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Research paper

MicroRNA regulation of stem cell differentiation and diseases of the bone and adipose tissue: Perspectives on miRNA biogenesis and cellular transcriptome

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ABSTRACT

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression through targeting and suppression of mRNAs. miRNAs have been under investigation for the past twenty years and there is a large breadth of information on miRNAs in diseases such as cancer and immunology. Only more recently have miRNAs shown promise as a mechanism for intervention with respect to diseases of the bone and adipose tissue. In mesenchymal stem cell (MSC) differentiation, alterations in miRNA expression patterns can differentially promote an osteogenic, adipogenic, or myogenic phenotype. This manuscript reviews the current literature with respect to miRNAs in the context of MSC function with a particular focus on novel avenues for the examination of miRNA associated with bone and adipose tissue biology and disease. Specifically we highlight the need for a greater depth of investigation on MSCs with respect to miRNA biogenesis, processing, strand selection, and heterogeneity. We discuss how these mechanisms facilitate both altered miRNA expression and function.

1. Introduction

Bone development arises from the interaction of a diverse array of mesenchymal and hematopoietic cells, including adipocytes, osteoblasts, and osteoclasts [1–4]. Together, these cells build, maintain, or reduce skeletal mechanical integrity by balancing the levels of bone formation, remodeling, and reabsorption. At the same time, bone cells are responsible for systemic calcium homeostasis in concert with the calcium-absorbing cells of the intestine and the calcium-secreting cells of the kidney. Endocrine and paracrine signal transduction pathways mediated in part by parathyroid hormone (PTH), bone morphogenetic proteins (BMPs), and transforming growth factor β (TGFβ) direct these complex interactions at the extracellular level. At the intracellular level, the binding of these hormonal factors, growth factors, chemokines, and cytokines to their surface receptors initiates kinase activities, phosphorylation of downstream target proteins, and second messenger cascades resulting in the modulation of transcriptional events.

microRNAs (miRNA) are small non-coding RNAs which regulate gene expression through the binding and inhibition of target mRNAs. The genomic DNA encoding these short RNAs are found within both intergenic and intronic regions [5] and deep sequencing modalities have revealed miRNAs that are expressed differentially between cell lineages [6–8]. miRNAs can modulate gene expression profiles by binding to common regulatory sequences found within both genomic DNA and mRNAs [9, 51]. As a

Abbreviations: PTH, parathyroid hormone; BMPs, bone morphogenetic proteins; TGFβ, transforming growth factor β; miRNA, microRNAs; BM-MSC, bone marrow-derived mesenchymal stem cells; UCP1, uncoupling protein-1; PRDM16, PR domain containing-16; MYF5, myogenic factor-5; MSC, mesenchymal stem cell; AGO2, Argonaute-2; UTR, untranslated region; pre-miRNAs, precursor-miRNAs; PPARγ, proliferator-activated receptor gamma; SMAD2, mothers against decapentaplegic 2; PTHLH, parathyroid hormone-related protein; TGFβR2, transforming growth factor beta receptor II; ASC, adipose derived stem cells; SNP, single nucleotide polymorphisms; APA, alternative polyadenylation; PAX3, paired box 3; Lin28, zinc finger CCHC domain-containing protein; EXPO5, exportin-5; MAPK, mitogen activated protein kinase; ERK, extracellular signal-regulated kinase; CDON, cell adhesion associated/oncogene regulated; FASL, fas ligand; FGF2, fibroblast growth factor 2; KSRP, KH-type splicing protein; BRCA1, breast cancer 1; TBI, traumatic brain injuries; HO, heterotopic ossification.

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result, a single miRNA can regulate the levels of downstream targets directly or indirectly.

This review focuses on miRNA mechanisms in relation to bone marrow-derived mesenchymal stem cells (also known as multipotent stromal cells) (BM-MSCs). The BM-MSCs were first recognized based on their ability to differentiate along multiple lineage pathways with the capability of adipogenesis, chondrogenesis, myogenesis, and osteogenesis [10–12]. Furthermore, BM-MSCs are characterized immunophenotypically by their surface expression of stromal markers (CD29, CD44, CD73, CD90, CD105) and the absence of hematopoietic markers (CD11, CD34, CD45) [12–16]. BM-MSCs, like their counterparts in adipose tissue (known as adipose-derived stem cells or ASCs [17]), exhibit these features in common with perivascular or pericytic cells [18–22]. Some investigators hypothesize that BM-MSCs and pericytes are one and the same population. Regardless of their precise cellular identity, these cells share many common biological properties [19].

An inverse relationship has been postulated to exist within the bone marrow between adipocytes and osteoblasts [23]. A substantial body of evidence suggests that signal transduction mechanisms promoting BM-MSC differentiation along the osteoblast lineage do so at the expense of adipogenesis and vice versa [4]; however, there is accumulating evidence indicating that this may be an over-simplification [24]. The body contains distinct types of adipocytes which are categorized as white, brown, or as a hybrid known as beige or brite (**br**own/wh**ite**). White adipocytes are most prominent in the adult and exist throughout the body, and are primarily responsible for energy storage and adipokine secretion. White adipocyte hyperplasia and hypertrophy manifests itself as obesity. In contrast, brown adipocytes are most prominent in new born infants where they serve a thermogenic role due to their expression of uncoupling protein-1 (UCP1). The UCP1 directly converts energy into heat within the many mitochondria that give the brown adipocyte its distinctive color. Recent studies of the transcriptional regulatory genes PR domain containing-16 (PRDM16) and myogenic factor-5 (MYF5) have demonstrated that brown adipocytes share closer developmental links to skeletal muscle cells than to white adipocytes [25–28]. While brown adipose tissue depots are less prominent with advancing age, they have been detected within the cervical and supraclavicular regions of lean adults using sensitive PET-based glucose uptake detection methods [29,30]. The hybrid beige/brite cell is a newly identified UCP1-expressing cell lineage found within white adipose depots; unlike brown adipocytes, beige/brite cells lack close developmental ties to the myogenic lineage [31–33]. Of importance to the current review article, cells expressing UCP1 and related proteins can be found within sites of new bone formation, suggesting that brown and/or beige/brite cells may play a role in osteogenesis and osteoblast differentiation [34–40]. Consequently, this review will explore how miRNAs can modulate BM-MSCs commitment to the various adipocyte, myocyte, and osteoblast lineage pathways. In the final summary section, we will evaluate how future investigations can use miRNA biology to understand, prevent, and treat bone- and adipose-related diseases such as obesity, diabetes, osteoporosis, osteosarcoma, and heterotopic ossification.

2. miRNAs

miRNAs are multifaceted small non-coding RNAs known to regulate cellular functions (proliferation, migration, epithelial to mesenchymal transition) and signaling pathways (estrogen receptors, TGFβ/SMAD, MAPK, WNT, PI3K/mTOR) through the suppression of target mRNA. Studies of mesenchymal stem cell (MSC) differentiation demonstrate a role for miRNAs in cell fate (Fig. 1) [59]. miRNAs regulate adipogenesis, myogenesis, and osteogenesis, and accordingly MSC undergoing differentiation along these pathways demonstrate altered miRNA expression profiles [41–43]. miR-489 was shown to be vital for osteogenesis; while expression of miRNAs (miR-143 and miR-1) is required for adipogenesis and myogenesis, respectively [44–46]. miRNA expression changes occur both intracellular and extracellular. In MSC, there is a release of miRNAs and their associated proteins to the surrounding cellular environment. Analysis of the BM-MSC's secretome has implicated the secreted microvesicles as a mechanism for the BM-MSC protective function on surrounding tissues. Released microvesicles contain the miRNA biogenesis associated protein Argonaute-2 (AGO2) as well as high levels of miR-125b, miR-222, miR-24, miR-99a, miR-100, miR-594, miR-31, miR-16, miR-125a, and miR-21; many of these miRNAs regulate osteogenic and adipogenic differentiation [47].

Expression levels of miRNAs change as cells are induced to differentiate down a specific lineage (myogenic, adipogenic, or osteogenic) (Fig. 2A and B, and Supplemental text S1 – describes full reference list and cell lines used). Interestingly, only a few miRNAs (miR-26a↑, miR-24↑, and miR-222↓) are similarly regulated during differentiation of all three lineages, suggesting that these miRNAs may be more closely associated with the maintenance of stemness as opposed to directing a specific lineage pathway. Alternatively, expression of some miRNAs is demonstrated to be lineage selective; specific examples are found in adipogenesis (miR-143, miR-378*), osteogenesis (miR-199a), or myogenesis (miR-206, miR-133a). Moreover, analysis of miRNAs altered between the three lineages suggests that miRNA regulation occurs both transcriptionally and during the processing of the primary miRNA transcript (pri-miRNA) transcript to a mature miRNA.

miRNA processing involves sequential maturation of a miRNA from the pri-miRNA transcript to the precursor miRNA (pre-miRNA), followed by processing of pre-miRNA to the mature miRNA [5]. In general miRNAs are transcribed as a pri-miRNA hairpin loop that contains two miRNAs, components denoted -5p and -3p. Of these two miRNAs one is denoted as the guide (or mature) strand and the other is the star (*) (or passenger) strand. One miRNA strand is degraded and the other is utilized for mRNA inhibition. The selection of the miRNA for degradation is determined by thermodynamic stability of the miRNA strands. In general, the star strand is degraded as it is frequently more thermodynamically stable while the guide strand is incorporated into RNA Induced Silencing Complex (RISC), a multi-protein complex which cleaves mRNAs to induce mRNA silencing [5]. Differences in expression of miRNAs which comprise the same miRNA duplex suggests that miRNA regulation may be occurring post transcriptionally, possibly during miRNA biogenesis. For instance, analysis of miRNAs altered during adipogenesis and osteogenesis reveals increased expression levels of star miRNAs during adipogenesis (miR-193a*, miR-22*, miR-378*, miR-423*, and miR-9*) and during osteogenesis (miR-199b*, miR-34b*, and miR-9*) without a subsequent increase in the mature counterparts (miR-193a, miR-22, miR-378, miR-423, miR-9, miR-199b, miR-34b, and miR-9). Conventionally the star strand is degraded and has low expression levels compared to the guide strand; the observed increase of only the star miRNA implicates a role for post-transcriptional regulation in addition to transcriptional regulation during cell fate. In accordance with this observation, recent studies show that some star miRNAs can be expressed in amounts equivalent to or exceeding their guide strand counterparts, thereby evoking phenotypic responses [48,49]. miRNA deep sequencing of multiple tissue types has documented alterations in strand selection with high expression levels of star miRNA [6–8]. Differences in strand preference for miR-24 have been previously described in breast