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MicroRNAs Regulation Modulated Self-Renewal and Lineage Differentiation of Stem Cells

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Stem cells are unique cells in the ability that can self-renew and differentiate into a wide variety of cell types, suggesting that a specific molecular control network underlies these features. To date, stem cells have been applied to many clinical therapeutic approaches. For example, hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) are the cells responding to ischemia or injury and engage in effective revascularization to repair within impairment regions. Transplantation of MSCs after stroke and hindlimb ischemia results in remarkable recovery through enhancing angiogenesis. MicroRNAs are a novel class of endogenous, small, noncoding RNAs that work via translational inhibition or degradation of their target mRNAs to downregulate gene expression. MicroRNAs have been strongly linked to stem cells, which have a remarkable role in development. In this study, we focused on the microRNA regulation in multiple stem cells. For example, miR-520h was upregulated and miR-129 was downregulated in HSC. MiR-103, 107, 140, 143, 638, and 663 were associated with MSCs while miR-302s and miR-136 were associated with ESCs. In NSCs, miR-92b, let-7, and miR-125 were the critical regulators. This overview of the recent advances in the aspects of molecular control of stem cell biology reveals the importance of microRNAs, which may be helpful for future work.

Key words: Posttranscriptional regulation; MicroRNAs; Stem cells

INTRODUCTION

Stem cells are immature cells with the ability to self-renew and differentiate into multiple cell types. The types of stem cells can be based on their developmental origin into cortiembryonic, fetal, and adult stem cells. Embryonic stem cells (ESCs) originated in the blastocysts are pluripotent cells and give rise to progenies of the embryonic germ cell layers. Hematopoietic stem cells (HSCs) give rise to all the blood cell types. Mesenchymal stem cells (MSCs), isolated from different adult tissue sources, have the ability to self-renew and to differentiate into multiple mature cells (29). Neural stem cells (NSCs) are stem cells that kept the ability to self-renew and possess the ability to differentiate to neuronal and glial cells. Induced pluripotent stem cells (IPSs) are pluripotent stem cells derived from a nonpluripotent cell artificially. Previously, our group has reported several papers related to the stem cell (43–45). In this article, we focus on the posttranscriptional regulation: micro RNAs (miRNAs).

THE IMPORTANCE OF miRNAS

miRNAs are found in many organisms and are single-stranded RNAs of 19–23 nucleotides that derive from a 70-nucleotide precursor (29). miRNAs are a novel class of endogenous and noncoding RNAs that via translational inhibition or degradation of their target mRNAs downregulate gene expression (55) (Fig. 1). The biogenesis shows the process of miRNAs. First, primary

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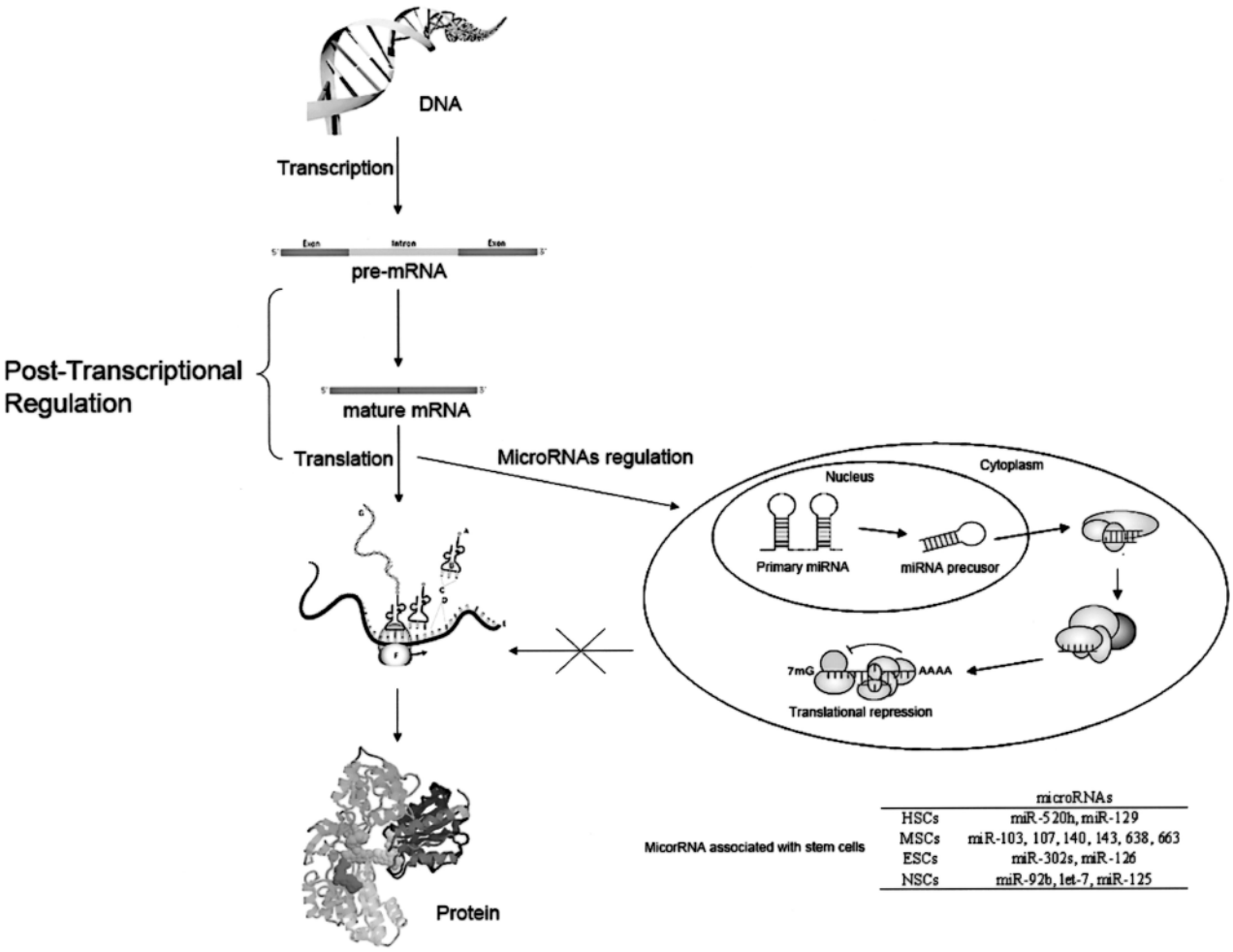

Figure 1. MicroRNAs regulation is shown in the figure. MicroRNAs are initially transcripted in the nucleus as pri-miRNAs. The pri-miRNAs are processed into pre-miRNAs (hairpin) and transferred to the cytosol. These microRNAs form incomplete base paring with their target mRNAs and direct the cleavage of these mRNAs or inhibit their translation.

miRNA (pri-miRNA) transcripts are produced by RNA polymerase II in nucleus (12). The pri-miRNAs are manufactured to hairpin precursors (pre-miRNAs). The pre-miRNAs are transported from nucleus to cytoplasm. In the cytoplasm, pre-miRNAs are processed to mature single-stranded miRNAs by Dicer. Finally, a single strand is preferentially loaded into the core binding protein: RNA-induced silencing complex (RISC). The miRNAs binds directly to the 3′-untranslational region of the specific mRNAs through complete or partial complementary pairing between mRNA and miRNA sequences to silence or direct cleavage of the mRNAs (52).

About 40–50% of mammalian mRNAs could be regulated by miRNAs at the translational level. Specific miRNAs are demonstrated to control neuronal cell fate, proliferation, development, apoptosis, differentiation, hematopoiesis, and exocytosis, as well as in diseases such as cancer and possibly neuronal disorders in mammals (27). miRNAs are also reported to be the key modulators of cardiovascular development and angiogenesis (55). Evidence indicates miRNAs are linked to stem cells. For example, ESCs were found to express a set of miRNAs, and it suggests a strong relationship between stem cell development and miRNAs (19,22). According to the combined expression profile information with miRNA, we identified that miRNA-mRNA pairs related with ES cell differentiation and pluripotency (18). We focused on the correlation between miRNA and stem cells, including HSCs, MSCs, ESCs, NSCs, and induced pluripotent stem (IPS) cells.

The Technologies for miRNAs Screening

miRNAs microarray analysis has been generally used to study the miRNAs expression profiling. miRNAs microarrays have been wildly used for the primary identifi-

cation of new miRNAs that were for the comparison of miRNA expression profiles from different tissues or cells. These arrays were also useful to evaluate the miRNA expression profiles during differentiation, development, oncogenesis, and processes of other disease (54). Moreover, miRNA arrays require only small amounts of total RNA to identify the expression of several hundred miRNAs in the sample at once. miRNA arrays will contribute to the basic and applied research. It also will be useful to change administering of medicine that potentially by providing the means for cancer detection, prognostic assessment, and personalized diagnosis (4,14). However, it is difficult to resolve mature miRNAs due to their small size and many miRNAs differ from one another by as little as one nucleotide. Using olid-phase primer extension step after target hybridization could enhance discrimination among closely related miRNA sequences (52).

There is a complementary approach with high sensitivity by using real-time PCR using TaqMan miRNA assays (9). Quantification of miRNAs by real-time PCR is a powerful tool to study the expression profiles of miRNAs. Using real-time PCR to amplify the miRNAs are challenging molecules because the mature miRNA is roughly the size of a standard PCR primer and the miRNA precursor consists of a stable hairpin. Successful real-time RT-PCR means had been developed despite these difficulties to amplify and quantify both the precursor and mature miRNA (40). Stem-loop reverse-transcriptional primers were used to distinguish between pri-miRNAs, pre-miRNAs, and mature miRNAs (40). The real-time PCR-based microarray had been developed and is a practical technique to performing high-throughput investigation of RNA samples (37). Real-time PCR technique has become the nice standard of nucleic acid quantification due to the sensitivity and specificity of PCR.

THE REGULATION OF miRNA IN HSCs

HSCs, a type of stem cells, give rise to all the blood cell types including lymphoid lineages (T cells, B cells, NK cells) and myeloid (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells) (34). HSCs could continuously differentiate into eight distinct lineages of mature blood cells, the hematopoiesis sustained for life (10). HSCs are found in the adult bone marrow, which includes hip, femurs, sternum, ribs, and other bones. The heterogeneity of the HSCs can be visualized by the kinetics of repopulation in transplantation assays. For example, primitive HSCs need several proliferation and differentiation steps to become the mature progeny and this causes a delay in the appearance of mature cells in the periphery.

To date, only a few of studies have addressed the role of miRNAs in HSCs biology, and hematological disease. According to Liao et al. (30), miRNAs play a important role in the development of human HSCs. They found that comparison to the control cells, hsa-miR-520h was up-regulated and hsa-miR-129 was down-regulated in HSCs (30). Hsa-miR-520h transduction into CD34+ cells lead to greatly increase number of different progenitor colonies by using colony-forming assays. It is suggesting that hsa-miR-520h may inhibit ABCG2 expression to promote the differentiation of HSCs into progenitor cells (30). In addition, the hsa-miR-129 was first characterized in mouse and homologues have been discovered in several other species (28). The genes, EIF2C3 and CAMTA1, related to miRNAs processing or transcription regulation, were proved to be real targets for hsa-miR-129 (28).Recently, Jin et al. reported that miRNAs upregulated in HSCs included hematopoiesis-associated miRNAs: miR-126, miR-10a, miR-221, and miR-17-92 (23). HSCs naturally segregated samples according to mobilization and isolation protocol and cell differentiation status by gene expression analysis. It suggests the regulation of miRNA was a very important mechanism in HSCs. miRNA microarrays may be useful for assessing differences in expression profiles of miRNAs in HSCs because HSCs have unique miRNA and gene expression profiles.

THE REGULATION OF miRNA IN MSCs

Mesenchymal stem cells, also called multipotent mesenchymal stromal cells (MSCs), isolated from several adult tissue sources, such as synovial fluid, placenta, adipose and dermal tissues, cord blood, amniotic fluid, and deciduous teeth, have the ability to self-renew and differentiate into multiple cells (29). MSCs represent a bone marrow (BM) population, defined by the following functional properties: extensive proliferation, and ability to differentiate into osteoblasts, chondrocytes, adipocytes, and stroma cells supporting hematopoiesis (46). In the previous studies, the ability of these cells to differentiate to several cell types of the connective tissue has been reported (5,6,11). Several heterogeneous MSC populations have been reported to maintain additional functional properties, such as the ability to heterotypically differentiate into hepatocytes and neurons, ability to homotypically differentiate into other mesodermic tissues as astrocytes, myoblasts, endothelium, or cardiomyocytes, and quasi-immortality (46). MSCs were used for many applications in cell replacement therapy (2,20).

The expression profile of miRNA in MSCs is different from that in pluripotent stem cells, such as human embryonic stem cells (16). During the differentiation into specific cell types, specific populations of miRNAs are regulated in MSCs. According to Lakshmipathy et

al., the miRNAs miR-103, 107, 140, 143, 638, and 663 were associated with MSC (29). MiR-103/miR-107 exists in vertebrate genomes within introns of the pantothenate kinase (PANK) genes. It is predicted by bioinformatics to affect the mRNA involved in the cellular acetyl-CoA and lipid levels pathways (53). In 2007, Iorio et al. reported that in comparison to normal ovary, miR-140 is markedly down expressed in human ovarian cancer (21). These data showed that miRNAs are mediators of key pathways during MSCs differentiation. In 2004, Esau et al., reported that the combination of expression data and functional assay results identified a role for miR-143 in adipocyte differentiation (13). The expression levels of miR-143 increased in differentiating adipocytes, and inhibition of miR-143 effectively inhibited adipocyte differentiation (13). The protein levels of ERK5, the miR-143 targeted, were higher in ASO-treated adipocytes. These results demonstrate that miR-143 may act through target gene ERK5 and is involved in adipocyte differentiation pathway (13).

In 2007, Greco and Rameshwar reported the miRNAs expression profile of human MSCs and MSCs-derived neuronal cells by using miRNA-specific microarray (17). In order to predict Tac1 mRNA targets, the miRNAs increased in neuronal cells and decreased after IL-1β stimulation were analyzed. They used the luciferase reporter system confirmed the miR-130a and miR-206 sites. The results showed that specific inhibition of miR-130a and miR-206 in the neuronal cells resulted in neurotransmitter substance P release and synthesis (17).

THE REGULATION OF miRNAs IN ESCs

ESCs are derived from the inner cell mass of mammalian blastocysts. They are pluripotent cells that have the ability to differentiate into all three germ layers (endoderm, mesoderm, and ectoderm) of the embryo (3). Because of ESCs distinctive ability to both self-renew and differentiate into a wide range of specialized cell types, they hold significant potential for clinical cell therapies (32). In the previous study, ESCs were developed for differentiating into functional dopamine neurons for cell therapy (26). Sharma et al. reported that murine ESCs-derived hepatic progenitor cells transplanted in recipient livers with limited capacity of liver tissue formation (41). Many evidences indicate that miRNAs contribute a key point to the control of early development. Specific miRNAs have been shown to be involved in the mammalian cellular differentiation and embryonic development pathway (47). miRNAs are important for the self-renewal and cellular differentiation of ESCs (32). According to Cao et al., 276 miRNAs were regulated in human ESCs. In the 276 miRNAs, 30 miRNAs had significantly changed expression levels during differentiation (7). For example, miR-302b in human ESCs could specifically downregulate the target mRNA (7). MiR-302 was also regulated by Oct4/Sox2 and targeted cyclin D1 in human ESCs (8). The expression level of miR-302a is dependent on Oct4/Sox2 in human ESCs. MiR-302a is predicted to target many mRNAs that are the regulators of cell cycle. In addition, the expression of miR-302a in primary and transformed cell lines promotes a decrease in G_1 phase cells and an increase in S phase (8). The results demonstrate that human ESCs differentiation is accompanied by changes in the expression of a unique set of miRNAs. It also provided a new molecular mechanism that may regulate early development in humans (8).

In 2008, Fish et al. (15) identified that miRNAs were enriched in developing mouse embryos and in endothelial cells derived from mouse ESCs. They also found that miR-126 regulated the response of endothelial cells to VEGF. In addition, knockdown of miR-126 resulted in hemorrhage and loss of vascular integrity during embryonic development in zebrafish. MiR-126 functioned in part by directly repressing negative regulators of the VEGF pathway. These findings demonstrated that a single miRNA can regulate angiogenesis and vascular integrity. It also provided a new target for modulating vascular function and formation (15). MiR-302-367 was also found to correlate with ESCs (1). Previous study found that miR-302-367 was important during embryonic development, but it is turned off later in development (1). From a hierarchical standpoint, its activity decays upon differentiation of ESCs, suggesting that its activity is restricted to the ESCs compartment and that the ESC-specific expression of the miR302-367 cluster is fully conferred by its core promoter transcriptional activity (1). These data demonstrated that miRNAs play a critical role in the regulation of multiple biological processes, the maintenance of stem cell pluripotency, and self-renewal in ESCs.

THE REGULATION OF miRNA IN NSCs

NSCs keep the ability to differentiate and to self-renew along neuronal and glial lineages (36). NSCs have the potential to revolutionize the treatment of neurodegenerative diseases (e.g., Alzheimer's and Parkinson's disease), but a large number of cells are required for this clinical application (56). Various new methods and protocols have been established and used to isolate, identify, and characterize live NSCs in terms of their capability to differentiate and proliferate (48). NSCs can be isolated by flow cytometric analysis based on physical properties such as granularity (33,35), size (forward scattering), or selected by surface antigens such as CD24 and CD133 (24,51). NSCs may be useful candidates for neural transplantation in neurological disorders (50).

According to Kapsimali et al., miRNAs have a wide

variety of different expression profiles in neural cells, including: expression associated with transition from proliferation to differentiation (e.g., miR-124); expression in neuronal precursors and stem cells (e.g., miR-92b); constitutive expression in mature neurons (miR-124 again); expression in both proliferative cells and their differentiated progeny (e.g., miR-9); regionally restricted expression (e.g., miR-222 in telencephalon); and cell type-specific expression (e.g., miR-218a in motor neurons) (25). In 2008, Rybak et al. reported that let-7 and mir-125 expressed in embryonic neural stem (NS) cells, and the posttranscriptional mechanisms contributed to the induction of let-7 (39). They demonstrate that the pluripotency factor Lin-28 binds the pre-let-7 RNA and inhibits processing by the Dicer ribonuclease in ESCs (39). In NS cells, Lin-28 is downregulated by the specific miRNAs, mir-125 and let-7, allowing processing of pre-let-7 to proceed. Suppression of let-7 or mir-125 activity in NS cells led to upregulation of Lin-28 and loss of pre-let-7 processing activity, suggesting that let-7, mir-125, and lin-28 participate in an autoregulatory circuit that controls miRNA processing of NS cells (39).

THE REGULATION OF miRNA IN IPSs

IPSs are a pluripotent stem cell artificially derived from a nonpluripotent cell (e.g., an adult somatic cell). Takahashi et al. first reported the use of adult human dermal fibroblasts to generate the iPS cells with four factors: Oct3/4, Sox2, c-Myc, and Klf4 (49). Human iPS cells are similar to human ESCs in proliferation, morphology, gene expression, surface antigens, epigenetic status of pluripotent cell-specific genes, and telomerase activity (49). This is an important advancement in stem cell research. This technique may allow researchers to obtain pluripotent stem cells, which are very important in stem cell research and potentially to the cell therapy.

In 2008, Lin et al. used mir-302 to reprogram human skin cancer cells into a pluripotent ES cell-like state (31). In slow-growing human ESCs, the mir-302 miRNA family (mir-302s) is expressed most abundantly, and quickly decreases after cell proliferation and differentiation (38). The mir-302s not only function to reprogram cancer cells into an induced pluripotent ES cell-like state but to maintain this state under a feeder-free cultural condition. It may offer a great opportunity for cell therapeutic intervention (31). Therefore, mir-302 was studied as one of the key factors to maintenance of ES cell pluripotency and renewal (31).

FUTURE DIRECTIONS

miRNAs are the novel field to study the regulation of mRNA. Because of their ability to simultaneously regulate many target genes, miRNAs are especially important candidates for regulating stem cell fate decisions and self-renewal (42). For this reason, miRNAs are the key regulators in stem cell biology. Previous study also demonstrated that iPSs could be generated by transfecting only one miRNA into the human skin cancer cell line (31). In the future, miRNAs will be very important regulators to study the stem cell therapeutic approach.

CONCLUSION

miRNAs, the cell-intrinsic regulators, are the emerging regulators controlling stem cell self-renewal and differentiation. These mechanisms are regulated the maintenance, self-renewal, development, and differentiation in stem cells. In order to better understand stem cell biology, demonstrating how individual signaling cascades integrate into the global regulatory networks will be essential. The future goals demonstrated the miRNA targeted genes and mechanism will be important for understanding stem cell biology. It will also facilitate developing the novel therapeutic approach by using stem cells for many diseases.

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