BSc 597: Special Topics- Maspardin effects on BMP Signaling Molecules (3 credit hours)

Summer II 2012

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Maspardin effects on BMP Signaling Molecules

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 phorphorylation 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 phosphylative 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 write an introduction about hereditary spastic paraplegias and specifically Mast syndrome based on previous research. Discuss what is known about BMP signaling and its proposed function. Write a methods section of what you did. Discuss your results. Be sure the statistical analysis is done properly. Discuss what you find and how it may participate in the behavior function of the SPG21 knockout mice. Propose further experiments.

1. Soderblom C, Blackstone C (2006) Traffic accidents: molecular genetic insights into the pathogenesis of the hereditary spastic paraplegias. Pharmacol Ther 109:42-56

2. Züchner S (2007) The genetics of hereditary spastic paraplegia and implications for drug therapy. Expert Opin Pharmacother 8:1433-1439

3. Simpson MA, Cross H, Proukakis C, Pryde A, Hershberger R, Chatonnet A, Patton MA, Crosby AH (2003) Maspardin is mutated in Mast syndrome, a complicated form of hereditary spastic paraplegia associated with dementia. Am J Hum Genet 73:1147-1156

4. Zeitlmann L, Sirim P, Kremmer E, Kolanus W (2001) Cloning of ACP33 as a novel intracellular ligand of CD4. J Biol Chem 276:9123-9132

5. Zhu P-P, Patterson A, Lavoie B, Stadler J, Shoeb M, Patel R, Blackstone C (2003) Cellular localization, oligomerization, and membrane association of the hereditary spastic paraplegia 3A (SPG3A) protein atlastin. J Biol Chem 278:49063-49071

6. Hanna MC, Blackstone C. "Interaction of the SPG21 protein ACP33/maspardin with the aldehyde dehydrogenase ALDH16A1." *Neurogenetics*, **2009,** 10(3), 217-28.

7. Soderblom, C., Stadler, J., Jupille, H., Blackstone, C., Shupliakov, O., and Hanna, M.C. **2010** Targeted disruption of the Mast syndrome gene *SPG21* in mice impairs hind limb function and alters axon branching in cultured cortical neurons. (*Neurogenetics*-accepted 6/30/10).

8. Wang, X., Shaw, W. R., Tsang, H. T., Reid, E. & O'Kane, C. J. Drosophila spichthyin inhibits BMP signaling and regulates synaptic growth and axonal microtubules. *Nature Neurosci.* **2007** 10, 177–185.
9. Blackstone C, O'Kane CJ, Reid E. Hereditary spastic paraplegias: membrane traffic and the motor pathway. *Nat Rev Neurosci*. **2011** Jan;12(1):31-42. Review.

10. Tsang, H. T. Edwards TL, Wang X, Connell JW, Davies RJ, Durrington HJ, O'Kane CJ, Luzio JP, Reid E. "The hereditary spastic paraplegia proteins NIPA1, spastin and spartin are inhibitors of mammalian BMP signalling." *Hum. Mol. Genet.* **2009***,* 18, 3805–3821.