論文の内容の要旨

論文題目： The roles of HSPB7 induction by p53 and HSPA5 glycosylation by GLANT6 in human carcinogenesis
（GLANT6によるHSPA５のグリコシル化とp53誘導分子HSPB7の発癌における機能解析）

氏　　名： 林嘉穎

Part I: Downregulation of the tumor suppressor HSPB7, involved in the p53-pathway, in renal cell carcinoma by hypermethylation

Introduction

Renal cell carcinoma (RCC) accounts for approximately 2% of all cancers worldwide and its incidence has increased by 2–3% in the last decade with higher in developed countries. However, the underlying mechanisms are largely unknown. For the advanced-stage patients, drugs for treatment are limited and the response rates are far from satisfaction. To better understand the molecular mechanisms of renal carcinogenesis and apply the information for the development of effective treatment and early diagnosis, we analyzed expression profile of renal cell carcinomas (RCCs) using microarray consisting of 27,648 cDNA or ESTs, and found a small heat shock protein, HSPB7, whose function in cancer is unknown, to be down-regulated in a great majority of human RCC samples. In this study, we attempted to address two key questions, (1) whether HSPB7 has the growth suppressive function and (2) how HSPB7 is down-regulated in RCCs. We here report for the first time that HSPB7 is likely to be a tumor suppressor which is frequently down-regulated by DNA methylation in RCCs and is involved in the p53 pathway.

Methods and result

We first confirmed down-regulation of HSPB7 in RCC by quantitative real-time PCR (qPCR) and immunohistochemical (IHC) experiments after the analysis of microarray data. qPCR result showed that HSPB7 mRNA expression was significantly down-regulated in 11 of 13 (85%) RCC tissues compared with the normal renal tissue (A, Figure 1), and in all of five RCC cell lines compared with normal HEK 293 and RPTEC cells (B, Figure 1). IHC analysis of a tissue array consisting of 11 pairs of human RCC sample revealed that the expression of HSPB7 was significantly higher in normal kidney tissues than that in RCC tissues (Table 1). We also detected HSPB7 expression mainly in the cytoplasm of normal renal tubular epithelial cells. To explore the expression patterns of HSPB7 in other normal tissues, we performed qPCR analysis using mRNAs isolated from 25 normal tissues. HSPB7 expression was detected ubiquitously in human tissues.

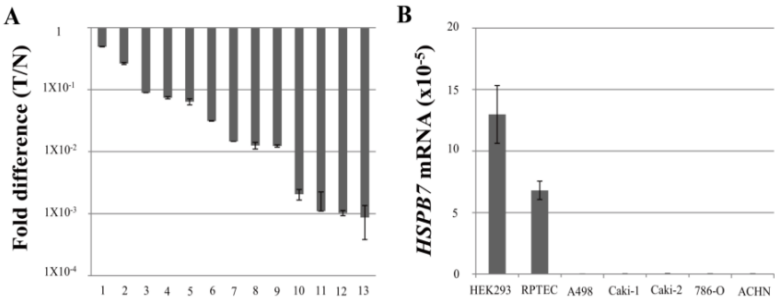

Figure 1. Down-regulation of HSPB7 in RCC.

Table I. Immunohistochemical expression of HSPB7 in RCC tissue array.

	Total	Low (-/+)	High (++/+++)	Fisher's t-test
Clear cell				
Cancer	9	7	2	P=0.015
Normal	9	1	8	
Papillary				
Cancer	2	1	1	-
Normal	2	0	2	
Total				
Cancer	11	8	3	P=0.008
Normal	11	1	10	

All tests were 2 sided and P<0.05 was considered to indicate a statistically significant difference.

Then, bisulfite sequencing of a genomic region of HSPB7 detected DNA hypermethylation of some segments of HSPB7 in RCC cells (region 4 in Figure 2) and concordantly a demethylating agent, 5-Aza-2'-deoxycytidine treatment of cancer cells restored HSPB7 expression significantly. In addition, we performed exon sequencing of HSPB7 in these five RCC cell lines, but no mutation or deletion/insertion was detected. This data indicated that suppression of HSPB7 in RCC was caused probably by DNA hypermethylation.

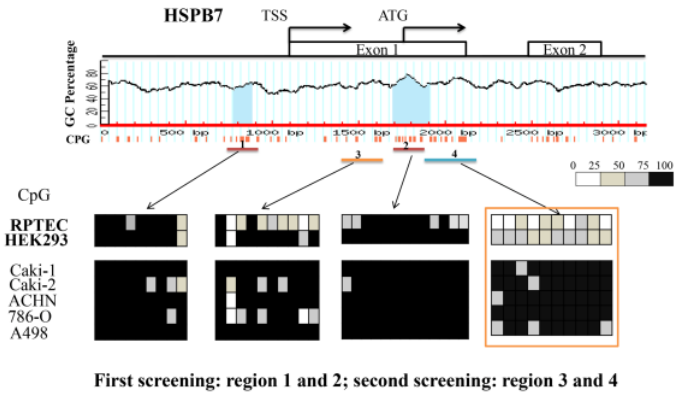

Figure 2. Epigenetic silencing of HSPB7 in RCC cell lines.

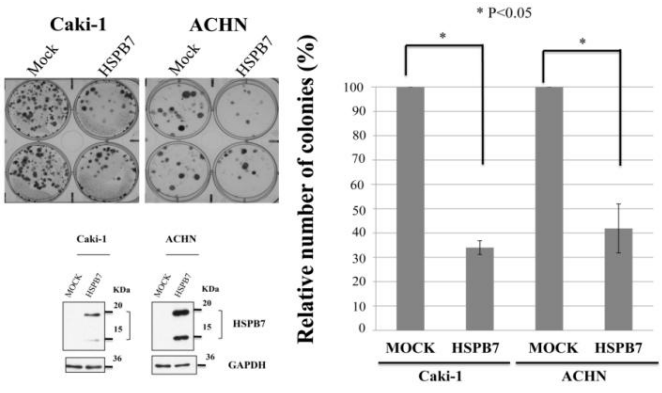

Figure 3. Ectopic HSPB7 expression suppressed RCC cell growth.

To study the effect of HSPB7 expression on tumor growth, Caki-1 and ACHN cells were transfected with HSPB7 expression vector, pCAGGSnHC-HSPB7-HA. Introduction of HSPB7 into these two cancer cell lines caused significant decrease in the number of colony, compared with corresponding mock-transfected controls (Figure 3). We also performed colony formation assay in other 3 RCC cell lines (Caki-2, A498, and 786-O) using the same vectors, and confirmed the similar growth-suppressive effects, implying that HSPB7 may function as a tumor suppressor gene.

To further elucidate the biological significance, we first investigated its possible involvement in the p53-pathway (Figure 4). We applied qPCR analysis to evaluate the expression of HSPB7 in NCI-H1299 (p53 null) cell lines with or without introduction of p53 using the adenovirus system. After the infection of Ad-p53, we observed induction of HSPB7 in a dose-dependent (A) and time-dependent manner (B), while no induction was observed in the control cells. Concordantly, DNA damage by adriamycin treatment induced HSPB7 expression in HCT116 cells with wild-type p53, but not in HCT116 cells without wild-type p53 (C and D), indicating that HSPB7 expression is regulated by wild-type p53. To further investigate whether HSPB7 is directly regulated by p53, we screened three possible p53-binding sites indicated by the p53-binding site search software developed by us, but neither of these candidate sites was confirmed to be a direct p53-binding site. Although there might be another site(s) that p53 might bind to, we are unable to conclude whether HSPB7 is directly or indirectly regulated by p53, it is certain that HSPB7 expression can be inducible by wild-type p53.

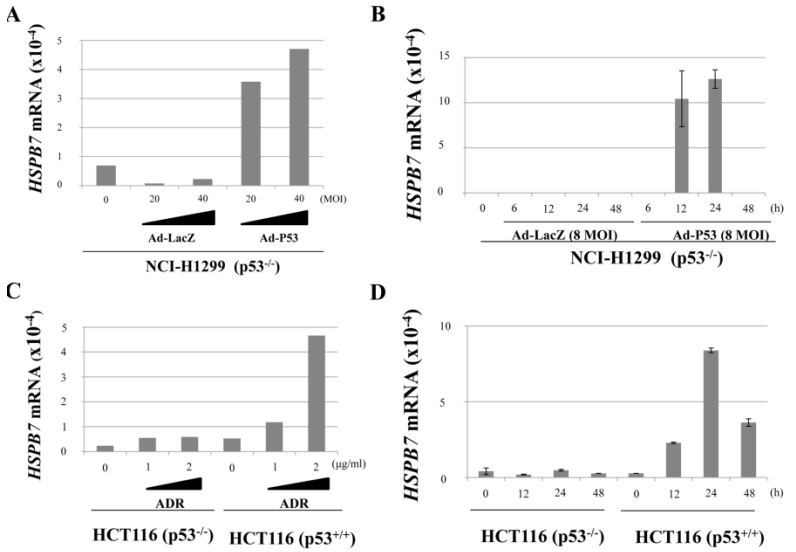

Figure 4. HSPB7 is regulated by p53.

Conclusion

In conclusion, we carried out a genome-wide gene expression analysis and identified HSPB7 to be a candidate tumor suppressor gene in RCC. We confirmed down-regulation of this gene caused by DNA hypermethylation, its growth suppressive effect in RCC cell lines and its p53-dependent expression, indicating the important roles of HSPB7 in renal carcinogenesis. Our finding could contribute to better understanding of the novel function of HSPB7 in cancer.

Part II: O-glycosylation and stabilization of HSPA5 by GALNT6

Abstract

We previously reported that overexpression of GALNT6 (polypeptide N-acetylgalactosaminyl transferase 6) which is a GalNAc-type (or mucin-type) O-glycosyltransferase, played a critical role in breast carcinogenesis. To further investigate the molecular function of GALNT6, we screened the substrates of GALNT6 through VVA (Vicia Villosa agglutinin) lectin (specific to GalNAc-Ser/Thr, called Tn-antigen) pull-down assay followed by mass spectrometry (MS) analysis. Here we report that HSPA5 (heat shock 70kDa protein 5 (also known as GRP78, glucose-regulated protein, 78kDa)) is a novel substrate of GALNT6. HSPA5 is highly expressed in cancers and involved in many cellular processes including in ER (endoplasmic reticulum) stress and autophagy, however whether O-glycosylation affects the function of HSPA5 and promote carcinogenesis is largely unknown. We found high expression level of exogenous HSPA5 drives Golgi-to-ER relocation of GALNT6 and they co-localize at ER in HeLa GALNT6 stable cells. We confirmed GALNT6 directly binds to and glycosylates HSPA5, and the ATPase domain of HSPA5 is important for their binding. During the process, GALNT6 itself is also auto-glycosylated. Six candidate O-glycosylation sites in HSPA5 were identified by mass spectrometry and four of them were located in the ATPase domain. Further study showed that GALNT6 stabilizes HSPA5 protein and mutation at one potential O-glycosylation site, T184A, affects the stability of HSPA5. Taken together, our findings imply that GALNT6 O-glycosylates and stabilizes HSPA5 protein, which may prolong the oncogenic effects of HSPA5 in breast cancer. Meanwhile, overexpression of HSPA5 can drive Golgi-to-ER relocation of GALNT6. The result may trigger O-glycosylation of multiple substrates of GALNT6 at ER. Our study revealed a novel mechanism of how GALNT6 and HSPA5 cooperate together to promote mammary carcinogenesis. And the O-glycosylated form of HSPA5 may be a good target for breast cancer therapy.