論文の内容の要旨

論文題目

Study on the role of *interleukin-11* in tail regeneration of *Xenopus laevis* tadpoles

(アフリカツメガエル幼生の尾再生において インターロイキン 11 が果たす役割に関する研究)

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The ability to regenerate lost organs varies depending on the animal species (*1*). Although mammals have poor regenerative ability, lower vertebrates have high ability to regenerate lost organs, such as limbs and tails. Understanding the molecular mechanisms of organ regeneration is not only interesting for basic biology, but also important for the advancement of regenerative medicine.

Xenopus laevis tadpoles possess remarkable tail regenerative ability, and are used as model animals to study the molecular mechanisms underlying organ regeneration. After amputation, specialised epithelium called the wound epithelium covers amputation plane, a mass of undifferentiated proliferating cells called the blastema appears, and these cells differentiate to form mature tail tissues, such as the notochord, spinal cord, or muscle. These tissues should coordinately reconstitute the whole organ, therefore the source of these tissues and the molecular mechanisms that regulate the proliferation or differentiation of the progenitor cells of these tissues are important issues for the study of organ regeneration. The source of these newly formed tissues are lineage-restricted tissue stem cells (*2*), but the molecular mechanisms that regulate these cells are not well understood. I hypothesised that the genes expressed in undifferentiated blastema cells have pivotal roles in regulating their proliferation and differentiation, and comprehensively identified proliferating

blastema cell-selective genes.

To determine when proliferating cells begin to accumulate at the blastema, I performed 5-Bromo-2'-deoxyuridine (BrdU) labelling, and found that proliferating cells began to accumulate at the regenerating tail blastema at 3 days post amputation. I isolated proliferating cells in the blastema, non-proliferating cells in the blastema, and proliferating cells in tail buds using a cell sorter based on their DNA contents. I compared their gene expression profiles by RNA-sequencing. I searched for genes selectively expressed in proliferating blastema cells compared to non-proliferating blastema cells and proliferating tail bud cells. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) confirmed that 10 genes (*interleukin-11* (*il-11*), *keratin 18, brevican, chromosome segregation 1-like* (*cse1l*), *lysyl oxidase, LINE-1 type transposase domain-containing protein 1-like (l1td1-like), cd200like-related, oocyte activation in Xenopus (oax)*, and two uncharacterised genes) among 36 candidate proliferating blastema cell-selective genes exhibited significantly higher expression in tail blastema compared to intact tail and tailbud (Fig. 1). When I performed whole mount *in situ* hybridisation (WISH), I detected signals for *il-11*, *keratin 18*, *brevican*, *cse1l*, and *lysyl oxidase*. *il-11* and *cse1l* were expressed in broad areas of the blastema, and *keratin 18*, *brevican* and *lysyl oxidase* were mainly expressed in the notochord bud (Fig. 2). Double-labelling with BrdU labelling and WISH confirmed that among these 10 genes, at least *il-11* and *keratin 18* were expressed in the proliferating cells of tail blastema (Fig. 3) (*3*).

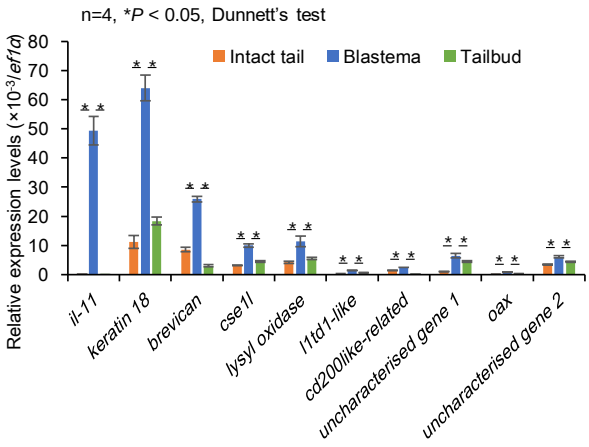

Fig. 1 Blastema-selective expression of the candidate genes.

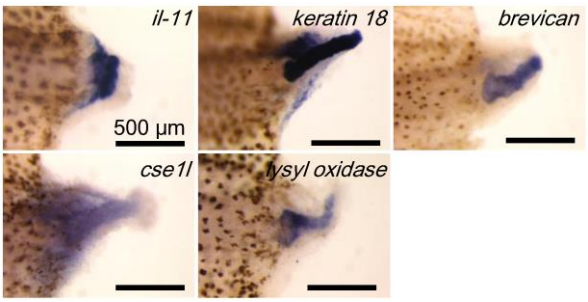

Fig. 2 Localization of proliferating blastema cell-selective genes.

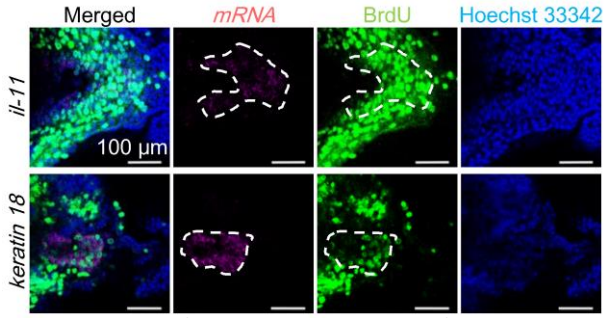

Fig. 3 Expression of *il-11* and *keratin 18* in proliferating blastema cells.

Among the 10 genes, I further analysed the role of *il-11*, which showed the most prominent blastema-selective expression. To reveal the correlation between *il-11* expression and the regenerative process, I performed qRT-PCR and WISH for *il-11* after tail amputation. To determine the function of *il-11*, I also created *il-11* knocked down (KD) tadpoles using the clustered regularly interspaced short palindromic repeat/CRISPR-associated 9 system. I also performed transcriptomic analysis of *il-11* KD tadpoles, and forced expression of *il-11* to reveal its role in tail regeneration.

L1td1 is an RNA-binding protein that is necessary for the self-renewal of human embryonic stem cells (*4*). It might also be required for the self-renewal of proliferating blastema cells of *Xenopus* tail. In mice, CD200 dampens overactivation of the immune response (*5*), and an isoform of CD200 inhibits immunosuppressive function (*6*). *X. laevis* tadpoles lose their tail regenerative ability during the refractory period (*7*), and administration of an immunosuppressant restores the ability (*8*), suggesting that an autoimmune reaction inhibits tail regeneration. It is possible that CD200 modulates the autoimmune response during the refractory period. *oax* is thought to have originated from tandem duplication of a short interspersed repetitive element, and B2 RNA, a transcript of the short interspersed repetitive element, represses the transcription of certain genes in mice (*9*). *oax* might also regulate gene expression during regeneration. *cse1l* is involved in cell cycle regulation in mammals (*10*), and might also regulate the cell cycle of proliferating blastema cells. Brevican is a component of the extracellular matrix (*11*), and lysyl oxidase catalyses crosslinking of collagen and elastin to stabilise the extracellular matrix (*12*). These genes might be involved in reconstruction of the extracellular matrix during tail regeneration. *keratin 18* is expressed in the blastema of regenerating

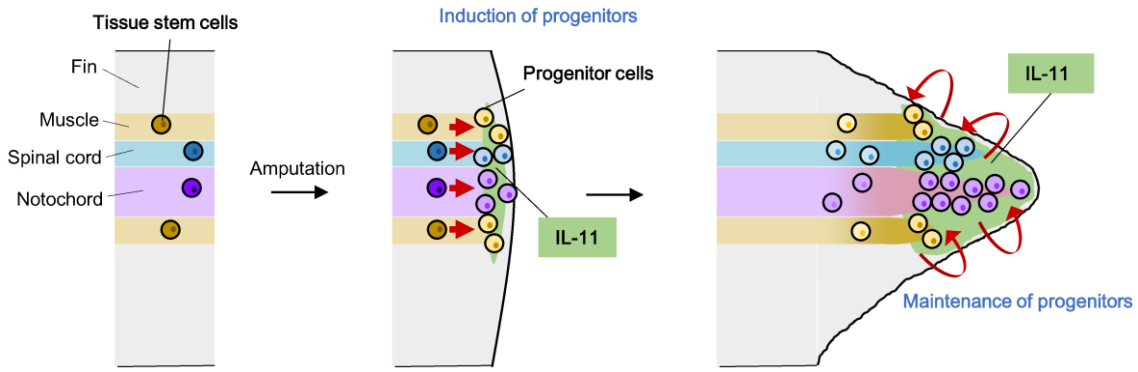

Fig. 4 Model of the role of *il-11* in tail regeneration of *X. laevis* tadpoles.

newt limbs and is necessary for cell proliferation (*13*); therefore, it might also be involved in the proliferation of blastema cells in *Xenopus*.

IL-11 is a member of the IL-6 family, and the IL-6 family transduces signals through the signal transducers and activators of transcription (Stat) 1/3 pathway, the mitogen-activated protein/extracellular signal-regulated kinase kinase pathway, or the phosphatidylinositol-3 kinase pathway in mammals (*14*). *il-11* is expressed in the regenerating heart of zebrafish, and forced expression of a dominant negative form of Stat3 inhibits the proliferation of cardiomyocytes and heart regeneration (*15*). This study is the first to investigate the precise role of *il-11* in organ regeneration, and suggests that *il-11* is involved in induction and maintenance of progenitor cells during tail regeneration (Fig. 4). The results of this study greatly advance our understanding of the molecular mechanisms specific to organ regeneration.

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