map04550: Signaling pathways regulating pluripotency of stem cells). In addition, signaling of Wnt, Hippo and PI3K-Akt, which have been reported to regulate maintenance of stemness (14-18), were identified. Pathways related to cancers were also found by the analysis. Cancer cells frequently have properties of stem cells and the stemness sustains cancer progression. These results indicate that the expression levels of stemness-related genes and miRNAs changed in the primary culture of parotid acinar cells. In the previous reports, miR-375, miR-30b, miR-29c and miR-148b increased during the development of parotid glands and are considered to be involved in stemness (19). Thus, their decrease in the primary culture may be related to change to undifferentiated state and acquisition of stemness.

Nestin has been shown to be associated with stemness of several types of cell (20). We have previously reported that
expression of nestin and cytokeratin-14, stem/progenitor markers, increased during the culture of parotid acinar cells (4, 6). In addition, ligation of parotid excretory duct induced expression of nestin and cytokeratin-5 (21, 22). After injury, various epithelial cells begin to express nestin, which may indicate a reversion to an immature phenotype. Pancreatic
acinar cells express nestin by stimulation with growth factors and trans-differentiate to duct cells (23, 24). Salivary acinar cells and pancreatic exocrine cells have been reported to have a capacity of self-renewal and trans-differentiation to duct-like cells (4, 23–26). These studies suggest that even fully-differentiated exocrine cells maintain multipotency. The ability allows exocrine acinar cells to dedifferentiate under cellular stress and re-differentiate to acinar cells after that.

In this study, we searched target gene candidates for miR-3473, which has been rarely studied so far. Among candidate genes, Sox10 was increased during the culture in an inverse manner. Sox10 is a member of the SRY-related HMG box family that function as transcription factors. It regulates embryonic development and maintains multipotency of neural crest cells (27, 28). Since Sox10 promotes expression of nestin (12, 13), down-regulation of miR-3473 may be one of the mechanisms to induce nestin expression via increase of Sox10.

The most up-regulated genes identified by mRNA expression analysis included inflammation markers and cell-cell attachment molecules (4, 11). Because cells were isolated from the parotid glands by digestion with enzymes, the tissue organization was destructed. This is the reason that the expression of such proteins remarkably changed. In contrast, combination analysis with miRNA array revealed that the expression of genes related to stemness also changed. There is a possibility that changes in miRNA expression in the primary culture of parotid acinar cells may be involved in acquisition of stemness.

Conflict of interest

The authors have no conflict of interests relating to this research.

Acknowledgments

This study was supported by JSPS KAKENHI Grant Number 17K17131 and research grants from Research Institute of Oral Science, Nihon University School of Dentistry at Matsudo.

References

1. Sreebny L. Xerostomia: diagnosis, management and clinical complications. In: Edgar W, O’Mullane D, editors. Saliva and oral health. 2nd ed. London: British Dental Association; 1996. p. 45–50.
2. Fujita-Yoshigaki J, Tagashira A, Yoshigaki T, Furuyama S, Sugiya H: A primary culture of parotid acinar cells retaining capacity for agonists-induced amylase secretion and generation of new secretory granules. Cell Tissue Res, 320: 455–464, 2005.
3. Qi B, Fujita-Yoshigaki J, Michikawa H, Satoh K, Katsumata O, Sugiya H: Differences in claudin synthesis in primary cultures of acinar cells from rat salivary gland are correlated with the specific three-dimensional organization of the cells. Cell Tissue Res, 329: 59–70, 2007.
4. Fujita-Yoshigaki J, Matsuki-Fukushima M, Sugiya H: Inhibition of Src and p38 MAP kinases suppresses the change of claudin expression induced on dedifferentiation of primary cultured parotid acinar cells. Am J Physiol Cell Physiol, 294: C774–C785, 2008.
5. Moriyama S, Yokoyama M, Katsumata-Kato O: Enhancement of Src family kinase activity is essential for p38 MAP kinase-mediated dedifferentiation signal of parotid acinar cells. Int J Oral-Med Sci, 14: 33–40, 2015.
6. Yokoyama M, Ogawa H, Katsumata-Kato O, Fujita-Yoshigaki J: Expression of the stem cell marker nestin in response to tissue injuries of parotid acinar cells. Int J Oral-Med Sci, 15: 93–97, 2017.
7. Hayashi T, Koyama N, Azuma Y, Kashimata M: Mesenchymal miR-21 regulates branching morphogenesis in murine submandibular gland in vitro, Dev Biol, 352: 299–307, 2011.
8. Matse JH, Yoshizawa J, Wang X, Elashoff D, Botschler JG, Veerman EC, Leemans CR, Pegtel DM, Wong DT, Bloemen E: Human Salivary Micro-RNA in Patients with Parotid Salivary Gland Neoplasms. PLoS One, 10: e0142264, 2015.
9. Fujita-Yoshigaki J: Analysis of changes in the expression pattern of claudins using salivary acinar cells in primary culture. Methods Mol Biol, 762: 245–258, 2011.
10. Kadota K, Nakai Y, Shimizu K: A weighted average difference method for detecting differentially expressed genes from microarray data. Algorithms Mol Biol, 3: 8, 2008.
11. Yokoyama M, Katsumata-Kato O, Fujita-Yoshigaki J: Comprehensive analysis of change in gene expression pattern during primary culture of parotid acinar cells. Nihon University Journal of Oral Science, 43: 21–26, 2017.
12. Feng W, Liu S, Zhu R, Li B, Zhu Z, Yang J, Song C: SOX10 induced Nestin expression regulates cancer stem cell properties of TNBC cells. Biochem Biophys Res Commun, 485: 522–528, 2017.
13. Flammiger A, Besch R, Cook AL, Maier T, Sturm RA, Berking C: SOX9 and SOX10 but not BRN2 are required for nestin expression in human melanoma cells. J Invest Dermatol, 129: 945–953, 2009.
14. Marson A, Foreman R, Chevalier B, Bilodeau S, Kahn M, Young RA, Jaenisch R: Wnt signaling promotes reprogramming of somatic cells to pluripotency. Cell Stem Cell, 3:
132–135, 2008.
15. Liu H, Jiang D, Chi F, Zhao B: The Hippo pathway regulates stem cell proliferation, self-renewal, and differentiation. Protein Cell, 3: 291–304, 2012.
16. Lee MY, Lim HW, Lee SH, Han HJ; Smad, PI3K/Akt, and Wnt-dependent signaling pathways are involved in BMP-4-induced ESC self-renewal. Stem Cells, 27: 1858–1868, 2009.
17. Armstrong L, Hughes O, Yang S, Hyslop L, Stewart R, Wappler I, Peters H, Walter T, Stojkovic P, Evans J, Stojkovic M, Lako M: The role of PI3K/AKT, MAPK/ERK and NFkappabeta signalling in the maintenance of human embryonic stem cell pluripotency and viability highlighted by transcriptional profiling and functional analysis. Hum Mol Genet, 15: 1894–1913, 2006.
18. Maimeta M, Rocchi C, Bron R, Pringle S, Kuipers J, Giepmans BN, Vries RG, Clevers H, de Haan G, van Os R, Copes RP: Long-Term In Vitro Expansion of Salivary Gland Stem Cells Driven by Wnt Signals. Stem Cell Reports, 6: 150–162, 2016.
19. Metzler MA, Appana S, Brock GN, Darling DS: Use of multiple time points to model parotid differentiation. Genom Data, 5: 82–88, 2015.
20. Wiese C, Rolletschek A, Kania G, Blyszczuk P, Tarasov KV, Tarasova Y, Wersto RP, Boheler KR, Wobus AM: Nestin expression—a property of multi-lineage progenitor cells? Cell Mol Life Sci, 61: 2510–2522, 2004.
21. Yokoyama M, Katsumata-Kato O, Sakurai H, Ogawa H, Fujita-Yoshigaki J: Expression of a neural stem/progenitor cell marker nestin in salivary glands. MOJ Anat Physiol, 4: 00133, 2017.
22. Yokoyama M, Katsumata-Kato O, Matsuki-Fukushima M, Fujita-Yoshigaki J: Cytokeratin-5 expression in salivary duct ligation. Nihon University Journal of Oral Science, 38: 96–100, 2012.
23. Means AL, Meszoely IM, Suzuki K, Miyamoto Y, Rustgi AK, Coffey RJ Jr., Wright CV, Stoffers DA, Leach SD: Pancreatic epithelial plasticity mediated by acinar cell transdifferentiation and generation of nestin-positive intermediates. Development, 132: 3767–3776, 2005.
24. Blaine SA, Ray KC, Anunobi R, Gannon MA, Washington MK, Means AL: Adult pancreatic acinar cells give rise to ducts but not endocrine cells in response to growth factor signaling. Development, 137: 2289–2296, 2010.
25. Aure MH, Konieczny SF, Ovitt CE: Salivary gland homeostasis is maintained through acinar cell self-duplication. Dev Cell, 33: 231–237, 2015.
26. Takahashi S, Nakamura S, Domon T, Yamamoto T, Wakita M: Active participation of apoptosis and mitosis in sublingual gland regeneration of the rat following release from duct ligation. J Mol Histol, 36: 199–205, 2005.
27. Kelsh RN: Sorting out Sox10 functions in neural crest development. Bioessays, 28: 788–798, 2006.
28. Bondurand N, Kobetz A, Pingault V, Lemort N, Encha-Razavi F, Couly G, Goerlich DE, Wegner M, Abitbol M, Goossens M: Expression of the SOX10 gene during human development. FEBS Lett, 432: 168–172, 1998.