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Identification of Commercially Available Antibodies that Block Ligand Binding by BMPR2

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Identification of commercially available antibodies that block ligand-binding by BMPR2

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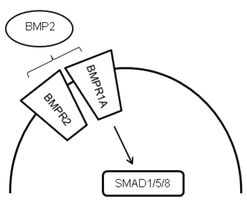
Osteoporosis, a disease of low bone mineral density, affects 10 million Americans and is a significant health problem and a considerable socioeconomic burden. Current treatments for osteoporosis have significant limitations, necessitating identifying new treatment strategies via building a better understanding of the endogenous mechanisms regulating bone mass. Recent research demonstrated that removal of the BMP type 2 receptor, BMPR2, in skeletal progenitor cells of Bmpr2-cKO mice leads to reduced age-related bone loss due to a sustained elevation in bone formation rate. The molecular mechanism underlying this phenotype is being pursued in other work. In the present study, we sought to advance the translational potential of the genetic model by identifying antibodies that neutralize the ligand-binding function of the BMPR2 extracellular domain (BMPR2-ECD). Using a modified, cell-free immunoprecipitation assay quantified by ELISA, we examined the neutralizing ability of 3F6, which is a mouse monoclonal antibody raised against the ligand-binding region of BMPR2, and found a dose-dependent inhibition of BMPR2-ECD ligand-binding. We then evaluated 1F12, which is another mouse monoclonal antibody raised against the ligand-binding region of BMPR2, and found that this antibody is also capable of neutralizing the ligand binding function of BMPR2-ECD. We extended the results by examining the ability of 3F6 to block endogenous BMPR2 function in the BMP-responsive HEK293T (human kidney embryonic 293 translation) cell line. Consistent with the results of our cell-free system, pre-treatment of HEK293T cells with 3F6 leads to reduced sensitivity to in response to BMP pathway activation by BMP2. These results provide proof-of-concept data for future studies evaluating inhibition of BMPR2 function in vivo as a means to reduce age-related bone loss.



Central Research Question:

Can ligand-binding ability of the BMPR2 extracellular domain (BMPR2-ECD) be blocked using commercially available antibodies?

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Laura Schoerning, Aaron Hudnall, Jordan Newby, Julia Hum, PhD, Warren Lawless

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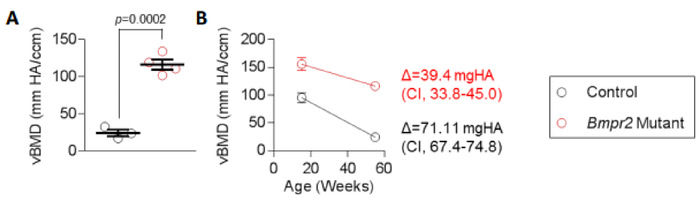

Figure. 1 High peak bone mass and reduced age-related decline in bone mass of female BMPR2 mutant mice. AB: Trabecular volumetric bone mineral density (vBMD) quantified by micro-CT in tibiae of females control and BMPR2 mutant mice at 55 weeks of age (A) and change between 15 and 55 weeks of age (B).

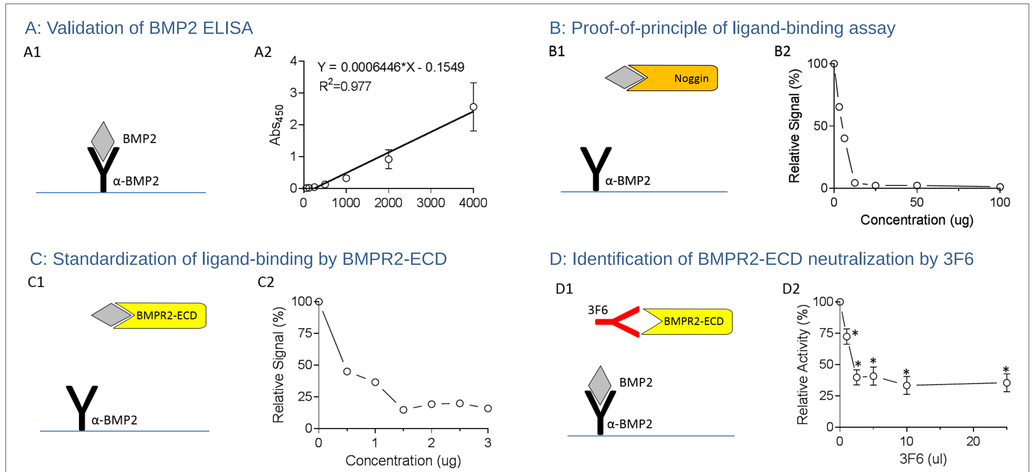

Figure. 2 Antibodies 3F6 and 1F12 inhibit BMPR2-ECD ligand-binding in a modified, cell-free immunoprecipitation assay quantified by ELISA. A: α-BMP2, bound to well, binds BMP2 (A1), so that quantitative ELISA showed dose-dependent increases in absorbance (A2). B: Noggin bound BMP2 (B1), resulting in a dose-dependent decrease in signal (B2). C: BMPR2-ECD also bound BMP2 (C1), resulting in a dose-dependent decrease in signal (C2). D: 3F6 blocked BMPR2-ECD (D1), resulting in a dose-dependent decrease in BMPR2-ECD activity (D2). E: Second lot of 3F6 verified previous findings (D1&2). F: 1F12 also bound BMPR2-ECD, resulting in a decrease in BMPR2-ECD activity. *indicates p<0.05 by unpaired t test versus control.

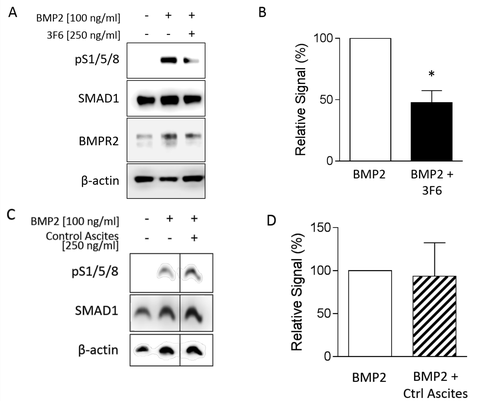

Figure. 3 Antibody 3F6 inhibits BMP2-induced pathway activation in HEK293T cells. HEK293T cells were treated with BMP2 for four hours +/- thirty minute pre-treatment with 3F6 or control ascites. A-B: BMP pathway activation level, indicated by phoshorylation of S1/5/8 (pS1/5/8), is reduced in the presence of 3F6. C-D: Ascites control does not inhibit BMP2-induced pathway activation. Data are expressed as mean+/- SEM relative to BMP2 alone. * indicates p<0.05 by unpaired t test versus BMP2 alone.

Conclusions:

Our results provide proof-of-concept data that BMPR2 function can be blocked using a neutralizing antibody approach. Specifically, the mouse monoclonal antibodies 3F6 and 1F12 block BMPR2-ECD ligand-binding in a cell-free immunoprecipitation assay. 3F6 was additionally shown to block BMP2-induced pathway activation in HEK293T cells. An incidental finding from our study is that BMPR2 is the major receptor for BMP2 in HEK293T cells.

Current and Future Directions:

1) Examination of 3F6 and 1F12 neutralizing activity of BMPR2 function in skeletal cells in vitro.
2) Examination of systemic delivery of 3F6 and/or 1F12 in vivo as a means of regulating postnatal bone mass.

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