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学位の種類	博士（医学）
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審査研究科	人間総合科学研究科
学位論文題目	TSC22D4/THG-1 suppresses cellular senescence in esophageal squamous cell carcinoma （TSC22D4/THG-1 は食道扁平上皮癌の細胞老化を抑制する）

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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, ZHANG XIN describes the role of TSC22D4/THG-1 in cellular senescence in esophageal squamous carcinoma. The summary is as follows:

（**目的**　Purpose）

Esophageal cancer is the seventh most common cancer worldwide and the sixth most common cause of cancer-related death. Although the significant development of diagnostic methods and combined therapy in recent years has led to a more prolonged survival of esophageal SCC (ESCC) patients, the overall survival rate is still poor in the advanced stages. TSC22 homologous gene-1 (THG-1) is a member of the TGF-β 1 stimulated clone 22 (TSC22) family, which is located at the basal layer in healthy cells but highly expressed in squamous cell carcinoma. The previous study in the applicant's lab had already found that THG-1 has an essential role in tumorigenic activity in ESCC. As knockdown of *THG-1* in ESCC shows a reduction of cell proliferation and the xenograft tumor size. However, the molecular mechanism of THG-1 in ESCC is still unknown. Therefore, the applicant aimed to generate *THG-1* KO cell lines in this study to investigate the role of THG-1 in ESCC, and find an effective molecular targeting therapy to treat ESCC.

（**対象と方法**　Materials and Methods）

In this research, molecular biological experiments were performed. CRISPR/Cas9 gene-editing system was employed to generate *THG-1* knockout (KO) in TE13 cell line. Senescence-associated β-galactosidase (SA-β-gal)

staining assay was performed to check the number of senescent positive cells. Western blotting was performed to examine the expression levels of proteins. Quantitative PCR (qPCR) was used to quantify mRNA expression levels. Immunofluorescence staining was used to investigate the localization of protein expression in each cell. Cell proliferation assay was used to check the cell growth condition. siRNA gene silencing was performed to knockdown *JUNB* in *THG-1* KO cells. Short hairpin RNA (shRNA)-mediated *THG-1* knockdown was used to confirm THG-1 function in ESCC.

（**結果**　Results）

The applicant successfully generated *THG-1* KO cell lines by CRISPR/Cas9 gene-editing system in TE13 cells. *THG-1* KO cells exhibited delayed cell proliferation and manifest accelerated senescent cell phenotypes such as cell size enlargement, SA-β-gal activity, and enhanced expression of P21(*CDKN1*A). Immunofluorescence staining detected P21 mainly in enlarged nuclei. Increased expression of *JUNB* was diffusely observed, and *JUNB* knockdown suppressed the expression of *CDKN1A* and SA-β-galactosidase activity. Besides, similar results were obtained by shRNA-mediated knockdown of *THG-1* in TE13 cells and another ESCC cell line KYSE 1260, as *THG-1* knockdown tended to stimulate the transcription of *CDKN1A* and *JUNB* and promote cellular senescence. Based on these results, the applicant concluded that *THG-1* suppresses cellular senescence at least partially through suppression of *JUNB* and subsequent suppression of *CDKN1A*.

（**考察**　Discussion）

Senescence is one of the critical tumor suppression mechanisms which limits cancer initiation and progression through various molecular pathways. In this study, the applicant found that *THG-1* KO can activate cellular senescence in cancer cells though inducing P21 (*CDKN1A*) transcription, leading to increased protein levels, in a manner which is independent of P53. The induction of P21 (*CDKN1A*) is thought to be crucial for the induction of cellular senescence in cancerous cells. P21 (*CDKN1A*) transcription is known to be regulated by JUN family proteins. In this study, JUNB was found to be involved in P21 (*CDKN1A*) induction and the induction of cellular senescence in *THG-1* KO cells. It is important to note that the possibility of additional involvement of other factors in this process cannot be ruled out. JUN family proteins, including JUNB, have been reported to act as transcriptional activators of P21 (*CDKN1A*) and P16 (*CDKN2A*), and to regulate cell proliferation, differentiation, and stress responses.

Furthermore, JUNB expression is found to be low in human squamous cell carcinomas. Exogenous expression of JUNB inhibited tumorigenesis in these tumor cells. Therefore, the activation of JUNB in cancer cells is an essential factor in the suppression of cancer cell proliferation. Although the applicant identified the senescence pathway present in *THG-1* KO cells, the mechanism that links THG-1 to JUNB has not been identified. It was confirmed that THG-1 mainly localizes in the cytoplasm, suggesting that THG-1 does not act as a direct transcriptional regulator. In the oncogene ($BRAF^{E600}$)-induced cellular senescence model, TSC-22/TSC22D1 play crucial roles in the induction of oncogene-induced cellular senescence. Since THG-1 can bind to TSC-22, it may be antagonized by THG-1 in tumor cells. It was previously reported that TSC-22 promotes P21 (*CDKN1A*) promoter activity through interaction with c-MYC in the nucleus. Further studies, including identifying THG-1 binding partners and the mechanism of senescence induction by TSC-22, are required to understand the molecular function of THG-1 completely.

審査の結果の要旨
Abstract of assessment result

（**批評**　General Comments）

In the present study, the applicant first successfully established *THG-1* KO in ESCC and found that THG-1 is involved in suppression of cellular senescence. Activation of JUNB-P21(CDKN1A) axis plays a crucial role on cellular senescence in *THG-1* KO cells. These findings provide a novel insight into the induction of cellular senescence in ESCC, providing the possibility for future therapy including combination or synthetic lethal therapy to induce cellular senescence in ESCC.

（**最終試験の結果**　Assessment）

The final examination committee conducted a meeting as a final examination on 15 January, 2020. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（**結論**　Conclusion）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.