学 位 論 文 の 要 旨

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学 位 論 文 名　Simian Virus 40 Large T Antigen Targets the Microtubule-Stabilizing Protein TACC2

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論 文 内 容 の 要 旨

INTRODUCTION

Cellular transformation is essential for progression toward tumor development. The proteins involved in this process have been identified as oncogene products and tumor suppressors. Simian virus 40 (SV40), a polyomavirus of rhesus macaque origin, is a DNA tumor virus that can induce tumors in rodents and transform many types of cultured mammalian cells, including those of human origin. The SV40-encoded replication protein, designated large T antigen and referred to as T antigen, is a viral oncoprotein that modulates diverse cellular activities in genome integrity and cell cycle regulation, thereby promoting the early steps of oncogenic transformation. Although many studies have reported that T antigen inactivates p53 and the RB family of tumor suppressors, it is suggested that other regulatory proteins are required for SV40-induced cellular transformation. Polyomaviruses are highly specific to their host species but encode a similar T antigen. For human, JC and BK polyomavirus were discovered to be responsible for progressive multifocal encephalopathy and renal nephropathy, respectively. Analogous to SV40, both viruses encode T antigens that transform the cells in culture and promote tumor formation in animals. More recently, Merkel cell polyomavirus, a new virus whose genome is integrated into cellular DNA in the aggressive human skin cancers. These lines

of evidence raise the essential question how their oncoproteins function in the cells. In addition to the known targets of T antigen, this protein was recently reported to interact with Bub1, which has dual roles in spindle assembly checkpoint and chromosome congression. Thus, T antigen of SV40 may target as-yet unidentified cellular proteins to cause cell transformation and oncogenesis. The aim of this study is to investigate the new target of T antigen for SV40-induced cellular transformation. I found that simian virus 40 (SV40) large T antigen interacts with the transforming acidic coiled-coil-containing protein TACC2 which is involved in stabilizing microtubules in mitosis. The results suggest that TACC2 is a key target of T antigen for promoting mitotic defects leading to abnormal chromosomal and nuclear inheritance.

MATERIALS AND METHODS

To identify factors that interact with T antigen, I performed yeast two-hybrid screening using the region containing amino acids 250-708 of T antigen as bait. From a screening of approximately 7 x 10^6 independent transformants of 17-day-old mouse embryo cDNA libraries, I isolated four cDNA clones encoding the carboxyl-terminal TACC domain of TACC2. To determine a direct interaction between T antigen and TACC2, I prepared glutathione S-transferase (GST)-fused T antigen (250-708) and His-tagged TACC domains of human TACC proteins, and subjected them to an *in vitro* pull-down analysis. To visualize the subcellular localizations of T antigen and TACC2, I carried out an immunofluorescence analysis in HeLa cells. To address the function of TACC2 in human cells, I performed knockdown of endogenous TACC2 using specific small inhibitory RNAs (siRNAs), and measured the distances between two centrosomes in mitosis. Also I performed a microtubule elongation assay using monastrol-treated cells. To further investigate the dynamics in cell cycle progression, I carried out a time-lapse imaging analysis of HeLa cells stably expressing GFP-fused histone H2B. Fluorescence and DIC time lapse analyses were performed using a microscope (Olympus XI70). The camera, shutters and filter wheel were controlled by the MetaMorph imaging software. Finally to demonstrate complex formation by T

antigen and TACC2 *in vivo*, I performed an immunoprecipitation analysis.

RESULTS AND DISCUSSION

By yeast two-hybrid screening and subsequent biochemical analyses, I found that T antigen interacts with transforming acidic coiled-coil (TACC) protein 2, designated TACC2. There are at least three TACC family proteins (TACC1, TACC2 and TACC3), all of which contain the conserved TACC domain and have been possibly implicated in tumorigenesis. TACC1 and TACC3 were found to be overexpressed in human cancers, while overexpression of TACC1 induced the transformation of primary mouse cells in culture. On the other hand, TACC2 was downregulated as breast tumors became more malignant. Overexpression of TACC2 in these cells reverted malignant phenotypes to benign phenotypes both *in vivo* and *in vitro*, suggesting that TACC2 may function as a potential tumor suppressor. My functional analyses showed that human TACC2 has a crucial role in microtubule stabilization in cultured cells, and that T antigen binds TACC2 for inducing mitotic defects and chromosomal instability. These mitotic defects are caused by amino-terminal-deleted T antigen which minimally interacts with TACC2, while T antigen-induced microtubule destabilization is suppressed by overexpressing TACC2. In addition to changes in the chromosome content, T antigen or the loss of TACC2 function causes nuclear structural abnormalities after cell division. My findings suggest that TACC2 is a key target of T antigen for promoting mitotic defects leading to abnormal chromosomal and nuclear inheritance. These findings may provide the first evidence that a viral oncoprotein can directly disrupt microtubule regulation, and shed light on the molecular basis of the initiation of cellular transformation.

CONCLUSION

The results of this study suggest that inactivation of TACC2 by T antigen promotes mitotic defects due to microtubule dysfunction, resulting in chromosomal instability and nuclear structural disorganization. Besides known anti-tumor proteins such as p53 and RB, T antigen selectively interacts with TACC2 and may lead to cell transformation.