論文の内容の要旨

Identification and Functional Analysis of Genes Critical to Tumorigenesis
（腫瘍形成に必須な遺伝子の同定と機能解析）

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Introduction

Cancer is a multi-faceted disease whose effective treatment relies on the exploitation of its addiction to certain gene products, the identity of which are generally elucidated through the mutational patterns present in a given cancer. However, it is not always immediately obvious what genes products a given cancer is addicted to. Even when considering mutations along pathway lines, certain cancer types show a surprising amount of variability in their mutation patterns. One such cancer is ovarian clear cell carcinoma (OCCC), with just over half of all OCCC cases showing little if any uniformity in mutations. Particularly in Japan, the proper treatment of OCCC is of great concern, owing not only to the 8% efficacy traditional treatment methods show against its higher staged cases, but also to the nearly three-times greater incidence rate of OCCC in Japan compared to of the west. The goal of my research is to better understand the genes that OCCC is addicted to, in order to allow for the

development of novel therapies against it.

Methods and Results

In chapter 1, in order to identify genes critical to the growth of OCCC, I carried out a comprehensive CRISPR-Cas9 knockout screen against cell growth using an OCCC cell line, JHOC5, with none of the mutations hallmark to OCCC, as well as a normal ovarian surface epithelium cell line, OSE3, as a control. Gene ontology analyses revealed that genes related to mRNA splicing were significantly enriched in the JHOC5-specific screening results. Furthermore, comparison of screening results with a public dataset containing CRISPR-Cas9 screening results against various cancer cell lines of various origins led me to identify one gene as an ovarian cancer-specific genetic dependency.

In chapter 2, I carry out functional analysis on this gene. RNAi-mediated knockdown in various OCCC and OSE cell lines confirmed the results of the CRISPR-Cas9 screening carried out in the previous chapter, namely that knockdown results in severe growth inhibition of OCCC cell lines, but minimal growth inhibition of OSE cell lines. Further analyses carried out to identify the cause of this OCCC-specific growth inhibition revealed that knockdown of this gene causes OCCC cells, but not OSE cells, to undergo apoptosis in a p53-independent manner. Further studies on the mechanism by which knockdown induces apoptosis revealed that the protein of interest binds to several proteins in implicated in the

regulation of apoptosis. Taken together, these results suggest that the gene identified in the CRISPR-Cas9 screening in chapter I may play a role in OCCC tumorigenesis, and is a promising therapeutic target for the treatment of OCCC cases independent of p53 mutational status.