（ 様 式 14 ）

最終試験の結果の要旨

報告番号	総研第657号		学位申請者	エリサ ハイラニ
審査委員	主査	後藤 哲哉	学位	博士（歯学）
	副査	杉村 光隆	副査	笹平 智則
	副査	松口 徹也	副査	比地岡 浩志

主査および副査の5名は、令和4年3月11日、学位申請者 エリサハイラニ 君に面接し、学位申請論文の内容と関連事項について、試問を行った。以下のような質疑応答がなされ、いずれについても満足すべき回答を得ることができた。

(質問1) What is the incidence of ameloblastoma in Indonesia?

(回答) Ruslin et al (2018) shown 37.5% were males, 62.5% were female, 87.6% located in mandible and 12.4% in maxilla. The most common type was solid/multicystic type (66%) and follicular was the most common histopathological subtypes.

(質問2)Why do you choose to investigate the interactions between ameloblastoma and osteoblast instead of osteoclasts?

(回答)In cancer, several reports showed the role of osteoblast in its disease progression, while in ameloblastoma, the effect of osteoblasts on ameloblastoma cells is not well understood, and there has been limited research on interactions between them.

(質問3)What did you mean by ameloblastoma inducing osteoclastogenesis? Do you think ameloblastoma cell itself caused bone resorption?

(回答)In the presence of ameloblastoma, osteoblast produced cytokines that have known to activate osteoclastogenic activity and might contribute to bone resorption. Previous study (Kibe et al., 2013) shown that AM-3 cells induced osteoclastogenesis by producing factors such as MMPs. So, it is not AM-3 cells itself that induce osteoclastogenesis but its derived factors that induce osteoclastogenesis. In addition, we would like to suggest the possibility that the MMPs produced by ameloblastoma cells could contribute to the bone resorption via their collagenase activities.

(質問4) How do you think to apply this study to clinical practice?

(回答) Neutralizing agents for IL-1 or MMPs may have therapeutic use to control ameloblastoma locally.

(質問5) Does ameloblastoma has malignancy? How do you think about that?

(回答) Based on WHO classification, most of ameloblastoma are benign tumor. However, there is a rare malignant type of ameloblastoma.

(質問6) Why did you use different species cell line (mouse and human)? Can you say that these data represent exact results in human?

(回答) It was very useful for the determination of the origin of cytokine and MMPs in the CM using human or mouse specific ELISA kit. It is not so strange to use difference species to investigate their reactions *(ref3,etc)*. Of course further studies are needed to whether our findings reflected pathological phenomena in human ameloblastoma.

(質問7) IL-1Ra inhibits these cytokines through indirect phenomena, is it OK to think that way?

(回答) No, AM-3 itself secrets IL-1α and it is reported that IL-1 induced MCP-1 in several cells.

(質問8) MMP-2 is the highlight in this study, what makes MMP-2 different from other MMPs?

(回答) It suggested the existence of MMP2-inducing signal pathway driven by osteoblast-derived soluble molecules.

(質問9) MCP-1 is a ligand for CCR2, and RANTES also binds to CCR2. What is the function of CCR2 in ameloblastoma?

(回答) Most chemokine receptors can bind to several chemokine. Based on these, we may assume that CCR2 may play a role in the ameloblastoma-bone microenvironment on bone remodeling process. Further study is needed to investigate this.

(質問10) What do you think about the interactions of ameloblastoma with T cell and Macrophage cell?

(回答) As a tumor, ameloblastoma will be recognized as a foreign material and activate the host defense. Macrophages detect the presence of microbes and initiate immune response. T cells recognize foreign antigens and carry out adaptive immune response. *(Abbas et al., Basic Immunology 6th ed, 2020, 1-4). In addition, ameloblastoma cells were reported to induce differentiation of macrophages into osteoclasts.*

(質問11) In supp. Fig.2 how do these cytokines act on osteoclasts?

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(質問 11) In supp. Fig.2 how do these cytokines act on osteoclasts?
(回答) IL-6 is regulating the differentiation of osteoclast progenitor cells into mature osteoclasts. MCP-1 was reported in the recruitment of mononuclear cells and participated on the fusion of preosteoclast to mature osteoclasts. RANTES has similar actions in promoting osteoclast fusion and survival. *(Lorenzo et al., 2016; Mulholland et al., 2019; Brylka et al., 2019; Siddiqui et al., 2017)*
(質問 12) What do you know about the relationship between ameloblastoma and other stromal cells (fibroblast, endothelial cells, growth factors)?
(回答) Several studies investigate the relationship of ameloblastoma and osteoclast *(Kibe et al., 2013, Sandra et al., 2010)*. Other reports were investigating ameloblastoma and stromal fibroblast *(Fuchigami et al., 2014, 2017)*, and ameloblastoma stromal cells and growth factors and its receptors such as FGF, VEGF and PDGF *(Zhong et al., 2011; Ibrahim et al., 2022; Kato et al., 2014; Kumamoto et al., 2002)*.
(質問 13) In supp. Fig.1 showed that IL-1α and IL-1β showed different pattern in culture supernatant. Why do you think it was different?
(回答) There are many processes between transcription and translation that might cause this. Other possibilities are post-transcriptional control mechanism and the possible interference role of miRNA, ncRNAs, etc. (http://www.ncbi.nlm.nih.gov/pubmed/22411467)
(質問 14) In fig. 1, RANTES production was still high even after treatment with IL-1Ra, what is the possible factor for this other that IL-1?
(回答) It is well known that most chemokines can bind to several chemokine receptors. This might be the possible factor that caused RANTES still showed relatively high expressions.
(質問 15) Do other ameloblastoma cell lines express IL-1α? How general is it?
(回答) We did not have the data of IL-1α production by other ameloblastoma cell lines. However, we have shown that the retention of IL-1α in the patient's cystic fluid. Considering several studies also showed data of IL-1α from ameloblastoma (*Ohta et al. 2017; Fuchigami et al., 2014; Zhong et al., 2011; Sandra et al., 2005)*, IL-1α might contribute to the general pathogenesis of ameloblastoma.
(質問 16) About the ELISA experiment, why did you pick only a few cytokines?
(回答) This study used an assay platform that simultaneously quantifies the concentrations of multiple cytokines. In the condition of this study, the results showed only these 3 cytokines (IL-6, MCP-1, and RANTES) were highly expressed.
(質問 17) Osteoblast also expressed RANKL, did you check the expression of RANKL from osteoblasts?
(回答) No, we did not check the expressions of RANKL from MC3T3-E1 osteoblast cells.
(質問 18) MC3T3-E1 cells shown to modulate tumor growth, and the use of neutralizing antibody of IL-6 did not show effect on the tumor growth, how about the other cytokines?
(回答) We have not checked the effect of MCP-1 and RANTES yet on tumor growth.
(質問 19) Why did ameloblastoma appear in the mandible rather than maxilla regarding this study?
(回答) It might be because of the differences of bone structure. Mandibular bone is denser than the maxilla, and it was shown that the dense quality of the bone effect the rate of bone remodeling process in the bone. *(Speight and Hunter, 2021. Diagnostic Histopathology of Tumors, chapter 6:273-300; Park et al., 2008 https://doi.org/10.1016/j.asodo.2006.01.044)*.
(質問 20) Previous report shown high expressions of MMP-9, while in this study, it was MMP-2. What do you think about it? And what is the role of MMPs in the osteoclastogenesis?
(回答) MMPs were known to be involved in the degradation of bone matrix. Supp. table 2. showed that the expressions of MMP-9 were constitutively high but not modulated in the presence of MC3T3-E1 osteoblast CM. AM-3 cells already produced high amount of MMP-9 in the basal condition. This study indicated the novel inter-cellular induction system of MMP-2 in ameloblastoma cells.
(質問 21) What do you think about RANK-RANKL with MMPs for osteoclastogenesis?
(回答) It was reported that RANKL modulate MMP-9 expression during osteoclast differentiation in macrophage cell line *(Sundaram et al., 2006)*. However, it is unclear whether MMPs are essential for osteoclastogenesis.
(質問 22) Why did migration capability was activated even though ameloblastoma is benign tumor?
(回答) Whether the cells are malignant or benign, cellular migration can occur both in physiological and pathological conditions.

以上の結果から、５名の審査委員は申請者が大学院博士課程修了者としての学力・識見を有しているものと認め、博士（歯学）の学位を与えるに足る資格を有するものと認定した。