学 位 論 文 の 要 旨

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GENE EXPRESSION PROFILE OF DBPA (DNA BINDING PROTEIN A) TRANSGENIC MICE

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論 文 内 容 の 要 旨

INTRODUCTION

We recently reported that the expression of dbpA (DNA binding protein A) is associated with advanced stages of human hepatocellular carcinoma (HCC), and that its transcription is positively regulated by E2F1 which is also implicated in hepatocarcinogenesis. To study the *in vivo* effect of dbpA on the hepatocarcinogenesis, we generated the dbpA-transgenic mouse that specifically expressed a transgene in hepatocytes. Here, we studied the effect of dbpA on the expression of other cellular genes by using microarray analyses. The expression profiles from livers of 31 and 32 week-old male transgenic mice (Tg (+)) that did not show any morphological changes and from livers of their male wild- type littermates (Tg (-)) were compared. Expression differences detected by microarray analyses were validated by reverse transcription-polymerase chain reaction (RT-PCR) using total RNA samples from livers of 3 pairs of Tg (+) and (-) mice. The 11 up-regulated genes included 7 carcinogenesis-related genes (Igfbp1, Tff3, Hpx, Orm2, Ctsl, Plg, Jdp1), and the 9 down-regulated genes included Car3 that is associated with the protection of cells from attack by oxygen radicals. Then we examined the *in vitro* effect of overexpression or knockdown of dbpA on gene expressions in the human HCC cell line Huh7. As for Igfbp1, we confirmed that its expression was reduced by siRNA targeting dbpA in Huh7

cells.

MATERIALS AND METHODS

The expression profiles from livers of 31 and 32 week-old male transgenic mice (Tg (+)) that did not show any morphological changes and from livers of their male wild-type littermates (Tg (-)) were compared. Total RNA was extracted from livers of 31 and 32 week-old male dbpA-transgenic mice (Tg (+)) and their male wild-type littermates (Tg (-)). For the microarray analysis, we used Agilent 22K 60-mer oligonucleotide microarray slides (Agilent, Palo Alto, CA) which covered 22,575 mouse genes. The result of microarray analysis was subjected to the validation by RT-PCR, using three pairs of male Tg(+) and Tg(-) mice including the two pairs used for microarray analysis. Reverse transcription (RT)-polymerase chain reaction (PCR) was performed using a Titan One Tube RT-PCR System (Roche) in accordance with the manufacturer's instruction.

To see the effect of overexpression or knockdown of dbpA *in vitro* on gene expressions that up-regulated or down-regulated in Tg (+) mouse, we transfected Huh7 cells with human dbpA expression vector or siRNA of human dbpA.

RESULTS AND DISCUSSION

The expression of 25 genes was significantly enhanced, whereas the expression of 20 genes was reduced in livers of 31 and 32 week-old male transgenic mice (Tg (+)) that did not show any morphological changes in common by microarray analyses. Expression differences detected by microarray analyses were validated by reverse transcription-polymerase chain reaction (RT-PCR) using total RNA samples from livers of 3 pairs of Tg (+) and (-) mice. The 11 up-regulated genes included 7 carcinogenesis-related genes (Igfbp1, Tff3, Hpx, Orm2, Ctsl, Plg, Jdp1), and the 9 down-regulated genes included Car3 that is associated with the protection of cells from attack by oxygen radicals. As for Igfbp1, we confirmed that its expression was reduced

by siRNA targeting dbpA in Huh7 cells.

It was unexpected for us to realize that as many as 7 out of 11 up-regulated genes were carcinogenesis-associated genes and that 1 out of 9 down-regulated was associated with the protection of cells from attack by oxygen radicals.

CONCLUSION

We showed that dbpA influenced the expression of carcinogenesis-associated genes. Our data suggested that dbpA could induce the steps of carcinogenesis by altering the cellular gene expressions.