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Review Article

ROLE OF CORE BINDING FACTOR BETA (CBF B) IN OSTEOPOROSIS

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Abstract

Osteoblasts, osteocytes and osteoclasts are essential for the remodelling of bone. Osteogenesis and chondrogenesis synchronise to develop the skeletal system. These mechanisms are gene mediated and an imbalance in remodelling is emblematic to a larger socio economical problem globally referred to as osteoporosis. In elderly osteoporotic individuals' dependence between adipose content in bone marrow and bone density was observed. Aging skeletal stem cells has greater proclivity to evolve into adipocytes than osteoblasts and this is mediated by transcriptional factors that are orchestrated by molecular signals. Runt related transcription factor 2 (Runx2) and Osterix (Osx) are principal regulators of osteogenic differentiation lineage and PPARγ (PGC-1α) and CEBPα/β/δ are essential for adipogenesis. A co transcriptional factor beta(cbfβ) binds to Runx2 and stimulates the expression of osteoblasts through Wnt/β-catenin and Wnt10b causing higher bone mass in mice. They increase the expression of AFT4 and Osx that increase osteogenesis. Thus, suggesting cbfβ as a pivotal co transcription factor in evaluating climacteric effect in bones and prevents osteoporosis.

Keywords: *Core binding factor beta, Runt related transcriptional factor, Osterix, Osteoblast, Adipocyte.*

Introduction

A human body comprises of a total of 206 to 213 bones (1). They are comprised of chondrocytes, osteoclast and osteoblastic cells. Throughout life each bone constantly undergoes remodelling to adapt to either biomechanical forces, environmental factors or healing damaged bone. An adult human bone is made up of 80% cortical bones and 20% trabecular bone. However, Osteoporosis is one such devastating skeletal disorder that literally contours the bone porous. The cortical to trabecular bone ratio is hampered in osteoporosis. The following ratio in vertebra is 25:75, femoral head is 50:50 and distal radius is 95:5 normally. However, it hinders the elderly population so much that a trivial, low-grade fall or a bump can cause a crack in the bone, thus decreasing their quality of life and increasing their social dependence (2). The most common fractures sustained in osteoporosis are, hip, vertebra and distal radius fractures. More than a million people, above the age of 50 are targeted. In osteoporosis there is a continuous, tremendous decline in the bone matrix and faulty microarchitecture of the bone. There are a number of risk factors causing osteoporosis, one of which is gene mediated (3). Molecular and cellular transcriptional factors have a vital role to play in osteoporosis. Recently researchers have developed interest in bone marrow mesenchymal stem cell that give rise to osteoblast and chondrocytes. Runx1 and 2 transcription factors both enhance osteoblastic activity (4). However, Core binding factor beta a co transcriptional factor of RUNX is the latest contributor in osteoblast regeneration. Cbf factor is located on chromosome 16q22 and is also known as polyomavirus enhancer binding protein and SL3 enhancer factor1. It has two isoforms with cbf α and cbf β. Cbf α is encoded by three mammalian genes, Runx1, Runx2, Runx3 (5). However, Cbf β a partner of Runt related transcription factor is encoded by a single gene that does not bind directly to the DNA but forms a Runx/cbf β complex that is more stable and enhances the activity of Runx through promoters and enhancers, thus stimulating osteoblastic activity. Alternate splicing renders two isoforms of human cbf-β, they differ at their C termini and share the same 165 amino acids at their N termini (6). cbfβ acts as a cofactor of cbf transcription factor complexes and play a vital role in female fertility (7). The exact role of cbf beta is still controversial and thus in this non-systematic review we aim to find the effective role of cbf beta in bone development and Osteoporosis.

1. **Theory behind the real cause of osteoporosis**

   The origin of bone cell progeny, the Skeletal stem cells (SSCs) have the ability to contribute to the osteoblast and adipocyte progenies in the adult bone marrow. In elderly osteoporotic individuals' correlation was observed between high bone marrow fat content and low bone density, thus increasing the risk for fractures. Bone marrow is the only tissue that synchronize fat and bone in the same microenvironment. Aging SSCs has a greater proclivity to evolve into adipocytes than osteoblasts. The fate of SSC is decided by transcriptional factors that are orchestrated by molecular signals and cues. Runx2 and Osterix are principal regulators of osteogenic differentiation lineage, whereas PPARγ (PGC-1α) and CEBPα/β/δ are considered essential for adipogenesis. Increased marrow adipose tissue (MAT) leads to faulty bone formation and in turn impairs osteogenic regeneration. The bone loss in osteoporosis is accompanied by MAT accumulation. Transcriptional coactivator with a PDZ-binding domain (TAZ) is an important switch for SSC fate suggesting inhibition of MAT accumulation through TAZ which is a co activator of Runx2 which has propensity towards osteogenesis differentiation (8). Adipocyte – Osteoblast lineage in osteoporosis is as shown in fig 1.

1.1 Signalling pathways

Most prominent signalling pathways associated with osteoporosis is Osteoprotegerin (OPG)/RANK/RANKL pathway. OPG are secreted from bone marrow stromal cells, regulatory T cells and most predominantly from osteoblasts. Bone resorption is inhibited by OPG +RANKL complex, this furthermore prevents the

subsequent binding to its receptor RANK, thus insinuating osteoporosis in the absence of OPG. Detrimental action to the bone is further accentuated, when the former RANK-RANKL, induce the transcription factor such as nuclear factor kappa B(NF-kB) in osteoclasts (9). Proinflammatory cytokines are elevated in osteoporosis, as they upregulate RANKL-RANK interaction and downregulate OPG. At the bone tissue liberates the helper T cells, Th17cells that produce IL17 which mediates osteoclasts by regulating the production of RANKL. Th1, Th2 and proinflammatory cytokines like interleukin 1, interleukin 6, interleukin 23, tumour necrotic factor -α all exhibit the same mechanism of bone resorption. Tumour growth factor βalso regulates osteoblast proliferation and development, at an early stage and constraints the maturation of osteoblasts at a later stage (10).

Wingless- related integration site (Wnt) are secreted polypeptides with a carbohydrate moiety. These bind to frizzled receptor and co receptor to form a complexed signalling pathway Wnt/β-catenin. This pathway plays an established role in osteoblastogenesis by suppressing adipogenesis by inhibiting its principal regulator PPARγ- induced gene. It directly enhances the osteoblastic maturation by stimulation OPG. WNT10 B polymorphism has an impact on bone regeneration. Wnt 5a, wnt 5b support osteoblast lineage, whereas β catenin deficiency inhibits osteoblastic differentiation at an early stage and targets the mineralisation of osteoblasts. Dickkopf-1 (DKK-1) is an inhibitor of wnt/β-catenin pathway and is expressed in high quantity in osteoporosis (11). Bone morphogenetic protein-2 (BMP-2) belongs to the family of TGFβ. BMP-2 and wnt/β-catenin pathway have synergistic effect on bone formation. BMP binds to their serine threonine kinase surface receptor and promotes Runx2 (12). Noggin and gremlin are two BMP antagonists that decrease the bone mass and are most predominantly expressed in osteopenia and osteoporotic fractures (13).

2.2 Transcription factors affecting bone formation

Runt related transcription factor 2 (Runx2)

Runx is a complex DNA binding domain with 128 amino acids, with three sub groups Runx1, Runx2 and Runx3 in mammals. They have a vital role in cell proliferation and differentiation and are tumour suppressor genes as well. RUNX proteins recognize a consensus binding sequence (5′-PyGPyGGTPy-3′) in the promoter or enhancer region of their target genes. They act as either a transcriptional activator or repressor, depending on the interaction with other transcriptional modulators. Runx 1 is present in the bone marrow and is frequently mutated in leukaemia and jointly expressed in osteoblasts (14). Runx 2 enhances osteogenesis as they are present in chondrocytes, osteoblasts, all lineage cells. They are additionally present in thymus and mammary glands. Whereas Runx3 causes cell proliferation in gastric epithelium. During skeletal development in mice, Runx2 is overexpressed by mesenchymal cells, osteochondroprogenitor cells of the perichondrium and bone marrow, which eventually have the capacity to become osteoblasts. Runx2 is important in skeletal morphology as they contribute to both intramembranous and endochondral bone formation such as in the case of cleidocranial dysplasia (CCD), a genetic skeletal disorder caused due to the deficiency of Runx2 (15). Runx2 is unique in comparison to other Runx family members as its structure has a QA (Glutamine/Alanine) domain which plays a role in the transactivation of Runx. Runx2 has two isoforms, derived from two promoters, P1 and P2. Enhancement of P1 promoter accounts for the expression of the 'MASNSL' isoform (type II *Runx2* mRNA), which is prevailing in osteoblasts and P2 promoter results in expressing T cells. Runx2 devoid mice have demonstrated to down regulate osteoblast related ColIA1 promoter and co transcriptional factor Cbf beta. Shn3 controls the regulation of Runx2 and stimulates osteoblastic cells and promotes bone formation. A zinc finger protein Schnurri-3 (Shn3) and transcription factor Twist bind directly to Runx2 and inhibit its binding to DNA implementing a negative effect on osteoblasts (16). Yes associated protein (YAP) reported to repress Runx2 and thus suppress osteoblastic differentiation, whereas TAZ was identified as a Runx2 coactivator that promotes bone formation and inhibits adipogenesis. SOX9 delays the action of this master osteogenic transcription factor. Ubiquitin ligase smurf1 degrades Runx2 and

supresses the osteogenic activity thus decreasing the bone mass (17). HDAC3 &4 deactivate the binding of Runx2 to the DNA in chondrocytes (18). However, a nuclear matrix protein SATB2 enhances the activity of Runx2 for osteoblast differentiation. ATF4, a substrate of RSK2, also known as prominent growth factor regulated kinase belongs to the ATF/CREB protein family and increases osteoblast differentiation. Runx2 and AFT4 are interdependent. This protein can promote chondrocyte and osteoblast proliferation (19). The mechanism of transcriptional pathway is as shown in Fig 2.

Osterix (Osx)

Osx also called as Sp7 is a transcription factor located on chromosome 12q13.13 and belongs to Krüppel-like family. It binds to the DNA at the C-terminus with three zinc finger moiety C2H2. The N terminal is proline rich and Osx is highly expressed in osteoblast progenitor cells, specific to convert pre osteoblasts into active osteoblasts of all developing endochondral and intramembranous bones. Osx is not expressed in Runx2-deficient osteoblasts thus confirming that Osx cause downstream of Runx2. However, Runx2 regulates the Osx expression (20). They act on all type of bone cells and directly control the overexpression of the adhesion protein, integrin β3. These are required for integration of bone cells to bone matrix during bone growth phase. The activation of Wnt/β-catenin signalling induces the expression of both Osx, at the early stage of proliferation and differentiation of osteoblasts and Osteoprotegerin an osteoclast inhibitory factor preventing bone resorption. This signalling pathway therefore plays a crucial role during both bone development and remodelling. Micro RNAs (MiR-335-5p) upregulates the expression of Runx2 and Osx synergistically and increases bone growth (21). Runx2 is released early on in prechondrogenic mesenchyme cells, which express high levels of Sox9 whereas at later stages Osx is stimulated which downregulates the levels of Sox9 (22). Kaback et al indicated that over expression of Osx increases the progression of osteoblast differentiation after the maturation of chondrocytes. A nuclear factor of activated T cells (NFATc) is inefficient in osteoblast differentiation, moreover they independently can bind to Osx and this complex in conjunction regulates the osteoblast differentiation. This interaction binds to DNA and regulates the release of Col1a1 promoter in primary osteoblasts (23).

1. **Role of core binding factor -β in various diseases**

Core binding factor -β is a key regulator of HIV-1 accessory factor i.e Virion infectivity factor(vif). It is an accessory gene which is required for the viral replication of normal non permissive cells. cbf-β and EloB-EloC together form a complex that upregulates the production of E3 ubiquitin ligase required to degrade the antiviral host proteins that eventually neutralise the apolipoprotein B mRNA editing enzyme catalytic protein (APOBEC3) (24). These proteins block the replication of retroviruses. Vif + cbf-β + Cullin 5 (Cul5)-RING ubiquitin ligase (CRL5) form a complex that disrupts this integrity and can be applied as an important strategy against HIV-1 (25).

Runx has an important role in transition of epithelial phenotype to mesenchymal cbf-β forms a functional complex with the family of Runx transcription factors. Therefore, a cbf-β induction can convert epithelial to mesenchymal phenotype and vice versa when deficient in cbf-β. Thus suggesting the importance of this transcription factor in metastasis. (26)

A recent study has shown that core binding factor Cbf β2 and promotor-1-transcribed Runt-related transcription factor 1 (P1-Runx1) are required for the differentiation of CD3-CD4+CD45+ lymphoid tissue inducer (LTi) cells thus reporting Cbfβ2 to display an essential role in nasopharengyl associated lymphoid tissue and mucosal associated lymphoid tissue (27).

CBFB gene produces a chimeric protein, smooth muscle myosin heavy chain (cbf β-SMMHC) when fused to the C-terminal of cbfβ. This complex protein is encoded by MYH11and binds the Runx1 with higher affinity to form dimers and multimers through myosin tails. This higher affinity provides a rationale for the dysregulation of hematopoietic development and also reported embryonic death due to failure in haematopoiesis causing leukaemia in cbfβ knockout mice. MYH11and cbfβ fusion (cbf β-SMMHC), furthermore inhibit the functional role of Runx1 (28). This change can block the maturation of blood cells and thus implying the importance of Runx1 in the pathogenesis of leukemia. cbf β

upregulates the production of Runx1 and mutations of RUNX1 is apparently exhibited in familial platelet disorder (FDP) thus predisposed to the development of Acute myeloid leukemia(AML) and acute lymphoid leukemia (ALL). (29)
An autosomal-dominant skeletal disorder cleidocranial dysplasia (CCD) is caused by deletion or missense mutations in Runx2 and cbf β is required for the execution of the action of Runx2.Thus showing the importance of this co transcription factor in the function of Runx2 (30).

2. Role of cbf-β in bone

Cbfβ does not interact with DNA by itself, instead it increases its affinity to Runx protein causing a change in conformation at loop11 and this conformation is known as S-switch. Runx/cbfβ interaction occurs between the N terminal 141 amino acids of cbf β and the Runt domain. Cbf β binds to the Runt domain at a site away from the DNA binding interface. Dimerization with cbfβ protects RUNX proteins through its stable conformation and prevents from ubiquitin-mediated degradation. Thus, cbfβ is essential for the functional activity of RUNX proteins (31). There are studies that demonstrate cbf β null (Cbfb−/−) mice die before bone formation due to hepatic and neurological disorders. Another study by Lim et al demonstrated a direct relation of cbf beta to skeletal development especially the cortical bone with a significant reduction in bone mass in cbf β-deficient mice from cbfβ-floxed mice expressing Cre from the collagen type Iα promoter. These promoters aided in deleting the mature osteoblasts and then the osteoblastic activity was observed during bone growth and maturity in a 5week and 12week old mice. However, osteoclast surface/bone surface activity were not affected by osteoblast-specific deletion of cbf β. While the ratio of Rankl/ Opg was increased (32).

Cbf β is a crucial co transcriptional factor that enhances the roles of Runx2 protein. A study displayed cbfβ is essential role in differentiation and proliferation of MSC origin tissue, with specific RUNX proteins exhibiting its unique and nonredundant role respectively. A finding by komori et al revealed faultless development of cartilage, instead complete absence of bone formation in homozygous mutation of Runx2 was observed. Furthermore, the specific targeting of this particular transcription factor to nuclear matrix-associated regions suggested precise activation of the osteocalcin gene (BGLAP gene) (33) (34). In osterix KO mice there is also no differentiation of osteochondro progenitor cells to immature osteoblast cells. However, these mice do have Runx2 expression in the mesenchymal cells, showing that Runx2 is upstream of Osx (Nakashima et al., 2002). Therefore, while Runx2 is required for MSC to differentiate into osteoprogenitor cells, both Runx2 and Osx are needed for osteochondro progenitor cells to differentiate into immature osteoblast cells. Further differentiation into mature osteoblasts requires down regulation of Runx2. This down regulation occurs in conjunction with Msx2, a homeobox protein transcription factor.

A study by WU et al executed the study by deleting Cbfβ gene at various stages of osteoblast lineage in mice using the Prx1-Cre, Col2α1-Cre and Osx-Cre, respectively. The Cbfβ f/f Prx1-Cre, Cbfβ f/f Col2α1-Cre, and Cbfβf/f Osx-Cre mice demonstrated severe osteoporosis with accumulation of adipocytes in the marrow mimicking the fate of SSC in aged bone. Indicating interconversion of osteoblasts and adipocytes respectively by programming and reprogramming of osteoblasts of the MSC. The adipocyte gene expression increased 5 to 70 folds in Cbfβ-deficient bone marrow mesenchymal stem cells. The study thus inclines towards cbf beta being an osteogenic inducer and also governs osteoblast–adipocyte lineage through β-catenin. Thus, indicating cbf β as a therapeutic indicator for osteoporosis (35).
Studies also exhibited role of cbf beta in chondrocyte proliferation, predominantly in cartilage and cbfβ deficiency showed impaired growth plate development (36) (37). Multiple studies have revealed the importance of cbf β in bone formation and osteoblast differentiation, accordingly they support the role of Cbf β in chondrocyte proliferation and differentiation. Cbfβ intrigued its role in postnatal cartilage and bone development by chondrocyte and osteoblast proliferation mechanism, pointing them out to be the key players for the maintenance of growth plates and trabecular bone in postnatal mice (38). Similarly, another study imlpicated that Cbfbeta null

mouse exhibited significant decrease in expression of proliferating cell nuclear antigen, type II collagen, alkaline phosphatase activity and mineralisation. This decrement in bone stature was due to inhibition of expression of Runx2, AFT4, osterix and osteocalcin and thus the inclusion of cbf beta could potentially contribute in fracture healing (39). In accordance to the production of post-natal skeletogenesis, a study revealed that Cbfβ null and collagen type I α 1 knockout mice dramatically decreased the expression of AFT4 and osterix and in turn decreased bone mineralization but proposed no radical effect on teeth, cartilage and mandibles (40).

Wnt/β-catenin signalling pathway is essential in proliferation of mesenchymal cells to activate osteogenic lineage as mentioned earlier and prevents adipogenesis by inhibiting PPARγ (PGC-1α) and CEBPα/β/δ. Wnt10b is the chief ligand , induced by cbf β causing higher bone mass in mice. Lopez-Camacho et al put forth that cbf beta is present in the centre of the midbody region and plays a crucial role in cortical bone formation (41). As mentioned earlier tumour necrotic factor β (TNF β) and TGF β plays a significant role in skeletal development by stimulating Runx2/osterix transcription factors and the knockout of these receptors in Osterix-Cre mice, pointed defects in bones and teeth due to suppressed proliferation and differentiation in pre-osteoblasts and pre-odontoblasts (42). Cbf β reduced drastically in aged mice and the switch of osteoblastogenesis to adipogenesis is the major contributor to osteoporosis.

Conclusion and future perspective

Taken together all these findings we are elucidated that cbf β is a major co- transcriptional factor for osteogenic differentiation and is essential in all the stages of skeletal development in embryonic as well as post-natal. They exhibit expression of transcriptional factors like Runx2, AFT4 and osterix which induce osteogenesis and inhibit osteoclast expression. Cbf β has the capacity to inhibit adipogenesis in bone marrow and governs the osteoblast- adipocyte lineage. They induce osteogenesis by stimulating wnt/ β-catenin and wnt10. Thus, helps in mineralisation and protect microarchitecture of bone and eventually prevents osteoporosis. However, these studies were conducted on animals and implications of cbf β on humans is still being researched. Majority of the treatment modalities either affect the bone matrix or the osteology independently. However, a scrupulous research of cbf β conducted on human skeleton can contribute to facts beyond horizon in treating and abolishing osteoporosis which is emblematic of a larger global problem.

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Figure 1. Adipocyte – Osteoblast lineage in osteoporosis

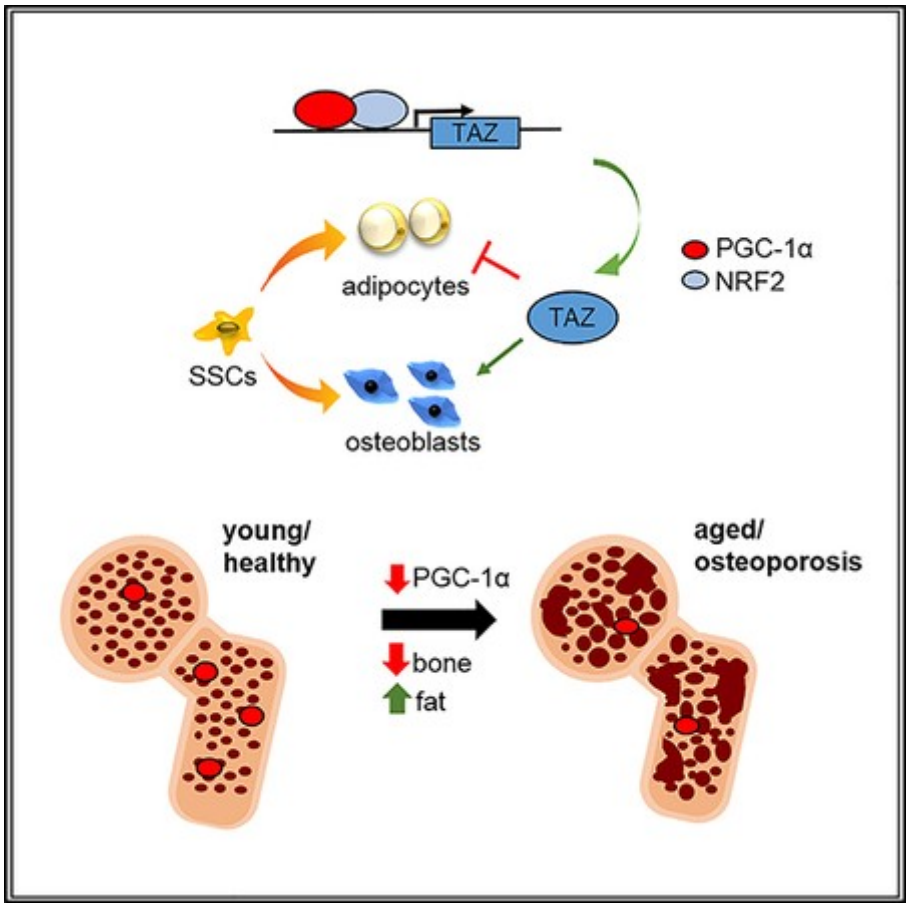

Figure 2. Signalling pathways and transcriptional factors for the differentiation of osteoblasts