Stem Cells and Tissue Regeneration

Program/Abstract # 364
Two different regenerations accomplish limb regeneration cooperatively
Satoh Akiraa, Susan V. Bryantb, David M. Gardinerb
aRCIS, Okayama Univ., Okayama, Japan
bDept. of Dev. & Cell Bio., UCI, CA, USA
We have developed a model called accessory limb model, which has a mystery. If an accessory limb is induced in a middle of stylopod, an induced limb possesses autopod and zeugopod, no stylopod. In theory, the induced limb should have had half of stylopod. However, none of the accessory limb possesses such a half of stylopod. If a stylopod was injured enough to damage a bone, an induced accessory limb exhibits half of stylopod. We found that this stylopod regeneration was AEC (apical epithelial cap) independent even though distal structures (autopod and zeugopod) were AEC dependent. These AEC dependent and independent mechanisms coordinate to achieve limb regeneration. This coordinated regeneration system also works in regular limb regeneration (amputated limb). Furthermore, we also found how to create the distal structure in AEC free environment. Fgf-signaling can trigger intercalary responses as reported in Xenopus. When we provide the distal in an amputated limb without AEC, no intercalation takes place, resulting in no intermediate structure. However, when we activated fgf-signaling with the same condition, intermediate structures were induced. Those findings solve the mystery of the accessory limb model and propose an entire new model for limb regeneration study. Those findings warn many of the running molecular analysis because all of them have not paid attention to an existence of those two regenerations. For a finer analysis of the epimorphic regeneration, we think that it is necessary to distinguish two types of regeneration.

doi:10.1016/j.ydbio.2010.05.373

Program/Abstract # 365
Diabetes mellitus impairs limb regeneration as analyzed in adult zebrafish
Michael P. Sarrasa, Ansgar Olsena,b, Robert Intinea,b
aChicago Med. School, Rosalind Franklin Univ. North Chicago, IL, USA
bScholl College of Podiatric Medicine, Rosalind Franklin Univ. North Chicago, IL, USA
The zebrafish (Danio rerio) has been established as a model organism for the study of developmental processes, human disease and tissue regeneration. We report that wound healing/limb regeneration is severely impaired in a newly developed diabetes model in adult Zebrafish. Intraperitoneal streptozotocin injection of adult, wild type zebrafish resulted in a sustained hyperglycemic state as determined by elevated fasting glucose values and increased glycated serum protein. Consistent with this, serum insulin levels were also found to be decreased and pancreas immunohistochemistry revealed a decreased amount of insulin signal in hyperglycemic fish and an increase in apoptosis in Islets as monitored by TUNEL analysis. In addition, the diabetic complications of retinal thinning and glomerular basement membrane thickening (early signs of retinopathy and nephropathy) resulting from the hyperglycemic state were evident in streptozotocin injected fish at three weeks. Most significantly, wound healing and limb regeneration, as assayed by caudal fin amputation, are severely impaired in adult diabetic zebrafish and this correlates with a decrease in cell proliferation in the regenerate blastema and epithelium. A broad range of control experiments indicated no non-specific effects of streptozotocin. This experimental system provides a broad spectrum of genetic and molecular approaches to study the regenerative processes in the diabetic background.

doi:10.1016/j.ydbio.2010.05.374

Program/Abstract # 366
Genetic/epigenetic controls of gene expression during limb regeneration in Xenopus
Koji Tamura, Tamae Maruoka, Akio Aruga, Takuya Higashidate, Hitoshi Yokoyama
Graduate School of Life Sciences, Tohoku University, Sendai 980-8578, Japan
Limb regeneration in amphibians is a representative process of epimorphic regeneration, in which a mass of undifferentiated cells referred to as the “blastema cells” proliferate, re-differentiate, and especially re-pattern to restore the lost part. We investigated cis-regulatory regions of Prx1 and Shh genes involved in this process. We have generated a transgenic Xenopus line that expresses GFP under the control of mouse Prx1 limb enhancer, which directs reporter gene expression in limb bud mesenchyme in mice, and found that GFP accumulated in Xenopus blastema cells after limb amputation. It is interesting that mouse Prx1 limb enhancer contains elements that are sufficient to drive the reporter gene in the amputated Xenopus limb. The enhancer can also drive the reporter in the healing process of back skin wound in Xenopus, but the enhancer activity is not detected in mouse skin wound. Whereas urodeles can reconstruct an exact replica of the amputated limb, the Xenopus froglet merely regenerates a cartilaginous spike, suggesting that froglet regeneration has some deficiencies in pattern formation. Our recent findings indicated that this incomplete patterning in Xenopus limb regeneration is highly related to deficient re-expression of Shh gene. We report the activity of limb-specific Shh enhancer during limb regeneration.

doi:10.1016/j.ydbio.2010.05.372