Summary of doctoral thesis review

Applicant's name: Mst Hajera Khatun

Department of Microbiology and Molecular Biodefense Research

Graduate School of Medicine

Yokohama City University

Examiner Panel

Chief examiner: Professor Yoshihiro Ishikawa, Cardiovascular Research Institute,Graduate School of Medicine, Yokohama City University

Co-examiner: Dr. Akira Nishiyama, Immunology,Graduate School of Medicine, Yokohama City University

Co-examiner: Dr. Yukie Yamaguchi, Dermatology,Graduate School of Medicine, Yokohama City University

Summary of doctoral thesis review

Title: Production and characterization of monoclonal antibodies specific for major capsid VP1 protein of trichodysplasia spinulosa associated polyomavirus.

Trichodysplasia spinulosa associated polyomavirus (TSPyV), has been indicated as a causative agent of trychodysplasia spinulosa (TS), a rare proliferative skin disease in severly immunocompromised hosts. VP1 is the major capsid protein of TSPyV, which functions as a potential target for diagnosis of TS disease. The monoclonal antibodies (mAbs) against specific antigens have provided the means for developing a number of specific immunological assays for rapid and accurate diagnosis of the diseases. So far no good mAb is available for diagnosis of TS disease. In this study we developed and characterized the mAbs specific for VP1 of TSPyV. We used wheat germ cell-free synthesized VP1 protein of TSPyV for immunizing BALB/c mice to generate hybridomas. Screening of the resultant hybridoma clones we selected five strongly positive clones producing mAbs reactive to the TSPyV-VP1 antigen. Next we sought to characterize these 5 mAbs. Epitope mapping and bioinformatic analysis showed that these mAbs recognized epitopes located within highly conserved C-terminal region of all clinical isolates of TSPyV-VP1. Further all the mAbs were highly effective for immunofluorescense and immunoprecipitation analysis. Among five mAbs, three clones did not exhibit any cross reactivity to VP1 of other related polyomaviruses. In addition, one of our mAbs (#14) provided immunohistochemical staining of skin tissue of a TS disease. It can be concluded that our newly developed mAbs might shed a new light for the study of TSPyV infection as well as the diagnosis of TS disease.

The questions and answers after presentation of the summary above are as follows:

1. Comments and questions by Dr. Akira Nishiyama:

(A) The TSPyV develops the highest seropositivity in TS patient. How is this seropositivity determined by antigen testing?

Answer: The previous study used VPI antigen based VLP to determine antibody against TSPyV by ELISA for determining the seropositivity. As VP1 is major immunogen to produce antibody, we selected VP1 to genereate mAbs. In case of TS disease VP1 based antibody is highest, so VP1 expression level is also highest. So, the generated mAbs would be able to detect the VP1 antigen expressed during TS disease.

(B) The TSPyV virus causes persistent infection in healthy individual as well as TS disease in immunosuppressed individual. Are your mAbs used to determine VP1 antigen in both cases?

Answer: Previous study demonstrated that VP1 is only detected in TS lesional sample but not in non lesional sample. So our mAb would not detect the VP1 from non lesional healthy individual.

(C) How does persistence develop and why VP1 is not detected in healthy individual?

Answer: The persistence mechanism of TSPyV is not well understood yet. But other human polyomaviruses like JCPyV and BKPyV develop persistent infection by the mechanism that these viruses express a single miRNA from late region. This viral miRNA regulate the expression of early gene product which ultimately regulate the replication of DNA genome and late gene expression. This miRNA also have immune evasion function from host cell. So viral miRNA mediated gene expression regulation and immune evasion contribute to the persistence. Some polyomaviruses also develop persistence as episomal DNA where no transcription occur. As VP1 is not expressed in non lesional skin tissue of healthy individual. it is not detected in such case.

(D) Why do you co-transfect VP1 and VP2 to the cell for immunoprecipitation analysis?

Answer: For immunoprecipitation analysis we co-transfected VP1 and VP2 to the cells to identify whether VP1 interact with VP2 to form the virus-like particle as well as to get idea whether VP1 can interact to host proteins to form virus-like particle.

(E) Why do not you choose VP3?

Answer: In case of human polyomaviruses, either VP2 or VP3 interact with VP1 to form virus like particle and infectious entry of the virus into the cell. Previous study about MCPyV demonstrated that VP1 interact with VP2 to form virus-like particle. As TSPyV is similar to MCPyV, we choose VP2, not VP3.

(F) There are two types of mAbs. Both types recognize C-terminal region of VP1. Is it possible to develop sandwitch ELISA using these mAbs?

Answer: Although the two groups of mAbs recognize the C-terminal region, but they recognize different regions without overlapping. So it is possible to develop sandwitch ELISA.

(G)For sandwitch ELISA, a pair of mAb is required. How can you choose the combination of mAbs?

Answer: After screening all the possible combinations we will choose the optimal pair which would show the highest reactivity. We have two groups of mAbs but one group showed slight cross reactivity. So for screening, we would choose cross reactive mAbs as immobilized antibody and specific mAbs for labeled antibody.

(H) How can you generate mAbs if you want to develop another specific group of mAbs which bind another epitope region for developing very specific sandwitch ELISA?

Answer: The same procedure of our previous mAb production method we will follow. After producing mAbs, we will perform epitope mapping. If another epitope region will be found, then other characterization will be done for selection of specific mAb.

2. Comments and questions by Dr. Yukie Yamaguchi:

(A) Why do you choose only one antibody for immunohistochemical analysis?

Answer: We generated five mAbs. Among them two (#3 and # 12) provided slight cross reactivity to JC and BK polyomaviruses. So we excluded these two. Another three (#1, #5 and # 14) mAbs bind the same epitope region. We selected only one (#14) because of its higher reactivity in immunoblotting and immunofluorescence assay.

(B) Do you perform other immunoassays using this mAb?

Answer: I performed the immunohistochemical analysis of healthy skin tissue using this mAb but did not perform other immunoassays.

(C) How does this virus contribute to the pathogenesis of TS disease?

Answer: TSPyV sT antigen interacts to the tumour suppressor protein PP2A. PP2A is an important protein to regulate MAPK mediated cell proliferation and growth. PP2A-sT interaction leads to deactivation of PP2A, rendering it incapable of dephosphorylation of MAPKs. So phosphorylated MAPKs (pMEK and pERK) are activated and translocated to the nucleus where they transactivate the transcriptional gene. Ultimately cell proliferation and growth is occurred.

(D) Where is this virus found in skin tissue?

Answer: Hair follicle.

3. Comments and questions by Professor Dr. Yoshihiro Ishikawa:

(A) The mAbs are used for diagnosis purposes. From where is the sample collected? Is blood or other sample used to detect virus using your mAbs?

Answer: As TSPyV VP1 expressed exclusively in the hair follicle of skin tissue of TS disease, immunohistochemical analysis would be performed using skin tissue to detect VP1 antigen. If sandwitch ELISA will be developed, blood, urine, nasopharyngeal swab and skin swab would be used to detect the antigen as TSPyV was detected in these sample previously.

(B) What is the concentration of your mAb to detect the antigen?

Answer: Actually the concentration of mAb to detect VP1 antigen of TSPyV was not optimized. In the future we will measure the concentration. We used culture supernatant (not purified mAb) as 1:50 dilution in immunoblotting analysis and 1:20 dilution in immunofluorescence analysis. But we used the purified mAb as 1:100 dilution in immunohistochemical analysis.

Several other questions were asked, all of which were responded appropriately by the applicant. In this study, it was judged that the newly developed monoclonal antibody would play an important role in the development of new diagnostic testing against TsPyV. Based on the above, the applicant was judged to be suitable for receiving a PhD degree in medical science.