最終試験の結果の要旨

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The chief examiner and four vice-examiners interviewed the applicant, Yasin Md Haider on 02/14/2024. The applicant was required to explain his thesis and respond to questions from the examiners. Our examiners asked him the following questions and received satisfactory responses to them.

Question 1: The secondary enamel knot (EK) appears after space is created during tooth morphogenesis. Is there any way to monitor the growth of tooth germ?

Response: There is no specific monitoring way for the size of tooth germs. Using histological sections, we can measure the size of them. But, a better way is to create the 3D reconstruction of the enamel-dentine interface and measure that size, which we have done in our study.

Question 2: Why did you focus on *Fgf4* among a lot of Fgf members?

Response: It has been found that the expression pattern of *Fgf4* is very concise and restricted within the EK. So, although other members of Fgf family are also expressed in the EK, we focused on *Fgf4* expression as an EK marker in our study.

Question 3: Why the metacone is higher than the paracone in case of upper molars of the house shrew?

Response: We can consider two points of this kind of phenomenon. From a developmental point of view, the paracone is a very small cusp, and we think the gene expression (such as *Shh*) from that cusp is also lower to inhibit the metacone formation. From the evolutionary point of view, the metacone is a much more important cusp than the paracone in the slicing/shearing function during mastication. So, the metacone of the upper molars has evolved to be larger than the paracone.

Question 4: You described that the EK is non-proliferative cells. Is there any apoptosis within the EKs?

Response: In the previous studies it has been found that apoptosis proceeds within the EK by the Tunnel analysis.

Question 5: You checked *Shh* expression in your experiment. Does any other hedgehog family members have any function in cusp formation?

Response: There has been no evidence other hedgehog family members, such as *Ihh* and *Dhh*, are involved in tooth development.

Question 6: What do you think about the relationship between *Shh* and *Fgfs* with their receptor in cusp formation?

Response: There have been several studies regarding receptors of *Shh* and *Fgfs* in tooth development. From these studies, when the expression levels of the receptors are altered, their phenotypes will be similar to those when the ligands are altered. For example, when *Ptch1*, the receptor of *Shh*, is downregulated, the number of cusps increases, and the same thing happens when *Shh* is downregulated.

Question 7: EKs secrete several growth factors. Why these growth factors do not work on EKs?

Response: Although EKs secrete several Fgfs which are the main mitogen during tooth development, their receptors are absent within the EK. So, the EKs remain non-proliferative whereas the surrounding epithelial and mesenchymal cells actively proliferate.

Question 8: In your study, *Fgf4* moves from the original position. Why can you tell that?

Response: We are now working to find the answer. At the present, we have two hypotheses. The first is, the EK cells migrate from the original position along with *Fgf4* expression. The second is, EK doesn't move, only the *Fgf4* expression transmits from cell to cell. As EK is a non-proliferative structure, we are now doing cell proliferative

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analysis to find the answer.

Question 9: Are there any similarities between tooth formation and odontogenic tumor formation?

Response: Fine-tuning between gene expressions is very important for both healthy tissue homeostasis and tumor formation. It has been found that higher expression of *Shh* disturbs tooth formation. In odontogenic tumors, *Shh* expression is also found higher.

Question 10: Are *Shh* and *Fgf4* the best markers for EKs?

Response: *Shh* is the most widely accepted marker for the EK. It starts to express very early and its expression level is very strong within the EK. However, in later stages, it becomes to express in the wider epithelial domains. So, we also used *Fgf4* because its expression is very confined within the EK at the later stages.

Question 11: Did you try the gain-of-function experiment of Fgf4?

Response: I have not done. But, in normal conditions, *Fgf4* expression is very weak and appears later than *Shh*. So, if we increase the expression of *Fgf4*, it will appear earlier than *Shh* and also it may disrupt the balance.

Question 12: What is the role of Shh and Fgf4 during cusp formation?

Response: During cusp formation, Shh acts as an inhibitor of gene network, whereas Fgf4 acts as a mitogen which promotes cell proliferation.

Question 13: Why is the *Shh* secreted to the wider areas in the later stage of tooth development?

Response: *Shh* is also necessary for the differentiation of ameloblasts. Although in the earlier stages, the expression is restricted, in the later stage it distributes along the inner enamel epithelium.

Question 14: Why doesn't cusp mineralization order follow the order of EK formation?

Response: I think the growth factor changed with the functional importance of the cusps in evolution. For mastication, the most important cusp for the upper and lower 1st molar are metacone and protoconid respectively. So, those cusps' mineralization started earlier than the others and they became the tallest.

Question 15: How did you contribute to the published paper?

Response: In the published paper, I studied the morphogenesis of the upper 1st molar, and Dr. Yamanaka studied the lower 1st molar morphogenesis.

Question 16: Why did you study the 1st molars instead of the 2nd or 3rd molars?

Response: Because the 1st molars of the house shrew are the least derived from the tribosphenic molars.

Question 17: What are the differences between the house shrew and other common experimental animals like mice or rats?

Response: Although rats and mice are the most common animals in tooth morphogenesis, these animals don't have canine and premolar teeth. In contrast, the house shrew has all sets of tooth type including canines and premolars.

Question 18: How did the insectivorous diet, as observed in the house shrew, change tooth morphology in evolution?

Response: Mesozoic mammals with tribosphenic molars are believed to have been insectivores. Those molars have two occluding functions of slicing and grinding. The house shrew's molars are the least derived from this condition, which are adapted for better chewing of foods like insects.

Question 19: In humans, the 3rd molars tend to degenerate. Why?

Response: During tooth development, proper space is needed for tooth growth both bucco-lingually and antero-posteriorly in the jaws. In humans, the jaws become smaller especially in the distal direction than in the older ages. So, the space has been getting not enough for the 3rd molars.

From the above results, the five examiners confirm that the applicant possesses the academic skills and knowledge required to complete the doctoral course and is qualified to receive the degree of Doctor of Philosophy in dental science.