Annotated bibliography

Final project: Effect of cis-regulatory element CNE14 on tissue-specific GLI3 expression in developing mammals

1. J. Briscoe, "The mechanisms of Hedgehog signalling and its roles in development and disease," *Nature*, vol. 14, no. July, pp. 416–429, 2013.

Sonic Hedgehog is a secreted protein that regulates pattern formation in limbs and CNS of mammals. GLI3 is important for formation of limbs and CNS in mammals, and is regulated by SHH. GLI3 can be found on two forms: truncated protein with a transcriptional repression function, due to the proteolytic removal of the C terminal activating domain, or a full-length protein with activating function. This processing is regulated by the signalling molecule Sonic Hedgehod (Shh). Shh signalling prevents to processing and leads to full-length GLI3 with activating function. There is evidence that a gradient of Shh throughout tissues induces a corresponding GLI3 activity gradient. Similar evidence is found for the temporal Shh expression. The correct spatiotemporal GLI3 concentrations and balance between repressor and activator forms determines the gene expression and thereby the patterning process.

Defects in this pathway leads to various phenotypes including polydactyly and severe congenital abnormalities.

Hh signalling is implicated in maintaining stem cell populations of many tissues and regeneration of organs after injury. As with many mechanisms involved in stem cell regulation, dysregulation of the pathway has also been associated with various cancers.

2. S. Anwar, R. Minhas, S. Ali, N. Lambert, Y. Kawakami, G. Elgar, S. S. Azam, and A. A. Abbasi, "Identification and functional characterization of novel transcriptional enhancers involved in regulating human GLI3 expression during early development," *Dev. Growth Differ.*, no. 57, pp. 570–580, 2015

GLI3 is a transcription factor important for mediating Shh signalling in development of CNS and limbs. Recently, two novel CNEs (conserved non-coding elements) were discovered in the intronic regions of the GLI3 gene. The functions of CNE14 was assayed in zebrafish and a mouse fibroblast

cell line. CNE14 induces reporter gene expression in the developing pectoral fin of zebrafish, suggesting an important role of this cis-regulatory element for GLI3 expression in limb development. The mouse cell line showed reporter gene expression driven by CNE14, indicating that CNE14 is active in mammals.

The authors conducted comparative genomic analyses and identified a range of putative binding sites in CNE14 for known transcription factors such as GATA1.

3. F. Demurger, A. Ichkou, S. Mougou-zerelli, M. Le Merrer, A. Delezoide, L. Faivre, C. Baumann, S. Nampoothiri, L. Pasquier, B. Isidor, D. Lacombe, M. Delrue, S. Mercier, N. Philip, E. Schaefer, M. Holder, A. Krause, F. Laffargue, M. Sinico, D. Amram, A. Liquier, M. Rossi, J. Amiel, F. Giuliano, G. Andre, O. Boute, A. Dieux-coeslier, M. Jacquemont, A. Afenjar, L. Van Maldergem, A. Munnich, O. Raoul, S. Romana, L. Devisme, and D. Genevie, "New insights into genotype – phenotype correlation for GLI3 mutations," *Eur. J. Hum. Genet.*, vol. 33, no. October 2013, pp. 92–102, 2015.

This paper investigates a number of different GLI3 mutations and how they correlate with phenotypes. I used the information to obtain background information about why GLI3 regulation is important, and how it is related to human disease. The paper focuses on Greig Cephalopolydactyly syndrome (GCPS) and Pallister-Hall syndrome. Symptoms of GCPS includes polydactyly, a phenotype that has been specifically correlated with GLI3 mutations, as GLI3 is the primary regulator of pattern formation.

4. B. Hall, A. Limaye, and A. Kulkarni, "Generation of Gene Knockout Mice," *Curr Protoc Cell Biol*, pp. 1–23, 2009.

This paper covers the process of creating knockout mice through different procedures, including the use of replacement vectors. The processes of positive and negative selection are mainly derived from this article.

5. E. Mcglinn and J. H. Mansfield, "Detection of Gene Expression in Mouse Embryos and Tissue Sections," in *Vertebrate Embryogenesis*, 2011, pp. 259–292.

This textbook section explains the procedures used for visualization – extraction of embryos, in situ hybridization and histochemical detection of mRNA.