School of Integrative and Global Majors
Ph.D. Program in Human Biology (HBP)

論文概要

Dissertation Abstract

Title of Doctor Dissertation:

Function of the interaction between THG-1 and NRBP1 in the esophageal squamous cell carcinoma development

(扁平上皮癌の進展における THG-1 と NRBP1 の結合の役割)

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Abstract

Esophageal cancer is the sixth leading cause of death by cancer, and eighth most frequent form of cancer in the world. Esophageal squamous cell carcinoma is the most common type of esophageal cancer in Asian countries. Due to the significant development of anti-cancer drugs in recent years, esophageal squamous cell carcinoma patients could survive longer, however, with a poor prognosis at advanced stages. In addition, it is difficult for esophagus cancer patient to feel the symptom at early stage because of absence of diagnostic marker, so most of esophageal cancer patients were diagnosed at advanced stage with a difficulty in swallowing. The partial esophageal resection following radiation therapy or chemoradiotherapy is the main treatment for esophageal cancer, which can result from an inaccurate prognosis and has, approximately, a 10% five-year survival rate due to severe metastasis – with likely recurrence by chemoradioresistant esophageal cancer cells. Therefore, understanding the biological events and molecular mechanisms of esophageal squamous cell carcinoma development could propose novel molecular targets, effective treatments and cancer prevention for esophageal squamous cell carcinoma patients.

THG-1 is localised in the basal layer of stratified squamous epithelium and ubiquitously overexpressed in squamous cell carcinomas (SCC) by unknown mechanism. Despite understanding the biological roles of THG-1 for a decade, the function of THG-1 in cancer development has not been discovered. We previously identified significant reduction of cell proliferation, anchorage-independent growth and cell migration from THG-1 deficient esophageal SCC cells. To determine the molecular mechanism how THG-1 functions in the development of esophageal SCC, we investigated THG-1 interacting molecules through proteomics approach. We found that THG-1

interacts with several factors regulating cell proliferation, metabolism and response to the microenvironmental factors including nuclear receptor binding protein 1 (NRBP1).

NRBP1 is a multidomain putative adaptor protein and recent studies demonstrated its tumor suppressive functions such as negative regulation of β-catenin. We therefore investigated the function of the interaction between THG-1 and NRBP1 to identify the role of THG-1 in tumorigenesis of SCC. This study conveys the role of THG-1 overexpression in the stimulation of β-catenin signaling through antagonizing NRBP1. Additionally, this study first discovered the molecular mechanism how NRBP1 downregulates β-catenin protein and its signaling. Thus, β-catenin targeting chemotherapy is promising to cure patients from THG-1 overexpressed esophageal SCC.

<Summary>

This study identified the function of THG-1 in the development of esophageal squamous cell carcinoma cells. THG-1 promoted cell proliferation, anchorage-independent growth and cell migration ability through promotion of β-catenin signaling pathway following interacting with two LXXLL motifs of NRBP1 and antagonization of NRBP1. NRBP1 negatively regulates β-catenin through interaction and ubiquitination as a E3 ligase, and THG-1 interrupts NRBP1-β-catenin interaction and rescue β-catenin protein expression, thereby leading to the activation of β-catenin target genes and promotion of tumorigenic development of cancer cells.

<Discussion>

We established that THG-1 has crucial role in tumorigenesis of esophageal squamous cell carcinoma cells including cell proliferation, anchorage-independent tumor growth and cell migration from this study. Also, we found out that the amount of nucleus-localised β-catenin protein was rescued by THG-1 overexpression through the interacting with E3-ubiquitin ligase NRBP1, a negative regulator of β-catenin. The overexpression of β-catenin in esophageal squamous cell carcinoma is consistent with the clinical database, and it implies that dysregulation of β-catenin by THG-1 overexpression would be one of major mechanism of esophageal squamous cell carcinoma development.

NRBP1 has been identified as a key tumor suppressor and E3 ubiquitin ligase in cellular homeostasis. *Liao et al*. revealed that NRBP1 negatively regulates colorectal cancer cell proliferation *in vitro* and *in vivo*. *Wei et al*. showed that NRBP1 suppressed the proliferation and negatively regulated β-catenin. However, the mechanism how does NRBP1 downregulate β-catenin was unknown. This study discovered the novel mechanism of negative regulation of β-catenin by NRBP1. It was shown that E3-ubiquitin ligase NRBP1 directly binds to β-catenin and leads to ubiquitination and proteasomal degradation of β-catenin. Destruction complex including APC has been identified as major β-catenin negative regulatory mechanism in colon cancer model, but there

has been no information regarding the mechanism of β-catenin dysregulation in esophageal squamous cell carcinoma. We also identified that NRBP1 targets even non-phosphorylated form of β-catenin. We found out that NRBP1 could target both phosphorylated β-catenin and non-phosphorylated β-catenin, compared with βTrCP which only targets phosphorylated form of β-catenin. It conveys that NRBP1 is a critical negative regulator of β-catenin. Considering this observation with recent studies showing fewer expression of NRBP1 in colorectal cancer cells, low expression of NRBP1 may synergically contribute the dysregulation of β-catenin as well as APC mutation or CTNNB1 mutation in colorectal cancer cells which are well-known character of colorectal cancer.

Liao et al. also identified the function of NRBP1 in the regulation of caspase-dependent intrinsic apoptosis mediated by JNK signaling pathway using colorectal cancer cells. They observed large amount of cleaved caspase-9 and cleaved caspase-3, which are factors leading to cell death, from NRBP1 overexpressing colorectal cancer cells. Also, NRBP1 overexpression suppressed anti-apoptotic factor Bcl-2 and increased pro-apoptotic factor Bax and cytosolic cytochrome *c*, an intermediate regulator of apoptosis. Consistently, NRBP1 knockdown promoted Bcl-2 and suppressed Bax and cytosolic cytochrome *c*. Dysregulation of apoptosis is also important oncogenic cellular process, so function of THG-1 overexpression in the regulation of apoptosis by NRBP1 requires further analysis in the future.

We identified the enhanced β-catenin signaling by THG-1 overexpression in squamous cell carcinoma cells. It suggests that β-catenin targeting molecular therapy would be considered for the treatment of esophageal squamous cell carcinoma patient. There are several β-catenin/TCF targeting molecules such as PNU-74654, iCRT3, iCRT5, iCRT14, 2,4-diamino-quinazoline derivative and BC21. With those molecules, it is promising to do *in vivo* mouse experiment with esophageal squamous cell carcinoma cells as a further study.