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Review

Molecular

TLE3 switch cell fate between osteoblast and adipocyte in bone marrow stromal cells

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Abstract

Introduction

Osteoblasts and bone marrow adipocytes originate from bone marrow stromal cells. In senile osteoporosis, the balance between adipogenesis and osteoblastogenesis in bone marrow stromal cells is disrupted such that adipogenesis is increased with respect to osteoblastogenesis resulting in decrease in bone mass.

In general, there appears to be a reciprocal relationship between adipogenesis and osteoblastogenesis in bone marrow stromal cells. Although several proteins have been reported to regulate this process, the exact nature of the signals regulating the balance between osteoblast and adipocyte formation within the bone marrow space remains to be determined.

Recently, transducin-like enhancer of split 3 (TLE3) was reported to regulate the balance between osteoblast and adipocyte formation. TLE3 enhances adipocyte differentiation and suppresses osteoblast differentiation of bone marrow stromal cells *in vitro*. In addition, canonical Wnt signalling, which plays important role in both adipogenesis and osteoblastogenesis, induces TLE3 expression, suggesting that TLE3 activity may be key to balancing adipocyte and osteoblast differentiation within the adult bone marrow microenvironment. The aim of this review was to discuss TLE3 switch cell fate between osteoblast and adipocyte in bone marrow stromal cells.

Conclusion

Currently, there is demand for new effective therapies that target the stimulation of osteoblast differentiation to enhance bone formation. Reducing TLE3 expression in bone marrow stromal cells could be a useful approach towards increasing osteoblast numbers and reducing adipogenesis in the bone marrow environment. Thus, the development of a therapy that combines small interfering RNAs against TLE3 with a local delivery system targeting bone marrow stromal cells could lead to novel bone formation agonist.

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Introduction

Osteoblast-lineage cells and marrow adipocytes are derived from a common progenitor, the bone marrow stromal cell (BMSC). In senile osteoporosis, the balance between adipocyte and osteoblast differentiation is disrupted in this cell population such that adipocyte differentiation is increased relative to osteoblast differentiation leading to a reduction in bone mass, an increased bone fragility and an increased susceptibility to fracture[1]. In 2004, 10 million Americans over the age of 50 had osteoporosis with another 34 million Americans at risk for the disease. In this population, it is estimated that 1.5 million fragility fractures occur each year, with an annual health care cost of 18 billion dollars[2]. By 2025, the health care expenditures for osteoporotic fractures will approach 25.3 billion dollars[3]. Therefore, the molecular mechanisms responsible for controlling the balance between osteoblastogenesis and adipogenesis in adult bone are of great significance.

A variety of studies have shown that there is a reciprocal relationship between adipogenesis and osteoblastogenesis in BMSCs. Several proteins such as tafazzin, Wnt5a, Wnt10b, Msx2, CCAAT/enhancer-binding family of protein β (C/EBPβ) and basic helix-loop-helix family member e40 (BHLHe40) and ID4 have been identified as regulators of this balance[4]. However, the exact nature of the signals regulating the balance between osteoblast and adipocyte formation within the bone marrow space remains to be determined. Recently, a novel factor, transducin-like enhancer of split 3 (TLE3), was reported to switch cell fate between osteoblasts and adipocytes in BMSCs.

In this critical review, we focus and discuss the function of TLE3 on the determination of cell fate in BMSCs.

Discussion

The authors have referenced some of their own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

TLE3 is member of Groucho/TLE family

TLE3 is expressed in the placenta, in brown and white adipose tissues and in bone marrow[4]. TLE3 is a member of the Groucho/TLE family. Groucho/TLE are present in all or almost all metazoans. While the Drosophila genome encodes a single Gro, the human and mouse genomes encode four members of each family: TLE 1–4 in human and Gro-related genes 1–4 (Gro1-4) in mouse[5]. TLE and Gro

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have often been used interchangeably for vertebrate orthologues in the literature and in sequence databases[6]. Groucho/TLE family proteins play critical roles in a wide array of developmental and cellular pathways[7]. They act as transcriptional co-repressor proteins that do not bind DNA directly but are recruited by a diverse profile of transcription factors, including the Hes family, Runx2, Nkx and Pax, and by downstream effectors of Wnt signalling such as cMyc and TCF/Lef[6].

TLE3 enhances adipogenesis

Adipogenesis is driven by a complex and well-orchestrated signalling cascade composed of several key transcription factors, most notably peroxisome proliferator-activated receptor (PPAR) γ and several members of the C/EBPs[8]. PPARγ is commonly referred to as the master regulator of adipogenesis because no factor has yet been identified that can induce normal adipogenesis in its absence[9]. Villanueva et al.[10] reported that TLE3 induces adipogenesis in preadipocytes. Kokabu et al.[4] confirmed that TLE3 enhances transcriptional activity of PPARg, thereby inducing adipocyte differentiation of BMSCs.

TLE3 suppresses osteoblastogenesis

BMSCs can not only differentiate into adipocytes but can also be induced towards osteoblast differentiation *in vitro*[11]. Bone morphogenetic proteins (BMPs) were originally discovered as factors that induce ectopic bone formation when implanted into muscle tissue and also stimulate osteoblast differentiation of various cell types including BMSCs.

In response to BMP signalling, several critical transcription factors for osteoblast differentiation, such as Runx2, Osterix, Dlx2 and Dlx5, are induced in target cells[12–15]. Runx2 is essential for the commitment of mesenchymal cells to the osteoblast lineage. Homozygous deletion of Runx2 in mice resulted in a complete lack of osteoblasts[12], whereas haploinsufficiency of Runx2 in mice or humans leads to hypoplastic clavicles and delayed closure of the fontanelles. These defects are characteristic of cleidocranial dysplasia in humans[16,17]. The expression of Runx2 is regulated, at least in part, by BMP signalling[18], and Runx2 controls osteoblast-related genes such as Osterix, collagen I and osteocalcin[13,19] and the Runx2 gene itself[20].

Runx2 interacts with several proteins including Groucho/TLE family proteins[21]. In turn, Groucho/TLE proteins work as co-repressors of Runx2[22]. TLE1 and TLE2 also repress Runx2-dependent activation of osteocalcin gene transcription[23]. Recently, Kokabu et al.[4] reported that TLE3 suppresses BMP2-induced osteoblast differentiation of BMSCs, by repressing Runx2 transcriptional activity[4].

Expression of TLE3 is regulated by canonical Wnt signalling

The Wnt family of 19 secreted glycoproteins has a critical role in regulating embryonic development, cell differentiation and cell fate determination[24]. Wnts transduce two types of intracellular signalling: canonical and non-canonical pathways. During the activation of canonical signalling, Wnts bind to a complex containing frizzled and low-density lipoprotein receptor-related protein (LRP)-5 or 6. This complex regulates the kinase activity of glycogen synthase kinase 3β (GSK3β). In the absence of Wnt, activity of β-catenin, a downstream effector of the canonical Wnt pathway, is blocked by a phosphorylation-dependent degradation mechanism induced by GSK3β. Canonical Wnt signalling stabilises β-catenin by inhibiting GSK3β activity. Stabilised β-catenin translocates to the nucleus and interacts with TCF/Lef to activate gene expression[25]. Canonical Wnt signalling has a key role in adult skeletal homeostasis and bone remodelling[26]. While the canonical Wnt signalling pathway is a major physiological inhibitor of adipogenesis[27], canonical Wnt signalling correlates with enhanced bone formation through increased differentiation and maturation of osteoblasts[28]. Indeed, in both humans and mice, loss of function of LRP5 leads to decreased bone formation[29,30] and a gain-of-function mutation in LRP5 results in a high bone mass phenotype[31,32].

The Groucho/TLE family acts as transcriptional co-repressor of Wnt signalling[6]. In the absence of Wnt ligand, TCF/Lef bound to Groucho/TLE transcriptional co-repressors inhibits Wnt target gene transcription[33,34]. According to Daniels and Weis[35], β-catenin that enters the nucleus on Wnt signalling directly competes with Groucho/TLE for TCF/Lef binding. Once bound to TCF/Lef on chromatin, β-catenin recruits a co-activator complex, thereby converting TCF/Lef into a transcriptional activator. Thus, TLE3 is able to suppress canonical Wnt signalling in BMSCs[5]. This repression of Wnt signalling by TLE3 may be part of the mechanism, together with suppression of Runx2, by which TLE3 suppresses osteoblast differentiation[4,30].

Most recently, and in contrast to the effects of TLE3 on Wnt signalling, Kokabu et al.[5](2014) identified Wnt responsive elements in the TLE3 promoter region through comparative genomic analysis, and then through functional analyses they also showed that expression of TLE3 is increased by Wnt signalling[5]. This provides a novel idea that induction of TLE3 by Wnt signalling is part of a negative feedback loop active during osteoblast differentiation and/or a part of a positive feedback loop during adipogenesis (Figure 1).

Conclusion

TLE3 suppresses osteoblast differentiation of BMSC *in vitro* while inducing adipogenesis. Moreover, canonical Wnt signalling, which plays

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an important role in both adipogenesis and osteoblastogenesis, induces TLE3 expression, suggesting that TLE3 activity may be key in balancing adipocyte and osteoblast differentiation in the adult bone marrow microenvironment.

The most commonly prescribed therapeutics for bone-related diseases are antiresorptives such as calcitonin, oestrogen and bisphosphonates that block osteoclast activity as a means to stabilise bone architecture. Recent data on the importance of continuous bone remodelling as a means to maintain the material and structural strength of bone caution against the overuse of antiresorptives as they may allow for accumulation of microdamage in bone and ultimately lead to fractures in some patients. Thus, the development of new effective therapies that target enhancing bone formation by stimulating osteoblast differentiation is required. The development of agents that reduce the expression of TLE3 may provide an additional benefit to antiresorptive therapies that block osteoclast activity as a way of stabilising bone architecture.

Abbreviations list

BHLHe40, basic helix-loop-helix family member e40; BMPs, bone morphogenetic proteins; BMSCs, bone marrow stromal cells; C/EBPs, CCAAT/enhancer-binding family of proteins; GSK3β, glycogen synthase kinase 3β; LRP, low-density lipoprotein receptor-related protein; PPARγ, peroxisome proliferator-activated receptor γ; TLE3, transducin-like enhancer of split 3.

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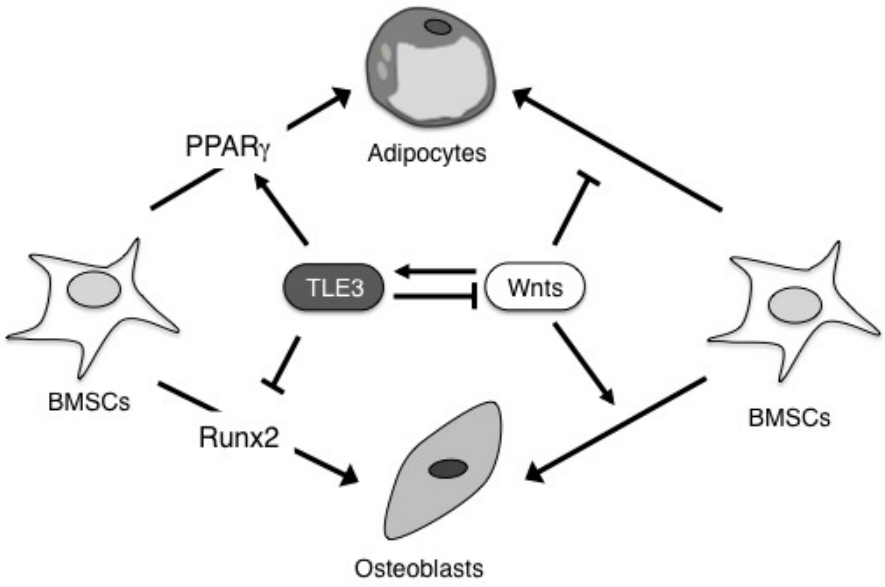

Figure 1: Model for the role of TLE3 in the bone marrow microenvironment. TLE3 directly induces adipogenesis and suppresses osteoblastogenesis of BMSCs by acting on PPARγ and Runx2, respectively. TLE3 also indirectly induces adipogenesis and suppresses osteoblastogenesis by repressing canonical Wnt signalling, which is capable of inducing osteoblastogenesis and inhibiting adipogenesis. In addition, canonical Wnt signalling induces TLE3 expression, suggesting that the induction of TLE3 by Wnt signalling may be part of a negative feedback loop during osteoblastogenesis and/or a positive feedback loop during adipogenesis in the adult bone marrow microenvironment.

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