最終試験の結果の要旨

報告番号	総研第 554 号		学位申請者	Muhammad Kamil
審査委員	主査	中川 昌之	学位	博士（医学・歯学・学術）
	副査	小林 裕明	副査	谷本 昭英
	副査	田川 義晃	副査	上野 真一

主査および副査の５名は、令和１年11月11日、学位申請者Muhammad Kamil君に面接し、学位申請論文の内容にて説明を求めると共に、関連事項について試問を行った。具体的には、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。

Q1) In figure 4, you showed the MMP activity assay only for 2 cell lines. Did you do the same assay for all cell lines?

A1) No, I did not do the same assay for all cell lines. We pick up one cell line each group to represent overexpression analysis and knockdown analysis group.

Q2) Basically, the cancer cell invasiveness consisted of 3 elements: cell attachment, proteolytic enzyme, and cell locomotion. In your study, did you check all of the three elements?

A2) No, I only did some experiments about proteolytic enzyme and cell locomotion. We did not check the cell attachment ability.

Q3) What are you thinking about cell morphology dynamic related to the FLNC signaling? Did you do experiment about that?

A3) No, I didn't check any morphological experiment. In our study, we assessed that FLNC was not working on physical/morphology regulation based on Rho activity assay. But I need to consider some experiments about morphological dynamic depending on FLNC in the future.

Q4) There are many MMPs other than MMP2, such as MMP9. Did you check MMP9 expression in your study?

A4) Actually I did zymography assay that is specific to the MMP2 and MMP9. But we didn't get appropriate result for MMP9. Also we didn't get the mRNA expression for MMP9 in our cell line. We assume it because our GBM cell lines does not express MMP9.

Q5) Did you check the tumorigenic level of your GBM cell lines you used?

A5) No, I didn't check it. But in the future we plan to check tumorigenic level of each cell line in vivo study.

Q6) Base on your result, do you have any idea about drugs?

A6) I think we will focus to the GBM invasive character, first we have to deeply understand about GBM invasiveness. For the next experiments, we plan to identify more about FLNC and we need to screen the drugs which target invasive character and of course we consider about the drugs penetration.

Q7) Why you are not using positive control for your IHC analysis?

A7) We used the same kind of antibody with our previous study in which skeletal muscle cell was used as a positive control.

Q8) You used four GBM cell lines which have high and low FLNC expression. In my opinion, you could use one cell line to modify the specific gene expression by genome editing. What do you think?

A8) Yes, that will be a good idea to consider in our future study.

Q9) Does FLNC regulate MMP2 expression and activity? transcription or translation or enzyme activation?

A9) We didn't do any experiment about the exact pathway how FLNC regulates MMP2, so we don't have any data to support it. We may establish this point in the future study.

Q10) You mentioned about unique structure of the brain environment in your article. Can you explain about extracellular matrix in the nervous system in detail?

A10) Major components of ECM in the nervous systems are proteoglycan and hyaluronic acid. And from our references, MMP2 is the major enzyme used by the cancer to invade brain parenchyma.

最終試験の結果の要旨

Q11) Does FLNC expression correlate with malignancy in GBM?

A11) In my opinion, we need to establish more study to make it clear. But, our study is the first study which states FLNC is a prognostic factor in GBM. Previously, only one study reveals that FLNC expression is a biomarker for glioma.

Q12) Is there any correlation between FLNC high expression cell lines group and low expression group about invasive character in resting state?

A12) In my opinion, there was very wide phenotype variety among each cell lines that makes it difficult to standardize and compare each other. Therefore we didn't check.

Q13) How does the FLNC overexpression occur? In primary GBM, various driver genes abnormality such as TP53 mutation, MDM2 amplification, and EGFR amplification are already known. Do you estimate that FLNC over expression occur by FLNC gene abnormality and that could be nominated as a new driver gene? Or, the FLNC overexpression is caused by the other mechanism such as abnormality of epigenetics?

A13) I don't know the mechanism of overexpression of FLNC. FLNC is highly expressed in heart muscle and skeletal muscle cells, it is also known to be important gene in the CNS cell differentiation stage. In related to malignancy, we need wider study to make it clear.

Q14)It is known that FLNC acts as a scaffold cytoskeleton. However, from the results in figure 3, FLNC silencing affected the invasion ability than migration ability. Does that means FLNC affects cancer invasion more through some kinds of networks than morphological change?

A14) FLNC affects the invasive ability of the GBM cell lines not via morphological changes. Our study findings show FLNC affected the MMP regulation network, but we still need more extended study to establish the exact relation pathway and interacting protein in this network.

Q15) After considering previous questions, can we take that GBM could gain the invasion ability associated with MMP2 in the growth process, rather than carcinogenesis by FLNC? And how are the relations of FLNC and MMP2 reported in other articles?

A15) First, our study showed the GBM regulate MMP2 to make more invasive to the surrounding tissue by degrading the ECM. This is the first study which shows the correlation of FLNC and MMP2 activity.

Q16) How about FLNA and FLNB roles in GBM?

A16) Some studies report that FLNA and FLNB have roles in glioma *in vitro*, but there was no evidence in the clinical data.

Q17) How about localization of FLNC protein in the GBM cell due to it invasive character?

A17) Some actin binding proteins may have a role to affect and localized on the invasive front of the cancer cells, for example on invadopodia, lamellipodia or filopodia. But in GBM cell lines, our findings showed that FLNC signaling has no effect to Rho family protein activity. So we assumed that FLNC doesn't work in this network. Otherwise we still need morphological study to make the localization of FLNC clear.

Q18) You mentioned in your article that GBM doesn't metastasize but invade to surrounding tissue. How does it correlate to your study?

A18) Our study shows that FLNC is working in the invasive character on GBM.

Q19) Did you do MMP gene expression modification experiment in your study?

A19) No, we didn't do it. We will consider to do in the future study.

Q20) In clinical settings, is there any impact to your GBM patients? For example if they have high FLNC expression in their tumor tissue?

A20) Yes, there is impact to the patients. Because our study shows that FLNC is a poor prognostic factor for GBM.

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（医学）の学位を与えるに足る資格を有するものと認定した。