最終試験の結果の要旨

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主査および副査の5名は，令和元年7月23日，学位申請者 ハリド ムハマド君に面接し，学位申請論文の内容につい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. Why South Korea has high incidence of pancreatic ductal adenocarcinoma (PDAC)?

A: High incidence of PDAC in South Korea are due to obesity, diabetes, spicy food or family history of genetic syndrome.

Q2. What are the risk factors for PDAC?

A: The exact risk factors for PDAC are still unknown but some factors are related to increase the risk of PDAC such as smoking, drinking alcohol, family history of PDAC, blood group, diabetes, and so on.

Q3. In Table 1, why is the incidence high in pT3 tumor?

A: Since it is very difficult to detect early PDAC, the incidence of pT3 is high.

Q4. How do you select patients for the neoadjuvant chemotherapy?

A: We could not prolong the prognosis of PDAC by surgery alone. Recently, we often adopt the neoadjuvant chemotherapy except for early PDAC.

Q5. What is the recurrence pattern of PDAC?

A: There are various types such as local recurrence, blood borne recurrence, dissemination and mixed recurrence. The most frequent recurrence is liver metastases.

Q6. In Figure 6, which are most important downstream genes?

A: Important downstream genes for RACGAP1 with a poor prognosis of patients with PDAC are MMP28 and ANLN as their p value is less than $p<0.0005$. So, these are considered as most important downstream genes.

Q7. In Figure 7, what is z-score?

A: Z-score is a sample which indicates the No. of standard deviations away from the mean of expression and the formula for z-score is, z=expression in tumor sample or mean expression in reference sample divided by standard deviation of the expression in reference sample.

Q8. What is your next step and any plans toward clinical application?

A: Our next step is to check the mechanisms including the malignant properties of PDAC and target genes will be searched for further treatment in the clinical application. Regrettably, the current status doesn't allow the use of microRNA-based therapy as a standard anti-cancer treatment. The clinical trials of miRNA-based therapies in cancer patients are ongoing.

Q9. In Figure 1A, why miR-204-5p is completely suppressed in both cell lines?

A: Because miR-2045p has pure PDAC cell lines, they are completely suppressed in PANC1 and SW1990.

Q10. What is the factor that both cell lines are suppressed?

A: The factors that both cell lines are suppressed are the expression of miR-204-5p in these cell lines. miR-204-5p was found to significantly downregulated in PDAC specimens.

Q11. In Figure 1B, C, D why in migration/invasion assay cell lines suppressed but in proliferation assay is not suppressed?

A: That is probably because miR-204-5p mainly related to cell motility.

Q12. In Figure 2, how do you define the high and low level of protein expression?

A: We defined the high and low expression as mean value according to the disease-free survival and overall survival of PDAC patients based on data from the TCGA database.

Q13. In Figure 3A, why normal cell lines were not used?

A: We couldn't get normal pancreatic cell lines. So, normal cell lines were not used here.

Q14. In Figure 3C, RACGAP1 expression was found in some cancer cells, but the others not? What is this difference?

A: One of the reasons in different expression of RACGAP1 was histologic type such as well-, moderately and poorly differentiated adenocarcinoma. Another reason is heterogeneity of tumor cells.

Q15. Was RGCGAP1 expression found in normal condition?

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A: In the normal condition, RACGAP1 has no or less expression.

Q16. What is the mechanism of upstream control in miR-204-5p?

A: The mechanisms of the upstream control in miR-205p suppress are unknown. Transcription factors and epigenetic effects might control it.

Q17. What types of inhibitors are target for RACGAP1?

A: Inhibitors such as HSP90 and 17AAG are currently entered as the phase 2 clinical trials an anti-cancer agent in breast cancer and Tangshan formula for diabetic kidney disease, is effective for the inhibiting of RACGAP1-stata-5 mediated cell proliferation.

Q18. In RACGAP1, what are the other GAP and what happens if the other GAP gene increase?

A: High RACGAP1 activity can move and the RAC affect is invasive and can be negatively regulate and the GAP protein can promote cancer cells, And RAC should be activated with GAP and GAP junction are involved in the cell growth control.

Q19. Is there early detection of the miR-204-5p level or RACGAP1 level in cancer patients?

A: Our present study showed that RACGAP1 was over expressed in PDAC and in this series, early detection of the miR-204-5 and RACGAP1 level was not found in PDAC patients. But RACGAP1 inhibits the cell migration and invasion, and the miR204-5p was downregulated in some cancers. At present, there is no specific biomarker for miR-204-5p.

Q20. What is the exact difference between microRNA and siRNA?

A: Formation process is the same, are processed inside the cycle by the enzyme called dicer and incorporated into RISC. siRNA is considered exogenous double stranded RNA, which is taken up by the cells, while miRNA is a single stranded.

Q21. What is the function of RACGAP1?

A: The function of RACGAP1 is that gene encodes a GAP, and a component of the central-spindlin complex. That protein plays an important regulatory role in the cell growth, cell differentiation and cytokinesis etc., and this protein binds activated form of Rho-GTPase and stimulates GTP hydrolysis, resulting in negative regulation of Rho-mediated signals.

Q22. Why GTPase is negatively control and why the prognosis is poor?

A: As a GTPase activating protein, the RACGAP1 binds to activated G proteins to stimulate GTPase hydrolysis and inactivates the G protein. So, this protein binds activated form of Rho-GTPase and in the results of negative controls. Thus, the patients with high RACGAP1 expression has poor prognosis according to the TCGA database of this study.

Q23. Are gene profiles of KRAS, TP53, CDK and SMAD 4 famous for PDAC?

A: Some studies showed that KRAS, TP53 and SMAD are driver oncogenes in PDAC. KRAS is mostly mutated in PDAC, which involved in cancer progression while TP53 is most frequently mutated genes in all cancers. KRAS mutation is found in about 70% in PDAC. CKD are tumor suppressor which regulates cell cycle progression by inhibiting cyclinD-CDK4.

Q24. Please explain Figure 2 again.

A: In this figure, we selected 7 targeted genes by miR-204-5p according to the clinical significance of the expression level from the TCGA. But we focus on RACGAP1 because its high expression level was most significantly associated with a poor prognosis in patients with PDAC, compared to other 6 genes in this figure.

Q25. Why the patients with high RACGAP1expression has poor prognosis?

A: From the TCGA database, the messenger RNA level is low, so the high RACGAP1 has the poor prognosis.

Q26. In Figure 3, what kind of tumor cells in nucleus site or any other site are stained?

A: RACGAP1 protein expression by IHC was mainly found in the nucleus in the PDAC clinical samples. RACGAP1 was strongly expressed in the PDAC lesions, but its expression was not recognized in noncancerous epithelial tissues.

Q27. Is RACGAP1 a promotor or inhibitors?

A: RACAGAP is a tumor suppressor but not activator. So, in PDAC, RACGAP1 is acting as a promotor, but not inhibitor.

Q28. In RACGAP1, what is the GTPase activity?

A: In RACGAP1, GTPase activity is to bind activated G proteins to stimulate the GTP hydrolysis, and the function of these GTPase activating proteins opposes the guanine nucleotide exchanged factors which stimulates the G protein activation. In a recent study, Yin C et al. (Oncology 2019;97:155–163), has revealed the potential of RACGAP1 as an oncogenic driver.

Q29. Can RACGAP1 be a therapeutic target?

A: The RACGAP1 and the RACGAP1 mediated genes are possible potential therapeutic targets for PDAC.

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。