BSc 489 Independent Studies (3 credit hours)

Fall 2012

Meng Sun

Alteration of BMP signaling using small peptide inhibitors

Instructor: Dr. Michael Hanna, Science 261 & 256

Purpose: Multiple HSP-associated proteins have been shown to be inhibitors of mammalian BMP signaling [Tsang et al., 2009]. Therefore experiments will be conducted to test the hypothesis that maspardin is involved in BMP-signaling events, more specifically as an additional BMP inhibitor. Binding of BMP to the type II BMP receptor, a serine/threonine kinase receptor, phosphorylates type I BMP receptors. This phosphorylation event further activates intracellular signaling molecules Smad 1, 5 and 8. These Smads bind Smad 4 and the entire Smad complex enters the nucleus to drive gene transcription, notably the target Id gene family [Wang et al., 2007]. Overexpression of NIPA1 results in diminished pSmad 1/5 response to the ligand BMP4. Importantly, knockdown of NIPA1, spastin and spartin each results in increased pSmad 1/5 expression [Tsang et al., 2009]. Therefore maspardin contribution to pSmad 1/5 activity will be sought. Investigation into maspardin overexpression and deletion on BMP signaling will begin with phosphorylative effects on Smad 1/5. GFP-maspardin will be overexpressed in Cos-7 cells and compared to control. In addition GFP-maspardin will be overexpressed in MEFs, thus direct comparison to maspardin deficient MEFs will be possible. If transfection efficiency is low, depletion of maspardin in Cos-7 cells will be warranted. Levels of phosphorlyated Smad1/5 (Cell Signaling Inc) will be examined by western blot analysis and compared to total Smad (Cell Signaling Inc) levels following BMP4 ligand (20ng/ml; R&D Systems) stimulation. Similar experiments will be conducted in primary neuronal cultures from wildtype and knockout mice. If variable or minimal effects on BMP signaling are found, focus will change to downstream BMP transcriptional responses. In addition other downstream activated and or/inhibitors will examined.

Objective:

1. Effectively culture primary neurons from P0 pups
2. Effectively culture mouse embryonic fibroblasts (MEFs) from E14 pups.
3. Effectively culture Cos-7 cells.
4. Transfect GFP-maspardin and determine transfection efficiency
5. Examine pSmad 1/5 differences
6. Write a publication worth report on any findings
7. Present findings at Pathways Symposium and at subsequent scientific meetings.
8. Supervision of at least one undergraduate researcher when possible

Grading Scheme:

Attendance and Participation 40 points
Culturing  20 points
Transfections  15 points
Western  10 points
Presentation  15 points

Report:
Your purpose is to aid in any presentation findings deem vital.

Requirements:
Meng will be working under the direct supervision of Ms. Lauren Bailey and will report to me secondarily. Meng will be required to attend all lab meetings and attempt at least 10 hours of laboratory work per week.


