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The Role of microRNAs Involved in Mesenchymal Stem Cell Differentiation

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= Abstract =

Over the past decade, microRNA has been emerging as a key regulator involved in various biological and physiological process including cell cycle, tissue development and pathogenesis. The effort to decipher the complicated machinery behind microRNA function is underway yet the complete understanding is far from being reached. However, various groups have made successful identification of the role of a specific microRNA involved in a specific process. This article summarizes the microRNAs identified in one such specific area, namely, differentiation of mesenchymal stem cell. Characterized by its unique potential to differentiate into three separate tissue cell types, adipocyte, chondrocyte and osteoblast, mesenchymal stem cells have been of immense interest for its possible applicability in regenerative medicine. Despite this interest, however, the study of microRNA function in MSCs differentiation remains rudimentary. The aim of this paper is to summarize the currently identified, experimentally validated and accepted by the scientific community as novel, microRNAs involved in MSC differentiation.

Key Words: MicroRNA, Mesenchymal stem cell, Differentiation

Introduction

The discovery of two small RNAs, lin-4 and let-7 during the study of genes involved in timing of larval development of Caenohabditis elegans was the official introduction of the microRNAs (miRNA) to the world[1,2]. Only 17 to 23 nucelotides in length, these small RNAs were soon recognized as a key regulator of the development of C.elegans and interestingly they were highly conserved across species. Following the initial discovery, numerous groups have began their own expedition onto the unknown territories of the world of miRNA, and have successfully identified that not only were they crucial for the larval development but also in cell proliferation[3] and apoptosis[4], immunoresponse[5], protein expression[6] and tissue development[7] as well as in various other biological processes. Currently, few

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miRNA are clearly identified and assigned to specific function in mammals. miR-181 promotes B cell development in mice[8], and it targets the homeobox protein Hox-A11 during mammalian myoblast differentiation[9]. miR-196 regulates several Hox genes while the brain-specific miR-134 regulates dendritic spine development[10]. miR-1, miR-133 and miR-206 are specifically induced during myogenesis[11] and miR-143 are known to regulate adiocytic differentiation[12]. However, despite the recent achievements in identification of miRNAs involved in various processes, the studies on the miR function in one particular field of physiology remain at primitive stage: the differentiation of mesenchymal stromal cells.

Mesecnhymal stromal cell, also known as MSCs, are plastic-adherent, fibroblast-like cells first identified and described by Friedenstein[13]. Following its initial discovery, various groups have identified the cells capacity to differentiate into multiple connective tissue cell types, including fat, tendon, cartilage and bone[14,15,16]. Their wide abundance in body coupled with their multipotent differentiation capacity has made the MSCs an ideal candidate for use in regenerative medicine, which led to the creation of its alias, mesenchymal stem cell, coined by Caplan. Despite this immense interest in dissecting the exact mechanism behind MSCs capacity to differentiate into multiple lineages, our understanding of all the regulators involved in MSC differentiation remains elusive. Until recently, MSCs differentiation was thought to be solely regulated by various transcription and growth factors, hormones, and signaling pathways provided in an optimal condition. However, with the discovery of miRNA, and considering its comprehensive impact on other biological processes, the regulatory role of miRNA on MSC differentiation seems convincing.

The functional mechanism of microRNA

Before attempting to understand the complex regulatory functions miRNA may have on the differentiation process of MSCs, sound knowledge in how miRNA are synthesized and, more importantly how they induce inhibitory effect on its target gene must be established. Currently, 10883 hairpin precursor miRNAs, expressing 10581 mature miRNA products, in 115 species have been identified in the Sanger database version 14.0. Being either intronic or exonic, and often located in non-coding regions between genes (inter-

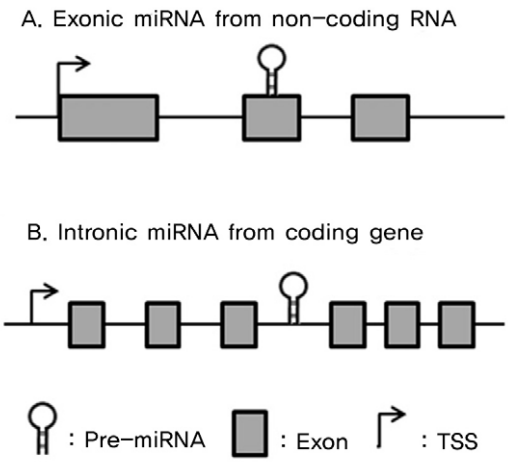

Fig. 1. Genomic locations of miRNAs. miRNAs may be located virtually anywhere on the genome. A. miRNA may be located within the exon of a non-coding transcript B. miRNAs may be located within the intron of a coding gene.
Figure obtained from Jeffrey M. Friedman and Peter A. Jones, MicroRNAs: critical mediators of differentiation, development and disease 2009 Swiss MED WKLY 139(33-34):466-472

genic) and sometimes within the coding region, miRNA expression may be induced by its own promoter (intergenic) or by that of its host gene (Fig. 1). The transcription product quickly folds back onto itself, forming a hairpin structure which is then cleaved by Drosha within the nucleus[17]. Once cleaved, it is exported to cytoplasm by exportin[18], secondary cleavage by Dicer before forming a functional complex called RNA-induced silencing complex (RISC)[19]. This complex recognizes the complementary sequence of its miRNA on the target genes 3' UTR region, which does not require a perfect match, and induce inhibition of gene expression by either of the two mechanisms: mRNA cleavage[20] or translational repression[21]. However, the complete identification of all regulators involved in miRNA expression and its functioning pathway remains to be fully identified and the data presented here only summarizes the knowledge established and accepted currently.

MSC differentiation

As previously mentioned, MSCs have trilineage differentiate potential to differentiate into adipoblast, chondroblast and osteoblast resulting in formation of fat, cartilage and bone, respectively. Recent advances in transcriptomics and proteomics have allowed us to study transcriptional regulation involved in these stage in more detail and have yielded valuable information. In the following sections, the differentiation process into three separate lineages will be examined closely involving identified transcription factors, genes and miRNA.

I. Osteogenesis

The long and complicated process of osteogenesis is initiated with cellular proliferation followed by extracellular maturation (ECM) which ultimately leads to matrix mineralization. Bone morphogenic protein (BMP), Wnt and Hedgehog signaling are accepted as the key signaling pathway involved during the process. These signaling pathways work symbiotically in a systematically regulated pathway and exert their impact on downstream transcription factors such as Cbfa1/Runx2, Msx2, Dlx5 and Osterix. Over the past decade, handfuls of miR-

Table 1. Identified microRNAs involved in osteogenesis and their targets

microRNA	Target	Function	Reference
miR-133	Runx2/Cbfa1	Negative	Zhaoyong Li, 2008
miR-135	SMAD5	Negative	Zhaoyong Li, 2008
miR-26a	SMAD1	Negative	Ettore Luzi et al, 2008
miR-210	AcvR1b	Negative	Yosuke Mizuno, 2009
miR-141	Distal-less Homeobox5	Negative	Tomohiro Itoh, 2009
miR-200a	HDAC4, TGFβ3,	Negative	Tomohiro Itoh, 2009
miR-29b	ACVR2A, CTNNB1P1, DUSP1	Postivie	Kristina Kapinas, 2009
miR-29	Osteonectin	Negative	Zhaoyong Li, 2009

List of the published microRNAs targeting genes involved in osteogenesis. Under function column, the microRNAs shown to inhibit osteogenesis are labeled as "negative" and the microRNAs shown to promote osteogenesis are labeled "positive."

NAs have been identified to inhibit or induce osteogenesis, which are summarized on Table 1. Specifically, Zhaoyong Li et al. have identified that miR-133 directly targets Runx2/Cbfa1 while miR-135 targets Smad5, which is a key transducer of the BMP-2 osteogenic signal, both of which had a complement sequence to respective miRNAs on their 3' UTR region[22]. SMAD1 was found to have two binding sites for miR-26a on its 3' UTR region[23] while miR-210 was identified to promotes osteogenesis via targeting AcvR1b and that miR-141 and -200a targets Distal-less Homeobox 5, respectively [24,25]. Kristina Kapinas et al. have discovered that miR-29 suppresses osteonectin in osteoblast[26]. Although majority of the data have identified the miRNAs which had an inhibitory effect on osteogenesis, some have also found the miR targeting the inhibitors of osteogenesis, which in turn promoted the differentiation. One particular study was carried out by Zhaoyong Li, the same group which have successfully identified that miR-133 targets Runx2/Cbfa1, in which they have discovered that miR-29b targets multiple inhibitors of osteoblast differentiations, HDAC4, TGFβ3, ACVR2A, CTNNBIP1 and DUSP2[27]. From these findings, one can understand that although the basic functioning mechanism of miR is to inhibit the expression of its target gene by binding to the 3' UTR region, based on which gene the miR targets, miR can either promote or inhibit the differentiation process. Although the majority of the data mentioned in this review have used mouse osteoblast cell lines, such as MC3T3 and ST2, and human adipose derive stem cell (hADSC), the repeating theme here is that the miRNA is highly conserved across species and as such the resulting data from these alternative cell lines are expected to be readily translated onto MSCs as well.

II. Chondrogenesis

Similar to osteogenesis, chondrogenesis also occur via multiple stages initiated by cell condensation driven by upregulated N-cadherin, neural cell adhesion moleucule (NCAM) and fibronectin, promoting cell-to-cell and cell-to-ECM interactions. Once the differentiating cells become committed into chondroblast phase, the cells switch the major components of the ECM from Type I collagen, which is a characteristic of an undifferentiated MSCs, to more cartilaginous make up involving Type-II, Type-IX and Type -XI collagen, Cartilage oligomeric matrix protein-1 (COMP-1) and Aggrecan. Ultimately, the chondrocyte reaches the hypertrophic state where the cells start to express Type-X collagen, unique to the hypertrophic stage of chondrogenesis. Eventually, as part of the natural bone growth and development, the hypertrophic chondrocytes undergo apoptosis and are replaced by bone. This long and complicated process of chondrogenesis are directed by number of signaling pathways involving TGFβ, FGF, Wnt and Indian hedgehog (Ihh) signaling to name a few. In turn, the aforementioned signaling pathways direct the expression of various transcription factors involved in chondrogenesis such as the SOX 5, SOX 6, SOX 9, also known as the SOX trio. Currently, handful of miRNAs have been experimentally validated as a key regulator in chondrogenesis. One study reported that miRNA18a targets CCN family protein 2/connective tissue

growth factor (CCN2/CTGF), which is a central player in endochondral bone formation[28]. Additionally, miR-140 was found to inhibit the expression of histone deacetylase 4[29], which may be involved in long bone development. miR-199a* had a profound inhibition on the early stages of chondrogenesis in pluripotent C3H10T1/2 stem cells grown on pellet culture. When treated with miR-199a*, the authors have observed significant reduction of key chondrogenic markers, namely; cartilage oligomeric matrix protein (COMP), sox 9 and Type II Collagen, whereas inhibition of miR-199a* resulted in increase of these genes[30]. Interestingly, unlike in studies which focused on the effect of miRNA during osteogenesis, several groups studying miRNA in chondrogenesis approached the subject with quite different aspect. Walter Dunn et al. have examined the miRNAs expressed on different areas of articular cartilage, specifically classified by the degree of weight-bearing. The authors have found that miRNA-221 and miR-222 were significantly up-regulated on higher weight-bearing region identified as M1, which is located on the anterior part of the cartilage, suggesting that this particular localization on anterior medial condyle may have a certain function on weight-bearing capacity of the cartilage[31]. Another group, have observed that miRNA-146a were gradually reduced as osteoarthritis progressed and have also found out that miR-146a targets matrix metalloproteinase 13 (MMP-13)[32].

III. Adipogenesis

Similar to the patterns shared by osteogenesis and chondrogenesis, adipogenesis also occur through multiple steps initiated by commitment to pre-adipocytes. The signaling cascade is initiated by the co-expression of CCAAT/enhancer binding protein β (C/EBPβ) along with C/EBPδ, which leads to the activation of downstream transcription factors C/EBPα and peroxisome proliferator-activated receptor γ (PPARγ). By the terminal differentiation stage, numerous genes are expressed which include hormone-sensitive lipase (HSL), fatty acid binding protein (FABPs) and glycerol-3-phosphate dehydrogenase (GPDH). During this period, various adipokines are also secreted including leptin (LEP) and adiponectin (ADIPOQ). Michael Karbiener et al. succeeded in identifying the miRNA targeting the key transcription factor during adipogenesis: PPARγ. By using human multipotent adipose-derived stem (hMADS) cells, the authors have found that when the cells were treated with miR-27b over-expressing vector, number of adipogenic marker genes showed notable decrease in expression, which included PPARγ, FABP4 and LPL[33]. Christine Esau et al. have also observed the increase of miR-143 in differentiating adipocytes, and that when miR-143 was inhibited so was the adipogenesis while BP Lewis et al. have suggested the possible target of miR-143 as the extracellular signal-regulated kinase 5 (ERK5)[34]. In last two years, number of groups has also successfully identified miRNAs involved in adipogenesis, including the positive effect of miR-103 and miR-19-92 cluster had on adipogenesis[35,36] and the negative effect induced by let-7 and miR-27[37,38]. In a study using ST2 cells, J.A. Kennell et al. have identified multiple miRNAs which

promoted adipogenesis, which were miR-200c/141 and miR-200b,a/429[39]. In C3H10T1/2 cells, F.Sun et al. have successfully identified that miR-24 enhanced adipogenesis while miR-31 enforced negative effect[40].

Conclusion

Despite being relatively recently identified, miRNAs are proving to be a critical mediators in various biological processes including tissue development, immunoresponse, protein expression, viral infection and cell proliferation, division, apoptosis and differentiation. However, despite the recent achievements in identifying the functioning pathway of miRNA and the impact of individual miRNA on a specific pathway, we are still at a primitive stage in understanding the whole picture that miRNA draws. One such aspect is our limited understanding of how miRNA actually recognizes its target and induce its effect. Although a computerized algorithm and simplified means of communications have yielded useful database such as TargetScan and PicTar, which predicts the potential target of each identified miRNA based on sequence complementarity, significant portion of the predicted targets remained independent of miRNA treatment. Such result makes us speculate the existence of additional regulatory mechanism that exists between the target mRNAs and the miRNA. Regardless, various groups are making progress in understanding the complete pathway of miRNA and more importantly, succeeding in applying miRNA in the field of medicine. For example, Gilad S et al. have developed a novel technique to isolate miRNAs from bodily fluids such as urine[41], which Mitchell et al. have utilized to identify miR-141 isolated from plasma as a novel indicator of prostate cancer[42]. One report suggested that more than one third of all protein coding genes in human genome may be regulated by miRNA[43], and considering the unparalleled advance in technology as well as the continued global effort to gain complete understanding of miRNA, its use and applicability seems, truly, endless. It seems, paradoxically, the potential for a molecule with such a tiny name, microRNA, has an endless potential.

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