Investigation of stem cell-specific microRNA expression as potential stem cell regulators, in rabbit embryos and embryonic stem cells

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1. Introduction and Aims

MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate multiple biological processes. They post-transcriptionally regulate gene expression mostly through binding to complementary regions within 3'UTRs of their target mRNAs leading to translational repression or cleavage. Increasing experimental evidence implies an important regulatory role of miRNAs during early embryonic development and in embryonic stem cell biology.

Regulation of gene expression in developmental processes is an important aspect of miRNA function. The first identified miRNAs, *lin-4* and *let-7*, were found to be critical for temporal control of larval development in *C. elegans.* Many experiments have been performed to dissect the miRNA pathway in various organisms demonstrating that miRNAs are essential for proper embryonic development. In *C. elegans*, *dcr-1* homozygote mutant worms, in addition to being sterile, show some partially penetrant developmental abnormalities, such as egg-laying defect (Egl) and burst vulva phenotypes. Dicer-deficient mice die very early in development, by embryonic day 7.5, with essentially arrest of development prior to gastrulation and depletion of pluripotent state. Recent studies demonstrate the embryonic stage-specific expression of miRNAs. Interestingly, several stem cell specific miRNAs such as miR-290 cluster were shown to be the first de novo expressed miRNAs in mouse embryos at 2-4-cell stage, with an increasing expression through the blastocyst stage. Moreover, miR-290 cluster deficiency in mouse embryos causes penetrant embryonic lethality and germ cell defects implying the important role of this cluster in embryonic development.

MicroRNAs have also been identified as important regulators of embryonic stem cell (ESC) properties. Dicer mutant mouse ESC lines expressing pluripotent markers show pronounced proliferation defect, failure of teratoma and chimera formation and loss of differentiation ability. DGCR8 knock out mouse ESCs have extended population doubling time and defect in embryonic body (EB) and teratoma formation. In addition to an overall investigation of the function of the miRNA pathway in ESCs, cloning and deep sequencing of miRNAs from stem cells have exhibited the identity of the ESC-specific miRNAs which may function in ESC self-renewal and differentiation. The mmu-miR-290 cluster has been reported as a ESC-specific miRNA which

expressed highly in undifferentiated ESCs and inhibited the ESC differentiation. This cluster has an important regulatory function in undifferentiated ESCs through direct control of de novo DNA methylation, cell cycle regulation and targeting cell cycle regulators and also modulates the pluripotency through the repression of canonical NF-κB pathway. In addition, the promoter of this cluster is a direct target of key ESC transcription factors, such as OCT4, SOX2, and NANOG. The human homolog of mmu-miR-290 cluster, miR-371 cluster was also identified to be expressed in human ESCs and does not reappear in any somatic lineage. This cluster shares the chromosomal region with C19MC cluster (Chromosome 19 miRNA Cluster) which is a highly repetitive region containing 46 members of the super-family and shows high sequence similarity to both hsa-miR-371 and mmu-miR-290 clusters. The miR-302 cluster is also highly expressed in both human and mouse ESCs and down-regulated upon differentiation. Similar to miR-290 family this cluster is a cell cycle regulator; cyclin D1 and Cdk4 are post-transcriptionally regulated by miR-302 cluster in human ESCs resulting in positive regulation of ESC self-renewal. Furthermore, this cluster might positively regulate the Nodal/Activin pathway, therefore contributing to the maintenance of pluripotency. The miR-302 gene is itself a downstream transcriptional target of NANOG, OCT3/4, REX1 and SOX2.

In the context of biomedical research, precise genetic engineering in rabbits (*Oryctolagus cuniculus*) would be precious model to generate genetically defined rabbit models of human diseases. To date, rabbit has been applied as an experimental model of atherosclerosis, Alzheimer's disease, eye research, osteoarthritis, tuberculosis and diabetes, as well as transgenesis for production of pharmaceutical proteins. The first live born ES-derived rabbit chimera was reported in 2010, albeit with low efficiency. Therefore, rabbit ESCs would be invaluable tool for both creating second-generation transgenic models of human diseases and testing stem cell therapies for human applications. Although several groups could generate rabbit ESCs with different derivation methods, it is still difficult to establish and remains poorly characterized. Since ECSs are derived from early blastocysts, they can reflect the potential characteristic of their founder embryonic population. Therefore it is important to compare the expression pattern of both miRNAs and proteins known to play regulatory roles during early lineage specification.

Bringing it all together, we aimed to explore ESC-specific miRNA expression pattern using SOLiD™ System Small RNA Analysis from early embryonic stages to early rabbit embryonic stem-like (ES-like) cell passages for the first time to get more insight into their potential regulatory mechanism in embryonic development and tuning stem cell properties.

2. Materials and Methods

2.1. Embryo collection

White New Zealand rabbits were superovulated with PMSG (30 IU/ kg) and hCG (45 IU/ kg). Embryos were collected at 1-cell, 2-cell, 4-cell and 8-cell stages, and at 2.5, 3.5, 4.5, 6, 7 and a 14 dpc embryo and its germinal ridges containing primordial germ cells. Likewise oocytes were collected after removal of cumulus cells with 0.03% Hyaluronidase. To investigate cell-specific gene expression trophoblast, embryoblast and its two cell layers (hypoblast and epiblast) were isolated based on the protocol described by Puschel *et al*. and stored separately at -80 °C.

2.2. Preparation of rabbit embryonic fibroblast conditioned media (CM)

The prepared rabEF were used to set up the conditioned media (CM). When rabEF cells reached 80–90% confluence, FM medium was replaced with basic rabbit ES media supplemented with Fetal Calf Serum (FBS) or KnockOut™ Serum Replacement (SRL). The conditioned FBS and SRL supplemented basic rabbit ES media (CM/ FBS and CM/ SRL respectively) were collected every 3rd day, then CM from parallel rabEF pooled together and stored at 4°C prior to use for ESC cultivation.

2.3. Rabbit ESCs derivation

The zona pellucida of 4.5 dpc rabbit embryos was removed by treating with 0.5 % pronase in PBS. Zona pellucida free embryos were cultured on mitomycin C treated CD1 mouse embryonic fibroblasts in three different conditioned media including: CM/FBS, CM/SRL/bFGF and CM/SRL/bFGF/rLIF. After embryo attachment, the fresh medium was changed every day. Colonies presenting a typical morphology of compacted ESCs were selected and subcultured every 5-7 days using accutase to disaggregate the cells.

2.4. SOLiD™ sequencing and small RNA analysis

Total RNA from 3.5, 4.5, 6, 7 and 14 dpc rabbit embryos, rabbit PGCs of 14 days embryo, rabbit embryonic fibroblast (rabEF), rabbit ES-like cells and mouse embryonic fibroblast (MEF) were processed into sequencing libraries using the Small RNA Expression Kit. Libraries were amplified onto beads using emulsion PCR, deposited on slides, and sequenced using the SOLiD™ 3 System. SOLiD data were first

analyzed by SOLiD System Small RNA Analysis Pipeline Tool (RNA2MAP, version 0.5). Annotation of sequences was performed on the basis of similarity to known human, bovine and mouse miRNA sequences downloaded from miRBase (Released 17: March 2011; http://www.mirbase.org/). To obtain the nucleotide sequences of rabbit miRNAs, color space SOLiD reads matching one of the known human, mouse or bovine mature miRNAs were converted to the nucleotide sequences and their consensus was generated after multiple alignment.

2.5. Real-Time Quantitative PCR

Mature miRNA quantification was performed on two step protocol including reverse transcription with miRNA-specific primers using TaqMan MicroRNA Reverse Transcription Kit, followed by quantitative real-time PCR with TaqMan MicroRNA Assays-TM using TaqMan Gene Expression Master Mix. For mRNA quantification, extracted total RNA samples were reverse transcribed into cDNA, using High Capacity cDNA Reverse Transcription Kit. Synthesized cDNAs were subjected to quantitative real-time PCR using SYBR® Green PCR Master Mix as a double-stranded DNA-specific fluorescent dye with rabbit specific primer sets. All reaction protocols were carried out according to the manufacturer's instructions. The reactions for both miRNA and mRNA were automated by Eppendorf Mastercycler ® ep realplex[4].

Fold change was calculated based on ΔΔCt method normalized to ocu-miR-191 (for miRNA expression analysis) and Gapdh (for mRNA expression analysis). Indeed, number of target cDNA molecules was calculated by comparison with standard curve generated from serial dilutions (10 to 10^8 molecule/µl) of primer-specific DNA probes generated from cDNA plasmid clones. Analysis was performed using GenEx qPCR data analysis software by MultiD.

For each sample, signal was averaged over replicates. Each biological replicates of embryos consisted of a pool of staged embryos. Results are expressed as the average ± SD. One-way ANOVA test (GenEx software) followed by Tukey-Kramer's test was used to document any statistically significant difference between multiple groups. The probability level of $P < 0.05$ was considered to be significant.

2.6. Transfection of rabbit ES-like cells with Anti-miR-302a inhibitor

Rabbit ES-like cells (2nd passage) in 96-well plate were transfected with anti-miR-302a inhibitor (100nM, 50nM and 30nM) and its corresponding negative control (50nM) using siPORT™ NeoFX™ transfection agent for 48 hrs according to the manufacturer's instructions. For global mRNA analysis, 48 hrs after transfection, cells were harvested in Trizol and subjected to real-time PCR analysis.

3. Results

3.1. Derivation of rabbit ES-like cells

To isolate rabbit embryonic stem cells, the whole blastocyst of 4.5 dpc embryos plated on mitomycin C inactivated MEF in conditioned media (CM/FBS, CM/SRL/bFGF, CM/SRL/bFGF/rLIF). A total of 100 blastocysts (4.5 dpc) were randomly allocated to three different ES culture media. The percent of established ES-like colonies at first passage was higher (71.4%) in CM/SRL/bFGF/rLIF media compared to the other culture conditions. The ES-like colonies at third passage in CM/FBS and CM/SRL/bFGF/rLIF media displayed higher ratio of 21.7% (5/23) and 21.4% (3/14) respectively. In contrast the ratio of ES-like colonies at third passage in CM/SRL/bFGF media was low 16.7% (5/30). Derivation efficiency was the highest in CM/SRL/bFGF/rLIF (18.8%). In contrast, ES-like cells in CM/FBS media presented lower derivation efficiency of 13.5%. The lowest derivation efficiency was observed in CM/SRL/bFGF culture condition (10.6%). We chose the rabbit ES-like cells derived in CM/SRL/bFGF/rLIF culture media for further analysis since they represented the highest derivation efficiency. They also could be maintained in a stemness state longer up to eight passages.

After the first passage rabbit ES-like cells formed flat colonies with clear border similar to the primate ESCs. The rabbit ES-like cells in CM/SRL/bFGF/rLIF retained the predominate morphology of undifferentiated cells up to eight passages. The rabbit ES-like cells expressed both Oct4 and Nanog transcripts at high level reflecting their pluripotent state; however, their expression was significantly diminished by the third passage. Immunohistological analysis underlined the expression of OCT4 in rabbit ES-like cells and attached ICM to the feeder layer (Fig. 7B and 7C). While they were weakly positive for SSEA1, no expression of SSEA4 was detected (data are not shown). After eight passage most of the colonies lost their typical ESC morphology and started to differentiate.

3.2. SOLiD small RNA cDNA library sequencing

We sequenced a small RNA library constructed with the SOLiD small RNA expression kit from total RNA of the rabbit 6 and 7 days dpc embryos and a Carnegie stage 17 embryo 14 dpc and its germinal ridges containing PGCs and also rabbit ES-like cells, rabbit embryonic fibroblast and MEF. Massively parallel sequencing of amplified cDNA fragments using the SOLiD 3 System generated

2664988, 901107, 3015131, 2551138, 2045460, 3049402 and 2448960 sequence reads corresponding to rabbit 6 dpc, 7 dpc, 14 dpc, PGCs, rabbit ES-like cells, rabbit EF and MEF, respectively.

To analyze the sequence data we identified sequence matches to known human, mouse and bovine miRNAs in miRBase representing 5.4 % of 6 dpc embryo reads, 15.8 % of 7 dpc embryo reads, 35.0 % of 14 dpc embryo reads and 16.6 % of rabES-like cell reads to known human miRNA database, 7.6 % of 6 dpc embryo reads, 21.7 % of 7 dpc embryo reads, 46.2 % of 14 dpc embryo reads and 27.1 % of rabES-like cell reads to known mouse miRNA database.

We defined similarity of the obtained ocu-miRNAs to the known human, mouse and bovine miRNAs as the fraction of all expressing rabbit miRNAs that corresponds to human, mouse and bovine miRNAs, respectively. Thus, ocu-miRNAs exhibited more similarity to known human miRNAs than to known mouse and bovine miRNAs.

From more than 14 million miRNA high quality reads obtained from constructed rabbit small RNA library, we could identify a total of 1693 expressing rabbit specific miRNAs representing more similarity to known human miRNAs. The number of reads obtained reflected the relative abundance and expression levels of the miRNAs. Among the frequently sequenced miRNAs we choose to analyze pluripotent associated miRNAs in rabbit ES-Like cells and during early embryogenesis.

3.3. Mature structure of rabbit miR-302 and miR-290 clusters

To analyze the mature structure of rabbit miRNA clusters, we performed a local alignment using UCSC Genome Rabbit BLAT Search by comparing mature miRNA query sequences obtained from SOLiD small RNA analysis with rabbit genome assembly. The miR-302 gene cluster was identified on the positive strand of the rabbit chromosome 15 in the same order as observed in human, mouse and bovine clusters. At the mature sequence level, seven miRNAs showed 100% identity to human mature miRNAs, while, ocu-miR-302a-5p, ocu-miR-302b-3p and ocu-miR-302b-5p had 95.8%, 95.7% and 95.4% identity, respectively. In contrast, similarity to mouse and bovine miRNAs was slightly lower. They all share the same AAGTGCT and CTTTAAC seed sequences with human, mouse and bovine homologs. However, ocu-miR-367 has a different seed sequence (ATTGCA) as its human and mouse homologs. Despite the high similarity of rabbit ocu-miR-302 cluster to its human

homolog, ocu-miR-290 cluster exhibited lower similarity to the mouse cluster. Rabbit homologs of the mmu-miR-290 cluster were located on the reverse strand of a short pseudo-chromosome chrUn0226 (scaffold GL018924). Among fourteen members of the mouse cluster, the rabbit ocu-miR290-5p, ocu-miR-292-3p and ocu-miR-294-3p presented 90.9%, 87.5% and 100% identity to their mouse mature miRNA homologs at mature sequence level, respectively. Interestingly, ocu-miR-294-3p was also found on chromosome 2 by mature sequence analysis with identity of 90.9% on this locus. Furthermore, homologs of mir-512-5p, miR-512-3p, miR-498 and miR-520e of human C19MC cluster were all represented in rabbit but we could not identify the rabbit homolog of miR-371 cluster. We found two repeats of rabbit ocu-miR-512-5p and ocu-miR-512-3p located 5´ to the rabbit miR-290 cluster on the reverse strand of chrUn0226. At the mature sequence level ocu-miR-512-5p repeats indicated 82.6 % and 78.2% (ocu-miR-512-I-5p and ocu-miR-512-II-5p respectively) identity to the human homologs. Both repeats of ocu-miR-512-3p which located within 2.8 kb from each other, displayed 72.2% identity to their human homologs. The rabbit miR-498 and miR-520e exhibited 82.6% and 95.2% identity to the human homologs.

3.4. MicroRNA expression during early rabbit embryonic development

In order to acquire a comprehensive perspective of stem cell specific miRNA expression level during early embryonic stages, miRNA expression in collected oocytes and early embryos at 1-cell, 2-cell, 4-cell, 8-cell, 2.5 dpc (morula), 3.5dpc, 4.5dpc, 6dpc and 7 dpc (blastocysts) were analyzed, using real-time RT-PCR analysis. We could observe up-regulation of rabbit miR-290 family began at 4-cell stage, and showed a steady increase through the 3.5 dpc blastocyst stage. The expression of ocu-miR-290-5p, ocu-miR-292-3p and ocu-miR-294-3p were significantly down-regulated at 6 dpc stage. Notably, the expression of ocu-miR-512-5p was restricted to a narrow developmental stage specific window, starting at 8-cell stage with extensively high fold change of 54 to 4-cell stage embryos and gradually increased through 4.5 dpc blastocyst stage. In contrast to rabbit miR-290 family, which was expressed in earlier embryonic stages, rabbit miR-302 family began to express later at 3.5 dpc stage, which could be considered as the reliable starting point for the expression of rabbit miR-302 cluster members. The expression level kept increasing up to 4.5 dpc; at 6 dpc was augmented by 3, 7.9 and

2.5 fold for ocu-miR-302a-3p, ocu-miR-302b-3p and ocu-miR-367-3p, respectively. These results suggest that miR-290 cluster might play more significant role during early rabbit embryogenesis compared to the miR-302 cluster.

Furthermore, we analyzed the cell-specific expression of both ocu-miR-302 and ocu-miR290 clusters in the trophoblast and the embryoblast of a 6 dpc fully expanded blastocyst to see whether there is difference in expression level of these embryonic cell types. While the expression levels of ocu-miR-290 cluster members were not significantly different among trophoblast and embryoblast cells, the expression levels of ocu-miR-302a-3p, ocu-miR-302b-3p and ocu-miR-367 were 3.9, 4.3 and 2.6 fold higher in the embryoblast cells. The ocu-miR-512-5p was the only miRNA, which its expression level was higher in trophoblast cells.

3.5. MicroRNA expression in rabbit ES-like cells

Subsequent to the ocu-miR-290 and ocu-miR-302 clusters expression analysis during rabbit embryonic development, we explored the expression analysis of both miRNA families in rabbit ES-like cells. We compared the miRNA expression level in attached ICM (ICM of attached blastocyst stage embryos to MEF) and 2^{nd}, 3^{rd}, 4^{th} and 5^{th} passages of rabbit ES-like cells. The expression of ocu-miR-290 cluster and ocu-miR-512-5p was not considerable in rabbit ES-like cell compared to their high expression level during early embryogenesis. The ocu-miR-290-5p, ocu-miR-292-3p and ocu-miR-294-3p were dramatically down-regulated after the second passage compared to the attached ICM. However down-regulation of ocu-miR-512-5p was not significant in contrast to ocu-miR-290 family. The second and third passages of rabbit ES-like cells showed nearly the same expression level of ocu-miR-290-5p, ocu-miR-292-3p and ocu-miR-294-3p, but the expression of ocu-miR-512-5p was diminished. The expression of ocu-miR290 family and ocu-miR-512-5p was reduced after third passages through the fifth passage of rabbit ES-like- cells. Unlike ocu-miR-290 family, ocu-miR-302 cluster was highly expressed in early passages of rabbit ES-like cells. The ocu-miR-302a-3p, ocu-miR-302b-3p and ocu-miR-367-3p exhibited up-regulation in the second passage of rabbit ES-like cells compared to the attached ICM. Expression of ocu-miR-302a-3p, ocu-miR-302b-3p and ocu-miR-367-3p was significantly elevated in third passage of rabbit ES-like cells

compared to the second passage. Likewise, both ocu-miR-302a-3p and ocu-miR-302b-3p showed significant increase in their expression level by the fourth passage, but ocu-miR-367-3p was down-regulated. By fifth passage ocu-miR-302a-3p and ocu-miR-367-3p were substantially down-regulated, whereas the expression of ocu-mir-302b stayed at the same level.

3.6. *Lefty* is post-transcriptionally targeted by ocu-miR-302a in rabbit ES-like cells

We chose to predict the putative targets of ocu-miR-302a miRNA from miR-302 family based on the fact that all -3p mature miR-302s are sharing the same seed sequence by which miRNA binds to the 3´UTR of its target mRNAs. Therefore, they may target the same mRNAs. LEFTY inhibits the expression of TGFβ/Activin/Nodal family proteins and is the main antagonist of Nodal signaling. It has also been demonstrated that *LEFTY* is negatively modulated by miR-302s in human ESCs. Based on this evidence, we first analyzed whether ocu-miR-302a may physically interacts with Lefty. The rabbit Lefty cDNA sequences available in databases do not contain the 3´UTRs; hence, the region coding for the 3´UTR was identified in the genomic sequence of chromosome 16 by similarity search against human LEFTY 2 mRNA (NM_003240.3). Sequence analysis of predicted 3´UTR of rabbit Lefty revealed the presence of two putative target sites for ocu-miR-302a.

In order to validate the bioinformatic predictions, we transiently transfected the rabbit ES-like cells (2^{nd} passage) with anti-miR-302a inhibitor. Mature miR-302a levels were decreased in anti-miR-302a transfected rabbit ES-like cells since the anti-miR inhibitors bind irreversibly to the miRNAs and inhibit endogenous miRNA molecules. Quantitative real-time PCR analysis showed a significant increase and dose-dependent in expression level of Lefty mRNA in anti-miR-302a transfected rabbit ES-like cells compared to the untransfected control rabbit ES-like cells. The rabbit ES-like cells transfected with 100nM of anit-miR-302a exhibited the highest level of expression or Lefty compared to the control ES-like cells by 3.5 fold change. Likewise, Lefty was up up-regulated in rabbit ES-like cells treated with 50nM and 30nM of anti-miR-302a in contrast to the control ES-like cells by 2.8 and 2.3 fold changes, respectively.

3. Conclusion

By applying SOLiD deep sequencing technique, we report here for the first time the miRNA expression profile during early rabbit embryonic development and in ES-like cells. SOLiD sequencing provides a powerful screening method enabling the simultaneous sequencing of up to millions of DNA or RNA molecules to discover novel miRNAs. The high throughput sequencing approach allowed us to identify rabbit specific miRNAs, which has not been annotated yet, and enabled a semi-quantitative estimation of the relative expression level of ocu-miRNAs. We could identify a total of 1693 expressing rabbit miRNAs based on comparison of obtained sequence reads to the known human, mouse and bovine miRNA databases (miRBase). The degree of homology of the identified rabbit miRNAs and the number of similar miRNAs to known human miRNAs was higher than known mouse and bovine miRNAs.

We identified rabbit specific ocu-miR-302, ocu-miR-290 clusters and three homologs of human C19MC cluster (ocu-miR-512, ocu-miR-520e and ocu-miR-498in rabbit preimplantation embryos and ES-like cells. The expression of ocu-miR-302 cluster members began at 3.5 dpc early blastocyst stage and stayed highly expressed in rabbit ES-like cells. In contrast, high expression level of ocu-miR-290 cluster members was detected during preimplantation embryonic development, but low level of expression was found in rabbit ES-like cells. Our results underlined the fact that the ocu-miR-302 cluster is ESC-specific, like in human ESCs, and can play a crucial role in the maintenance of rabbit stem cell pluripotency and self-renewal. Obtained results also lead us to speculate that ocu-miR-290 cluster might play an essential role during early rabbit embryogenesis, which is consistent with the notion that knockout of the mmu-miR-290 cluster lead to early embryonic lethality. We observed the highest expression of ocu-miR-290 cluster at 3.5 dpc embryonic stage, while the ocu-miR-302 cluster began to express at this embryonic stage. It seems that 3.5 dpc stage might be a crucial time point of embryonic development, where ocu-miR-290 and ocu-miR-302 expressions are evidently altered.

The murine ESCs representing the “Naïve” state, predominantly express the miR-290 cluster, whereas human ESCs and EpiSCs represanting “Primed” state, display predominant expression level

of the miR-302 cluster. Thus, high expression level of ocu-miR-302 cluster compared to low expression level of ocu-miR-290 cluster in rabbit ES-like cells might reflect their primed state.

We also found that Lefty has two potential target sites in its 3´UTR for ocu-miR-302a and its expression level augmented by ocu-miR-302a inhibition. We propose that the ocu-miR-302 family negatively modulates Lefty mRNA expression in rabbit ES-like cells and maintains their pluripotency through targeting the signaling pathways.

The thesis is based on the following publications:

Pouneh Maraghechi, László Hiripi, Gábor Tóth, Babett Bontovics, Zsuzsanna Bősze and Elen Gócza, (2013): **Discover of pluripotency associated microRNAs in rabbit preimplantation embryos and embryonic stem-like cells.** *Reproduction,* 2013 Feb 20. [Epub ahead of print]. IF: **3.090.**

Zs. Tancos, Cs. Nemes, Zs. Polgar, E. Gocza, N. Daniel, T.A.E Stout, **P. Maraghechi**, M. K. Pirity Osteil,Y. Tapponnier , S.Markossian, M. Godet, M. Afanassieff, Zs. Bosze, V. Duranthon, P. Savatier, A. Dinnyes. (2012): **Generation of rabbit pluripotent stem cell lines.** *Theriogenology,* 2012 Nov; 78(8):1774-86. Epub 2012 Aug 24. IF: **2.045.**

Other publication not closely related with the topic of the thesis:

Lichner Z., Páll E., Kerekes A., Pállinger E., **Maraghechi P.**, Bősze Z., Gócza E.,(2011): **The miR-290-295 cluster promotes pluripotency maintenance by regulating cell cycle phase distribution in mouse embryonic stem cells.** *Differentiation*, 2011 Jan; 81(1):11-24. Epub 2010 Sep 22. IF: **3.069**.

Other publication not associated with the topic of the thesis:

Sági B, **Maraghechi P**, Urbán VS, Hegyi B, Szigeti A, Fajka-Boja R, Kudlik G, Német K, Monostori E, Gócza E, Uher F., (2011): **Positional Identity of Murine Mesenchymal Stem Cells Resident in Different Organs is Determined in the Post-Segmentation Mesoderm.** *Stem Cells Dev.*, 2012 Mar 20; 21(5):814-28. Epub 2012 Jan 17. IF: **4.791.**