日本財団 The Nippon Foundation 助成事業

財団法人日中医学協会

2008年共同研究等助成金—在留中国人研究者—報告者

2009年3月10日

財団法人　日 中 医 学 協 会　御中

貴財団より助成金を受領して行った研究テーマについて報告致します。

添付資料：研究報告書

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1. 助 成 金 額：　600000　円
2. 研究テーマ

Comprehensive Analysis of Aberrantly Suppressed Genes due to Promoter Hypermethylation in Hepatocellular Carcinoma

3. 成果の概要

Aberrant DNA methylation is deeply involved in human carcinogenesis through inappropriate gene silencing. Previous studies have reported that some tumor suppressor genes were silenced by aberrant promoter hypermethylation and the mRNA expression of these genes were reversible by 5-Aza-dC treatment. In this study, we performed genome-wide analysis of both transcriptional and epigenetic profile of hepatocellular carcinoma (HCC) using microarray-based technologies. After treatment of HCC cell line Hep3B with 5-Aza-dC and/or TSA, changes of mRNA levels were measured by microarrays. Then, we compared upregulated genes to the list of candidate promoter hypermethylated genes identified by MeDIP-chip analysis. Array-based analysis revealed the capability of re-expression by pharmacological treatment and DNA hypermethylation in promoter region showed close relationship not only in the limited cases but also in a genome-wide manner. Our data provide the evidence that aberrant promoter methylation of the candidate genes is more frequently seen in HCV-positive HCC than HBV-positive HCC.

4. 研 究 業 績

（1）学会における発表　　有　67th Annual Meeting of the Japanese Cancer Association (JCA)

（2）発表した論文　　無
今　投稿の準備

—日中医学協会助成事業—

肝癌における DNA 異常メチル化癌抑制遺伝子の網羅的な解析

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Abstract

HCC, the major type of primary liver cancer, is one of the most common cancers worldwide and a leading cause of death in many countries. Although many of the major viral and environmental risk factors for hepatocellular carcinoma (HCC) development have been unraveled, the genetic and epigenetic pathways leading to malignant transformation of liver cells have remained obscure. Epigenetic instability characterized by methylation of multiple cancer-related genes is gaining recognition as a key mechanism of tumor suppressor gene silencing in many human cancers, including HCC. Aberrant DNA methylation is deeply involved in human carcinogenesis through inappropriate gene silencing. Previous studies have reported that some tumor suppressor genes were silenced by aberrant promoter hypermethylation and the mRNA expression of these genes were reversible by 5-Aza-dC treatment. In this study, we performed genome-wide analysis of both transcriptional and epigenetic profile of HCC using microarray-based technologies. After treatment of HCC cell line Hep3B with 5-Aza-dC and/or TSA, changes of mRNA levels were measured by microarrays. Then, we compared upregulated genes to the list of candidate promoter hypermethylated genes identified by MeDIP-chip analysis. 2016 genes among 3266 hypermethylated genes showed expression level less than 50 in gene chip score in control cells (i.e., without 5-Aza-dC/TSA treatment), which were upregulated after 5-Aza-dC treatment in 336 genes (expression level more than 50 in gene chip score). And 274 genes among 3266 hypermethylated genes showed 2.5 fold expression than the genes without treatment. 451 genes were upregulated after 5-Aza-dC+TSA treatment. And 346 genes showed 2.5 fold expression than the genes without treatment. Array-based analysis revealed the capability of re-expression by pharmacological treatment and DNA hypermethylation in promoter region showed close relationship not only in the limited cases but also in a genome-wide manner. The presence of hepatitis viruses, especially HCV, could play a role in accelerating the methylation process that is involved in HCC development than HBV. Our data provide the evidence that aberrant promoter methylation of the candidate genes is more frequently seen in HCV-positive HCC than HBV-positive HCC.

Keywords: methylation, hepatocellular carcinoma, 5-aza-2'-deoxycytidine (5-Aza-dC), Trichostatin A (TSA)

Background/Purpose

Aberrant DNA methylation is deeply involved in human carcinogenesis through inappropriate gene silencing and chromosomal instability. Previous studies have revealed that some tumor suppressor genes were silenced by aberrant promoter hypermethylation and the genes were re-expressed by 5-aza-2'-deoxycytidine (5-Aza-dC) treatment. However, the genome-wide analysis of DNA methylation were poorly understood in hepatocellular carcinoma (HCC). In this study, we examined silencing caused by the aberrant methylation of those promoters in HCC and re-expression of the genes

in hepatoma cell line (Hep3B) after 5-Aza-dC treatment.

Materials and Methods

Liver cancer cell line, Hep3B cells was treated with 5-Aza-dC and/or Trichostatin A (TSA) to analyze the effect of pharmacological reversal of aberrantly DNA hypermethylation and/or histone deacetylation. Changes of mRNA levels after these treatments were measured by Affymetrix HG-U133 Plus 2.0 microarrays. Then, we compared re-expressed genes by 5-Aza-dC/TSA treatment to the list of candidate promoter hypermethylated genes identified by MeDIP-chip (methylated-DNA immunoprecipitation with high-resolution tiling array) analysis. A total 105 liver tissues, including 50 pairs of HCCs and the matched noncancerours liver tissues, and 5 normal liver tissues, were analyzed for promoter methylation status of the candidate genes by MassARRAY system.

Results

Relationship between gene expression and promoter methylation

The relation of upregulated genes after the treatment (5-Aza-dC or 5-Aza-dC+TSA) and hypermethylation-detected genes by MeDIP-chip analysis were determined. 2016 genes among 3266 hypermethylated genes showed expression level less than 50 in gene chip score in control cells (i.e., without 5-Aza-dC/TSA treatment), which were upregulated after 5-Aza-dC treatment in 336 genes (expression level more than 50 in gene chip score). And 274 genes among 3266 hypermethylated genes showed 2.5 fold expression than the genes without treatment (Fig. 1A). 451 genes were upregulated after 5-Aza-dC+TSA treatment. And 346 genes showed 2.5 fold expression than the genes without treatment (Fig. 1B).

MeDIP-chip

274 genes or 346 genes in Fig.1 included gene SFRP1, SOCS1 and the other candidate gene1, gene2 of HCC-specific and gene3 of HCV(+) HCC-specific hypermethylated genes were selected by MeDIP-chip analysis (HBV(+) HCC-specific hypermethylated genes couldn't be selected). We observed that SFRP1, SOCS1 and gene1, gene2 and gene3 showed significantly higher levels of methylation in Hep3B cells.

GeneChip score

In this study, SOCS1, gene1 and gene3 showed upregulation after the treatment. SOCS1 and gene1 which were hypermethylated in HCC and hypomethation in liver cirrhosis (LC) and normal liver tissue (NL) in MeDIP-chip analysis, and gene3 were only hypermethylated in HCV(+) HCC. Their expression showed silencing.

MassARRAY

SFRP1, SOCS1, gene1 and gene2 were frequently methylated, Gene3 was preferentially methylated in HCV(+) HCC (p=0.002, x^2-*test*).

Discussion

HCC, the major type of primary liver cancer, is one of the most common cancers worldwide and a leading cause of death in many countries. Although many of the major viral and environmental risk factors for HCC development have been unraveled, the genetic and epigenetic pathways leading to malignant transformation of liver cells have remained obscure. Epigenetic instability characterized by methylation of multiple cancer-related genes is gaining recognition as a key mechanism of tumor suppressor gene silencing in many human cancers, including HCC. In this study, we performed a detailed quantitative methylation analysis of a large number of methylation in a total 105 liver tissues, including 50 pairs of HCCs and the matched noncancerours liver tissues, and 5 normal liver tissues. The results presented herein clearly demonstrate that higher degree of methylation occurs in the

promoter region of hepatocellular carcinoma, and it is likely that methylation of some gene may sequentially progress and participate in the development of carcinogenesis. Another important observation is that the presence of hepatitis viruses, especially HCV, could play a role in accelerating the methylation process that is involved in HCC development than HBV.

Conclusion

Our data provide the evidence that aberrant promoter methylation of the candidate genes is more frequently seen in HCV-positive HCC than HBV-positive HCC.

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作成日：2009 年 3 月 10 日