Comparative Regenerative Biology

We use animals with high regenerative abilities, such as planarians and newts, to understand the principle of regeneration. In particular, we investigate the difference between regenerative and non-regenerative animals to evoke said abilities from non-regenerative animals. We have already succeeded in achieving this with planarians, which were able to regenerate their heads through RNAi (Umesono et al., 2013 Nature) in addition to accomplishing functional joint regeneration in frogs through the activation of reintegration systems (Tsutsumi et al., 2016 Regeneration).

We are trying to induce limb-regeneration ability in frogs, as they lose the capability to achieve complete limb regeneration after metamorphosis. Thus, we are focusing on the Sonic hedgehog (Shh) enhancer MFCS1 (mammals-fishes conserved sequence 1), since it was suggested that the loss of MFCS1 activity after metamorphosis might cause a failure to achieve the aforementioned limb regeneration in adult frogs (Yakushiji et al., 2009). When we compared the MFCS1 sequences between newts (C.p. and P.w.) and frogs (X.l. and X.t.), newts were found to possess several specific sequences (Figure 1). Thus, we subsequently planned to swap the MFCS1 sequences between newts and frogs using CRISPR/Cas9 technology.

Figure 1. Comparison of the MFCS1 sequences between newts and frogs.

We then prepared various guide RNAs to swap these sequences, and succeeded in the production of mosaically targeted Iberian ribbed newts (Pleurodeles waltl: P.w.) and frogs (Xenopus tropicalis: X.t.), newts were found to possess several specific sequences (Figure 2). Thus, we subsequently planned to swap the MFCS1 sequences between newts and frogs using CRISPR/Cas9 technology.

Figure 2. Deletion patterns of the newt-MFCS1 region (ca. 1.6 kb) obtained by microinjection of several different cocktails of gRNAs with CRISPR/Cas9.

Trial for the cultivation of planarian embryonic and adult pluripotent stem cells

We developed an isolation method of adult pluripotent stem cells (aPSC) from planarian using FACS and tried to cultivate them under in vitro culture conditions, but we have yet to succeed in propagating these cells. LCDM and EPSCM media have recently been developed to cultivate mouse EPS (Expanded Potential Stem) cells, which can differentiate both embryonic and extra-embryonic cells. Thus, it is expected that these media might work to cultivate embryonic pluripotent stem cells (ePSC) derived from non-mammalian species. Thus, we tried to cultivate planarian embryonic cells by LCDM and EPSCM media and got healthy planarian ePSC-like-aggregates in vitro (Figure 3). We also attained similar healthy cell-aggregates from planarian aPSC after isolation using FACS. However, we unfortunately could not detect the active proliferative activity of these cells in these culture media so far.

Figure 3. planarian ePSC-aggregates formed in EPSC (left) and EPSCM (right) media.

Publication List:

- **Original Papers**

  
  

- **Review article**