Exploiting the key binding determinants of the BMP2/BMPR1A interaction: Using mutagenesis to probe a key binding hotspot of BMP2/BMPR1A.

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Abstract: In clinical settings, the need for a more potent but physically smaller BMP2 dosage in surgical implantation applications is necessary due to adverse immune events. Implantation failure is often the result of the large physical doses of BMP2 that are necessary to elicit a biological effect. These large doses induce adverse immune events which result in clearance of the implanted BMP2, antibody production, pain and swelling at the site of implantation. These adverse immune effects might be reduced with smaller physical doses of implanted BMP2. However, in order to administer physically smaller doses of BMP2, engineering a more potent molecule would be necessary. One key binding hotspot of the BMP2 molecule, the Leucine 51 position, may be a key position to scan for high binding efficacy. This residue position is highly conserved among analogous members of the TGF-Beta superfamily as well as related TGF-Beta superfamily molecules across metazoan phyla. Thus, examining the Leucine 51 position via scanning mutagenesis may yield a mutant that has the ability to bind the cognate BMP type 1 receptors with greater affinity, hence may prolong the stability of the signaling complex effecting transduction of greater signals. We hypothesize that a subset of mutant BMP2 molecules may be able to have greater efficacy in eliciting bone formation than wild type BMP2. Further, we wish to express such a subset of molecules in rice as it is able to provide a hypoallergenic expression platform as well as provide all the essential post-translational modifications of a biologically functional BMP2. Our objectives are 1) To create a mutant collection of BMP2 molecules, 2) To screen through candidate mutants to find those which have greater biological efficacy than the wild type, and 3) Express such mutants using the rice 'protein farming' platform for recombinants.

Material and Methods: *In silico analysis*. We used the crystal structure of the BMP2/BMPR1A ligand-receptor complex (PDB ID: 1REW), mutated the Leucine 51 position to the other amino acids using Sybil and ran a minimization using AMBER. Minimized runs were assessed using Python Molecular Viewer (PMV, The Scripps Institute) and hydrogen bonds between ligand and receptor were examined for *bona fide* hydrogen bonding using criteria by Xu, D et al. (1997). *Expression of mutant proteins*: We expressed the mutant proteins using a protocol formerly used for expression of wild type BMP2 by Long, S et al (2006).

Results: *In silico analysis*: We found that amino acid residues with bulkier side chains were unable to form bona fide hydrogen bonds within the ligand-receptor interface.

Table: Leucine 51 substitutions that are compatible within the BMP2/BMPR1A receptor interface. The residues listed are those hypothetical mutants which were found to be sterically compatible using AMBER minimization runs and assessed using hydrogen bonding criteria from Xu, D et al. (1997).

Leucine 51 mutants that are sterically compatible with the BMP2/BMPR1A binding interface via AMBER minimization runs
L51G L51A L51V L51I L51D L51E L51T L51S L51F L51N L51Q

Expression of mutant proteins: We were able to express mutant BMP2 protein as per the method of Long, S et al. (2006).

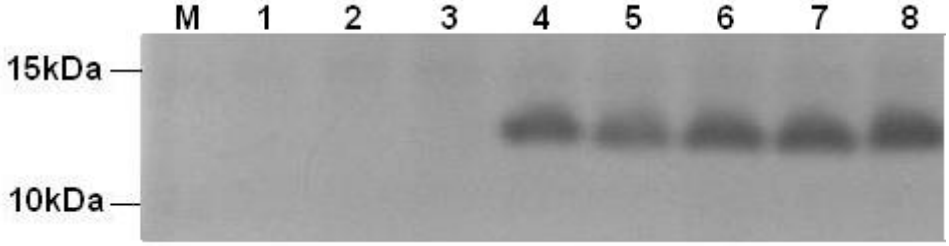

Figure: BMP2 Leucine 51 mutants. Mutants were expressed following the methods of Long et al. 2006. M- Markers, 1-Rosetta Gami, 2-pET, 3-pET reverse, 4-wt BMP2, 5-L51V BMP2, 6-L51A BMP2, 7-L51G BMP2, 8-L51P BMP2.

Discussion: The Leucine 51 position of BMP2 is a highly conserved residue position across the TGF-Beta superfamily as well as across this protein family in all metazoan phyla. Substitutions within Cnidarians are only limited to the other branched chain amino acids, Isoleucine and Valine, thus suggesting that this residue is highly conserved for ligand-receptor binding function. Whether the Leucine 51 at this position is resistant to evolutionary drift may be suggested by its high conservation but this has not yet been shown. As this residue is situated within a highly mobile domain, which only becomes fixed and immobile upon binding the BMP2 receptor, the contributions of the other adjacent residues within this mobile domain may be underappreciated with this approach. Additionally, if a mutant were to demonstrate higher biological activity than wild type BMP2, this may not hold true for a molecule that is fully glycosylated, as our model system in *E. coli* is not able to provide the complete post-translational modifications that may be present in our ultimate objective of expressing the candidate set of mutants in rice. Rice is able to provide post-translational modifications that may be close to human post-translational modifications. However, the glycosylation may induce intramolecular steric effects and a mutant expressed in *E. coli* may not be equivalent in structure or function to one expressed in rice, even with an identical primary sequence. In the next step in this study, a conventional assay, the alkaline phosphatase assay, used by Keller, S et al (2004), will be used to screen for biological activity of the mutant BMP2 collection. From this step, mutants displaying higher biological activity than wild type BMP2 will be expressed in transgenic rice and further assayed to confirm higher biological activity than that of wild type BMP2.

References:

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