Bone 50 (2012) 189–199

Contents lists available at SciVerse ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Altered plasma membrane dynamics of bone morphogenetic protein receptor type Ia in a low bone mass mouse model

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ARTICLE INFO

Article history:
Received 22 July 2011
Revised 26 September 2011
Accepted 10 October 2011
Available online 22 October 2011

Edited by: David Burr

Keywords:
Image cross correlation spectroscopy
Caveolae
Caveolin
Osteoblast
Mineralization
Family image correlation spectroscopy

ABSTRACT

Bone morphogenetic proteins (BMPs) are growth factors that initiate differentiation of bone marrow stromal cells to osteoblasts and adipocytes, yet the mechanism that decides which lineage the cell will follow is unknown. BMP2 is linked to the development of osteoporosis and variants of *BMP2* gene have been reported to increase the development of osteoporosis. Intracellular signaling is transduced by BMP receptors (BMPRs) of type I and type II that are serine/threonine kinase receptors. The BMP type I a receptor (BMPRIa) is linked to osteogenesis and bone mineral density (BMD). BMPRs are localized to caveolae enriched with Caveolin1 alpha/beta and Caveolin beta isoforms to facilitate signaling. BMP2 binding to caveolae was recently found to be crucial for the initiation of the Smad signaling pathway. Here we determined the role of BMP receptor localization within caveolae isoforms and aggregation of caveolae as well as BMPRIa in bone marrow stromal cells (BMSCs) on bone mineral density using the B6.C3H-6T as a model system. The B6.C3H-6T is a congenic mouse with decreased bone mineral density (BMD) with increased marrow adipocytes and decreased osteoprogenitor proliferation. C57BL/6J mice served as controls since only a segment of Chr6 from the C3H/HeJ mouse was backcrossed to a C57BL/6J background. Family of image correlation spectroscopy was used to analyze receptor cluster density and co-localization of BMPRIa and caveolae. It was previously shown that BMP2 stimulation results in an aggregation of caveolae and BMPRIa. Additionally, BMSCs isolated from the B6.C3H-6T mice showed a dispersion of caveolae domains compared to C57BL/6J. The aggregation of BMPRIa that is necessary for signaling to occur was inhibited in BMSCs isolated from B6.C3H-6T. Additionally, we analyzed the co-localization of BMPRIa with caveolin-1 isoforms. There was increased percentage of BMPRIa co-localization with caveolae compared to C57BL/6J. BMP2 stimulation had no effect on the colocalization of BMPRIa with caveolin-1. Disrupting caveolae initiated Smad signaling in the isolated BMSCs from B6.C3H-6T. These data suggest that in congenic 6T mice BMP receptors aggregation is inhibited causing an inhibition of signaling and reduced bone mass.

1. Introduction

Osteoporosis is a bone disease affecting an estimated 10 million Americans. It is characterized by a "compromised bone strength predisposing to an increased risk of fracture" [1,2]. Factors influencing osteoporosis and associated fractures are bone strength, rate of bone turnover, structural integrity of the bone, and the largest risk factor—bone mineral density (BMD) [1,2]. Directing these osteoporotic factors are two cell populations: osteoblasts and osteoclasts. Growth factors such as BMP2 (bone morphogenetic protein 2) and IGF-I (insulin-like growth factor 1) are crucial for osteoblast differentiation. BMP2 is needed for early osteoblast lineage commitment, while IGF-I is necessary for late osteoblast differentiation [3]. Since IGF-I serum levels are decreased in osteoporotic patients and linked to low BMD [4–6], a congenic mouse model with low serum IGF-I was created. The B6.C3H-6T mouse carrying C3H alleles on mouse Chr 6 was made by backcrossing the C3H/HeJ mouse with C57BL/6J mice for ten generations. Previously Chromosome 6 (Chr6) was found to contain the strongest QTL for serum IGF-I between the two strains. The C57BL/6J mice were chosen as a control since only a segment of Chr6 from the C3H/HeJ mouse was backcrossed to a C57BL/6J background [2,7,8]. In comparison with (C57BL/6J) femurs, B6.C3H-6T has low serum IGF-I, reduced volumetric BMD, smaller periosteal circumference, decreased trabecular bone volume fraction of the distal femur, decreased osteoblast progenitor cells, as well as increased marrow adipocytes. The

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doi:10.1016/j.bone.2011.10.016

age-accelerated phenotype of this mouse at 16 weeks was remarkably similar to B6 mice at 24 months of age [2,7–9].

BMP2 drives early osteoblast differentiation as well as adipocyte differentiation yet the mechanisms resulting in differentiation of one lineage over the other are unknown [10–14]. BMP2 has been linked to the development of osteoporosis although recent studies have not resolved the genetic link [15,16]. Analysis of a case-control study of 705 individuals with osteoporosis showed *BMP2* as a candidate gene involved in regulating the initiation and/or progression of osteoporosis and variants of *BMP2* can almost triple the probability of developing osteoporosis [17]. Furthermore, an independent replication study of two groups of postmenopausal Danish women found comparable results, with a higher incidence of the *BMP2* variants in the affected women compared to the controls [17].

BMP2 signaling occurs through serine/threonine kinase receptors, BMP type I and BMP type II receptors. Crucial to BMP2 signaling is the BMP receptor (BMPR) aggregation and co-localization to the distinct plasma membrane domain, caveolae. Caveolae are large, flask-shaped invaginations enriched with cholesterol and Caveolin (Caveolin-1, -2, and -3). Caveolin-1 (Cav-1) is found in two isoforms, alpha and beta. Two caveolae populations have been observed, one enriched in isoforms of Cav1 alpha and beta (Cav1 alpha/beta) and the second enriched in only Cav1 beta [18–20]. BMPRIa is co-localized and activated within caveolae [21,22]. For the initiation of the signaling pathway BMP2 binds with high affinity to BMPRIa that is localized in caveolae compared to other membrane domains; this leads to BMPRIa aggregation [21,23]. BMPRIa is then activated by BMPRII and initiates downstream signaling pathways. The most studied is the Smad pathway. Aggregation of BMPRIa is needed for BMP2 signaling to occur [23].

BMP2 is a significant factor for osteoblast and adipocyte differentiation [12]. Its signaling is dependent upon BMPRIa aggregation; however the role of BMPRIa aggregation on low BMD and possibly osteoporosis is unknown. Therefore the B6.C3H-6T mouse model provides a model system with decreased BMD and an osteoporotic phenotype. Using the family image correlation spectroscopy (FICS) in this manuscript we showed that BMP2 stimulation failed to induce an aggregation for BMPRIa; however it induced a dispersion of caveolae in BMSCs isolated from the B6.C3H-6T. Further there was increased co-localization of BMPRIa with caveolae at the cell surface of BMSCs isolated from the B6.C3H-6T mice compared to the C57BL/6J (Summarized in Fig. 1). The loss of BMPRIa aggregation and increased localization with caveolae resulted in decreased BMP2-induced Smad signaling for the BMSCs isolated from the B6.C3H-6T mice. Disruption of caveolae was able to induce Smad signaling in BMSCs isolated from B6.C3H-6T. Mineralization, a downstream effect from BMP2 signaling [24], was not significantly increased in the BMSCs isolated from the B6.C3H-6T mice, although BMP2 did significantly increased mineralization of the BMSCs isolated from the C57BL/6J. Our results demonstrate the shuttling and localization of BMPRIa at the cell surface facilitate BMP2 induced Smad signaling. The lack of BMPRIa shuttling between caveolae populations or aggregation of BMPRIa results in decreased Smad signaling and mineralization. For the first time a regulatory mechanism of BMPRIa signaling at the plasma membrane of BMSCs is linked to low BMD and an age related osteoporosis congenic mouse model.

2. Materials and methods

2.1. Materials

Recombinant BMP2 was obtained from R&D Systems (Minneapolis, MN). The polyclonal goat anti-sera against the BMP receptor BMPRIa, the Alexa 546 red X conjugated donkey anti-goat antibody and Alexa 488 goat anti mouse antibody, mouse antisera against Caveolin alpha and alpha/beta, and siRNA against Cav1 were from Santa Cruz (Santa Cruz, CA). Mice strains C57BL/6J and 6T were both housed at The Jackson Laboratory and the University of Delaware and protocols were approved by the appropriate Institutional Animal Care and Use Committee (IACUC).

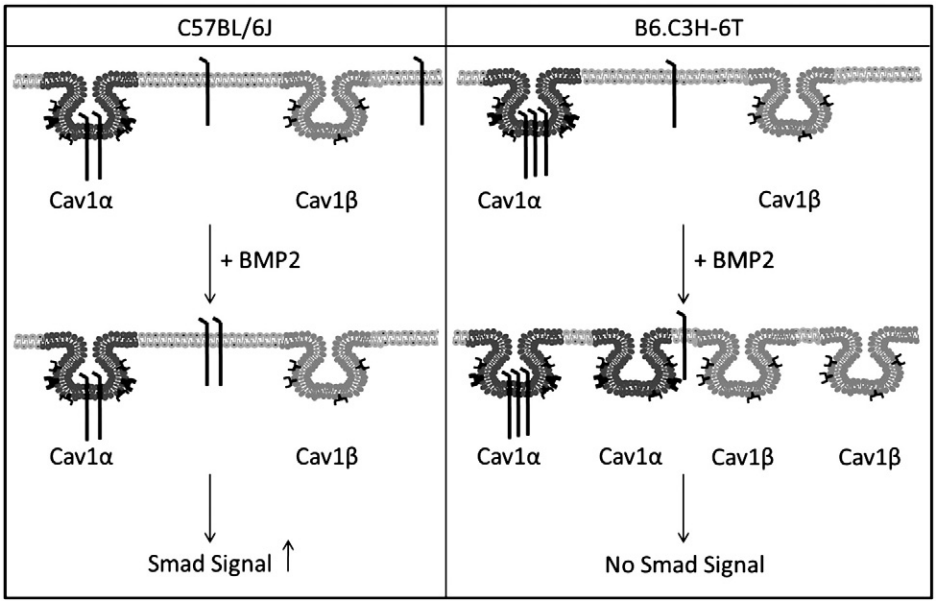

Fig. 1. Summary of BMP2-induced BMPRIa and caveolae dynamics. The summary of the plasma membrane dynamics and signaling in response to BMP2 for the BMSCs isolated from the C57BL/6J (left panel) and B6.C3H-6T (right panel). For the C57BL/6J model, BMPRIa is localized to caveolae enriched with Cav1 α and Cav1 β, while some receptors are not localized to these domains (located at the cell surface). The addition of BMP2 decreased CD for BMPRIa. No change was observed for caveolae. The amount of BMPRIa localized to caveolae enriched with Cav1 α or Cav1 β did not change in the C57BL/6J. These dynamics led to increased Smad signaling. For the B6.C3H-6T, BMPRIa is localized predominately to caveolae enriched with Cav1 alpha/beta. There is a small percentage that was not localized to caveolae. BMP2 stimulation led to increased CD for caveolae resulting in increased caveolae. There was no change with the CD for BMPRIa. There was no change in the co-localization of BMPRIa with caveolae. This led to no Smad signaling.