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Review

Instruction of mesenchymal cell fate by the transcription factor C/EBPβ

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ABSTRACT

The transcription factor CCAAT/enhancer binding protein beta (C/EBPβ) plays a role in the differentiation of a large variety of cell types. C/EBPβ was initially described as an early inducer of adipocyte differentiation, however, recent data have shown that this is not the only mesenchymal cell lineage where C/EBPβ has an instructive function. Mouse models and tissue culture studies have now established a regulatory role of C/EBPβ in osteoblast and in chondrocyte differentiation. These three different cell lineages are derived from the same precursor, the mesenchymal stem cell (MSC). This review will focus on the emerging role of C/EBPβ and its different protein isoforms in various mesenchymal cell lineages and its function in adipocyte, chondrocyte and osteoblast differentiation. Moreover, the mesenchymal stem cell has attracted the attention of regenerative medicine in recent years, and the possible role of C/EBPβ in this respect will be discussed.

Contents

1. Introduction

In healthy subjects, various bone cell types concertedly regulate bone development as well as bone homeostasis in adulthood. Chondrocytes are responsible for the growth of the long bones during endochondral ossification, which occurs in the initial phase of bone development. Osteoblasts are the bone forming cells, which deposit the bone extracellular matrix. Osteoblasts eventually differentiate into osteocytes, which are located within the bone matrix and act as mechanical sensors to adjust bone formation on demand. Bone formation is kept in balance by bone resorbing osteoclasts, which degrade bone matrix and thereby mobilize calcium and matrix-embedded hormones, cytokines, and growth factors. During development, bone formation exceeds bone resorption resulting in an increase in bone mass. After this initial phase of bone formation, remodeling continues and homeostasis is maintained by continuous

Abbreviations: ATF4, activating transcription factor 4; BAT, brown adipose tissue; bZIP, basic leucine zipper; cAMP, adenosine 3′,5′-cyclic monophosphate (cyclic AMP); CBP, CREB binding protein; CD-RAP, cartilage-derived retinoic acid sensitive protein; C/EBP, CCAAT/enhancer binding protein; Ebf, early B cell factor; GR, glucocorticoid receptor; HDAC1, histone deacetylase 1; KLF, Krüppel-like factor; LAP, liver activating protein; LIP, liver inhibitory protein; MAFbx, F-box protein 32; MEF, mouse embryonic fibroblasts; MSC, mesenchymal stem cell; mRNA, messenger RNA; mTOR, mammalian target of rapamycin kinase; Myb, myeloblastosis oncogene; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; Sox9, SRY-box containing gene 9; SWI/SNF, switching/sucrose nonfermenting; uORF, upstream open reading frame.

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adjustment of bone formation and bone resorption (Zaidi, 2007; Karsenty, 2008; Karsenty et al., 2009). This delicate equilibrium requires a tight control to prevent bone loss, as observed in many age-related lytic bone diseases, such as osteoporosis, or in inflammation-induced or cancer-induced bone loss (Zaidi, 2007). The balance between bone formation and resorption is in a large part regulated by a strict control of the differentiation of osteoblasts and osteoclasts from their respective precursor cells. Whereas osteoclasts are derived from monocytic precursors as part of the hematopoietic system (Novack and Teitelbaum, 2008), osteoblasts are derived from mesenchymal stem cells, MSC (Zaidi, 2007; Karsenty, 2008; Karsenty et al., 2009).

In addition to osteoblasts, also chondrocytes, adipocytes, fibroblastoid cells, and myocytes can be derived from mesenchymal stem cells (MSC). The differentiation process of mesenchymal precursor cells into differentiated and fully functional cells is orchestrated by key transcription factors and their co-factors. Although each specific mesenchymal cell lineage has their own unique transcription factor signature, they also have several transcription factors in common. Specificity is achieved by context and adjustment of the functional collaboration between different transcription factor networks (Karsenty, 2008; Karsenty et al., 2009; Baek and Kim, 2011). C/EBPβ has emerged as a transcription factor that plays a key role in at least three of the mesenchymal cell lineages. Although first identified in adipocytes as a transcriptional activator, C/EBPβ evidently is a regulator of growth and differentiation in chondrocytes and osteoblasts (Tominaga et al., 2008; Hirata et al., 2009; Smink et al., 2009) and might function as both, repressor or activator in context dependent fashions. The diverse functions of C/EBPβ are connected to different protein isoforms and post-translational C/EBPβ modifications that define and confine the contextual C/EBPβ interactome and its functional consequences (Nerlov, 2008; Zahnow, 2009; Leutz et al., 2011).

Multi-lineage differentiation capacity of MSC is an important focus in regenerative medicine. Especially in degenerative bone diseases, which affect a large portion of the aging population, regenerative medicine is being developed as an alternative treatment. This mainly involves osteoblasts and chondrocytes, which are the most affected cell types in these bone diseases. Although myocytes and fibroid lineages (including tendon, ligament and stromal cells) are also derivates of MSCs, a possible function of C/EBPβ has not been rigorously addressed in these cell types and therefore these cell lineages will not be covered in detail in this review. We will discuss how C/EBPβ may implement both, repressor and activator functions during different stages of differentiation of mesenchymal cells. Increasing the understanding of mesenchymal differentiation mechanisms is of great importance for the development of therapeutic strategies in regenerative medicine, in particular, for cartilage or bone regeneration by directing mesenchymal stem cell differentiation. Therefore, implications of regulated C/EBPβ functions in mesenchymal cell lineages for tissue engineering will also be discussed.

2. The transcription factor CCAAT/enhancer binding protein β

The CCAAT/enhancer binding protein β (C/EBPβ) belongs to the group of basic leucine zipper (bZIP) transcription factors. The family of C/EBP bZip proteins consists of 4 highly related members (C/EBPα, β, δ, ε) that probably reflect gene quadruplications of an earlier C/EBP precursor and two distant relatives (C/EBPγ, ζ) of different origin. Single and compound C/EBPβ gene deletion studies and gene replacement experiments suggested the importance of C/EBPs in cell proliferation vs. differentiation and compensatory or redundant functions of various C/EBPs, with C/EBPα and β playing dominant roles as early as in trophectoderm differentiation (Tanaka et al., 1997; Chen et al., 2000; Jones et al., 2002; Begay et al., 2004).

C/EBPβ is downstream of several signaling cascades and appears to be highly regulated. C/EBPβ plays a role in many different physiological and pathological processes, including cell survival, apoptosis, inflammation, metabolism, and tumorigenic transformation. Moreover, C/EBPβ is a crucial regulator of differentiation in a large variety of cell types, including keratinocytes, hepatocytes, mammary epithelial cells, ovarian luteal cells, adipocytes, B cells, macrophages and osteoclasts (Ramji and Foka, 2002; Nerlov, 2007; Zahnow, 2009; Smink et al., 2009; Smink and Leutz, 2010).

C/EBPβ is encoded by a single exon mRNA, yet distinct N-terminally truncated C/EBPβ protein isoforms are generated by a process of alternative translation initiation that involves a highly conserved small upstream open reading frame (uORF). The generation of two long isoforms LAP* and LAP and the short isoform LIP depends on the uORF, which mediates signaling through mTOR and additional pathways affecting translation initiation (Fig. 1A, B) (Calkhoven et al., 2000; Wethmar et al., 2010). The rapamycin sensitive TORC1 branch

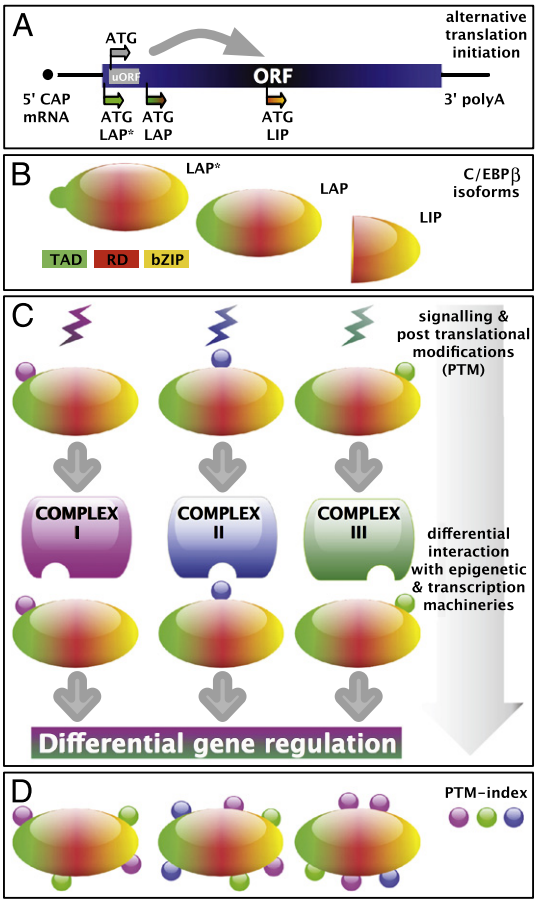

Fig. 1. C/EBPβ modifications, alternative translation initiation and indexing by post-translational modifications (PTM).(A) An upstream open reading frame (uORF, gray) in the single exon mRNA of C/EBPβ directs translation initiation to alternative start sites (gray arrow). This results in the generation of the different protein isoforms of C/EBPβ, termed LAP*, LAP, and LIP. (B) LAP*, LAP, and LIP proteins differ in their N-terminal length causing the differential presence of N-terminal transactivation (TAD, green) and regulatory domains (RD, red), but common C-terminal DNA-binding bZip domains (bZip, yellow). (C) Various signals cause different post-translational modifications (PTM; indicated by colored marbles). C/EBPβ and its PTMs specify differential recruitment and/or assembly of alternative co-factor complexes (complex I, II, III, etc.) of the epigenetic and transcriptional machinery to assist in differential gene activation. Note that for simplicity only one C/EBPβ isoform and only three alternative modifications are shown. (D) C/EBPβ is decorated with multiple PTMs (shown only for one isoform, as indicated by colored marbles) that constitute a PTM-index to determine alternative/sequential functions. Various modifications may represent functional patterns of signaling crosstalk to orchestrate co-factor recruitment during differential gene regulation. PTMs entail lysine acetylation and methylation (mono-, di-, trimethylation), arginine methylation (mono-, asymmetric or symmetric di-methylation), and/or phosphorylation (on threonine, serine, or tyrosine) to generate different PTM patterns that may direct co-factor interactions.