Identification of DNA methylation alteration during the course of lung adenocarcinogenesis

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論文概要

論文題目　Identification of DNA methylation alteration during the course of lung adenocarcinogenesis (肺線癌発生過程における DNA メチル化変化の解析)

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Abstract

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

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

My first study is about DNA Methyltransferase 3 alpha (DNMT3a), which is a key enzyme of the methylation pathway. I used IHC to demonstrate DNMT3a expression pattern in lung adenocarcinoma, and also Western blot to confirmed the specificity of the antibody used for IHC. Consequently, I found that low DNMT3a expression is associated with histologically invasive type and a poor prognosis.

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

For that purpose, 3 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. From the results, I 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 later, we finally selected 6 CpG sites located in SFN, GORASP2, CD1D, ZYG11A, LOC10099657, and Mir656 and validated the result using 21 cases of lung adenocarcinoma by pyrosequencing. As a result, SFN, GORASP2, and ZYG11A showed stepwise demethylation tendency from normal lung, AIS to invasive adenocarcinoma as I expected. Moreover, its methylation rate is conversely correlated with the protein expression, suggesting that hypomethylation at those sites might lead to their overexpression. I next performed immunohistochemistry of GORASP2 and ZYG11A to clarify their clinicopathological implication for lung adenocarcinoma. I demonstrated that GORASP2 and ZYG11A show high expression in lung adenocarcinoma and were associated with histologically invasive subtype, and a poor prognosis. I also found that GORASP2 and ZYG11A are independent prognostic factors for lung adenocarcinoma.

In this study, I made the status of methylation alteration in lung adenocarcinoma clear. DNMT3a expression decreased in invasive adenocarcinoma and methylated genes have been widely changed in the course of malignant progression. Even though the number of hypomethylated genes in invasive adenocarcinoma was limited compared to those of hypermethylated genes, I found overexpression of GORASP2 and ZYG11A induced by DNA demethylation other than SFN. The epigenetic changes of the two genes may have an important function in the progression of lung adenocarcinoma. GORASP2 and ZYG11A might be clinically applicable as an indicator of prognosis and potential novel target molecule for drug development.