Identification of DNA methylation alteration during the course of lung adenocarcinogenesis

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論文の内容の要旨
Abstract of thesis

（目的　Purpose）

Lung cancer is still the leading cause of cancer death with varied survival rate. Among the histological subtypes, adenocarcinoma is observed most frequently. Lung adenocarcinoma has been known to show a stepwise progression from precancerous lesion, adenocarcinoma *in situ* (AIS), and finally to invasive adenocarcinoma, and also to be correlated closely with gene aberrations. While many new treatments, targeted against specific genetic aberrations, have been developed for advanced stage adenocarcinoma, patients treated with them eventually acquire resistance against them, which fails to decrease the mortality rate.　Although studies on early stage lung adenocarcinoma are still scarce, previous studies have shown that overexpression of particular genes are correlated with progression of lung adenocarcinoma and most of them do not have genetic aberrations, which means that the abnormality of their expression might be induced epigenetically.

DNA methylation status has been reported to be correlated with the progression of adenocarcinoma. A previous study demonstrated that overexpression of Stratifin (SFN, 14-3-3 sigma) in invasive adenocarcinoma is triggered by DNA demethylation at the SFN promoter region. Based on this finding, the author determined to focus on DNA methylation.

The aims of this thesis are 1) to reveal expression alteration of DNA methyltransferase 3 alpha (DNMT3a), which is a very important component of the methylation pathway in lung adenocarcinoma, and 2) to screen differentially methylated genes during the course of lung adenocarcinogenesis.

（対象と方法　Materials and Methods）

One hundred thirty five lung adenocarcinomas were selected and used for immunohistochemistry (IHC). A 1:100 dilution of rabbit polyclonal DNMT3a antibody was used.

Three samples of invasive adenocarcinoma, 3 samples of AIS, and 2 samples of normal lung tissue were subjected

to Infinium methylation array to screen extensive DNA methylation profiles across the whole genome. After making candidate genes selection, the author validated the results using 21 cases of lung adenocarcinoma by pyrosequencing. Next, the author used IHC to find correlation between methylation rate and protein expression, and also clinicopathological implication for lung adenocarcinoma.

（結果　Results）

From the IHC results, the author found that low DNMT3a expression is associated with histologically invasive type and poor prognosis.

Based on the fact of DNMT3a dysfunction, the author expected that there would be many oncogenes that turn to demethylated status and facilitate tumor progression besides SFN. Thus the author searched for another differentially methylated genes that have the same tendency as SFN, which is correlated with early stage adenocarcinogenesis.

Consequently, the author found that 583 CpG sites showed more than 10% higher methylation rate in invasive adenocarcinoma compared to AIS and normal lung. On the other hands, only 23 CpG sites including SFN locus showed more than 10% lower methylation rate in invasive adenocarcinoma relative to AIS and normal lung. Among the latter, the author finally selected 5 CpG sites located in SFN, GORASP2, CD1D, ZYG11A, and LOC10099657.

As a result of pyrosequencing validation, SFN, GORASP2, and ZYG11A showed stepwise demethylation tendency from normal lung, AIS to invasive adenocarcinoma as expected. Moreover, its methylation rate is inversely correlated with the protein expression, suggesting that hypomethylation at those sites might lead to their overexpression.

Additionally, the author demonstrated that GORASP2 and ZYG11A show high expression in lung adenocarcinoma and is associated with histologically invasive subtype, and poor prognosis. The author also found that GORASP2 and ZYG11A are independent prognostic factors for lung adenocarcinoma.

（考察　Discussion）

In this thesis, the author showed lack of DNMT3a expression and diverse methylation alteration in invasive adenocarcinoma. Even though the number of hypomethylated genes in invasive adenocarcinoma was limited as compared to those of hypermethylated genes, overexpression of GORASP2 and ZYG11A induced by DNA demethylation appear to have important function in the progression of lung adenocarcinoma. Although further analyses are required to understand and elucidate the function of both genes in the lung adenocarcinogenesis, the author’s data suggest that these two genes are possibly new prognostic indicators and might have potential for new target molecule for lung adenocarcinoma.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

The author analyzed the lung cancer samples derived from patients and showed that demethylation of CpGs in SFN, GORASP2, and ZYG11A occurs in a stepwise manner as the cancer progresses. His research also revealed. GORASP2 and ZYG11A show high expression in lung adenocarcinoma and are associated with histologically invasive subtype and poor prognosis. These investigations by the author provide novel findings about how some genes become activated by demethylation of CpGs during progression of cancer. These results link abnormal epigenetic regulation and the progression of cancer, and his findings raise important questions to be addressed in future research in this field.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on December 27, 2018. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（結論　Conclusion）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.