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学位論文題目	Roles of TIF1β Phosphorylation in Colorectal Cancer （大腸がんにおける TIF1β のリン酸化の役割）

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論文の要旨
Abstract of thesis

Transcription Intermediary Factor 1 beta (TIF1β) is an intermediary factor of transcription and epigenetic modulator of gene expression involved in various biological processes, including embryonic development, cellular differentiation and cancer initiation/progression.　TIF1β contains numerous phosphorylation sties, in which serine 473 has been well-studied. TIF1β was shown to act as a molecular switch to regulate the chromatin remodeling and expression of particular genes through its phosphorylation status at serine 473. Protein phosphatase 4 (PP4) is a serine/threonine phosphatase known to function in DNA double-strand break repair and cell cycle by regulating phosphorylation of its target proteins. Although the human PP4 phosphatase complex (PP4C) directly dephosphorylates TIF1β phosphoresidue serine 473 (TIF1β-Ser473), the molecular mechanism of phosphorylation/dephosphorylation of TIF1β and its roles in colorectal cancer (CRC) are unknown. Hydrogen peroxide (H_2O_2), one of reactive oxygen species (ROS), induces oxidative stress and acts as a signaling molecule involved in the growth of tumor cells. However, the effect of oxidative stress on TIF1β and how it alters the biological functions of CRC cells have not been determined. The overall goal of the applicant is to understand whether the homeostatic balance of phosphorylation of TIF1β is critical in the multistep processes of colorectal carcinogenesis. The thesis project is divided into two parts, through which the applicant proposed to achieve her goal.

Project I: PP4 dephosphorylates growth factor induced TIF1β phosphorylation in colorectal cancer

1.1. Purpose To elucidate the biological correlation of TIF1β phosphorylation with PP4 in CRC

1.2. Materials and Methods

i. The expression of the PP4C protein and the phosphorylation level of TIF1β were detected by immunohistochemistry multiple-staining on CRC patients' tumor tissues.

ii. The upstream signaling pathway of TIF1β phosphorylation was examined under EGF induction with kinases inhibitors (AG1478 and U0126) and analyzed by Western blot.

iii. PP4C regulated dephosphorylation of TIF1β at Ser473 was investigated under EGF induction with transient PP4C expression in HEK293T by Western blot and in vitro phosphatase assay. Also, it was further confirmed by PP4C expressing cells under EGF induction compared with control cells.

iv. The functions of PP4C were evaluated by cell proliferation assay, colony formation assay, sphere formation assay and *in vivo* tumor formation assay.

v. To examine the potential of anticancer drug resistance in PP4C expressing cells, cells were treated with anticancer drugs. Cell survival was measured by MTT assay.

1.3. Results

i. High expression of PP4C was found in high grade of CRC tissues.

ii. The phosphorylation level of TIF1β exhibited a negative correlation with PP4C in CRC.

iii. EGF induced TIF1β phosphorylation through ERK signaling pathway.

iv. PP4C dephosphorylated TIF1β phosphorylation.

v. PP4C suppressed proliferation and tumorigenesis and increased anticancer drug resistance.

1.4. Discussion

The data showed a negative correlation between PP4C expression and TIF1β phosphorylation in CRC. TIF1β phosphorylation was transiently induced through ERK signaling pathway and was suppressed in the presence of PP4C. In addition, PP4 suppressed cell proliferation, tumor growth and promoted anticancer drug resistance. These findings provided evidence supporting that TIF1β can be post-translationally modified upon growth signal in cancer. The potential value of PP4C in the regulation of TIF1β phosphorylation on CRC aggressiveness suggested that inhibition of PP4C might be an option to enhance the chemotherapeutic effect of conventional anticancer drugs toward CRC.

Project II: Oxidative Stress-induced phosphorylation in TIF1β

2.1. Purpose To investigate the effect of oxidative stress on TIF1β in CRC cells

2.2. Materials and Methods The effects of H_2O_2 and the kinases responsible on TIF1β in CRC cells were examined and analyzed by western blot and MTT assay.

2.3. Results H_2O_2-induced cell death was enhanced by TIF1β knockdown in DLD-1 cells. In addition, phosphorylation of TIF1β was induced by H_2O_2 and mediated through MAPKs in DLD-1 and HCT-116 cells.

2.4. Discussion The data indicated that H_2O_2 activated MAPK, particularly p38 MAPK, and stimulated phosphorylation of TIF1β-S473 to faciliate more efficient DNA repair to assist tumor cell survival against exogenous ROS at both physiological levels and chemptherapeutic levels.

In summary, the applicant directly demonstrated that TIF1β can be phosphorylated upon growth signal (such as EGF) for tumor growth, and oxidative stress for stress defense in CRC through different MAPK pathways. Future studies include how phosphorylation of TIF1β-S473 is regulated by the multiple pathways and whether feedback regulatory mechanism takes a central or secondary role in ROS-mediated, MAPK-driven activation in cancer cells.

審査の要旨
Abstract of assessment result

【批評 Review】
The applicant demonstrated the importance of TIF1β-S473 phosphorylation through a homeostatic balance between PP4C and Ras/MEK/ERK signaling pathway, which regulates cellular proliferation and tumor aggressivenss in human colorectal cancer. Furthermore, the applicant establishes S473 as the common activation hub for TIF1β to excert its functions on chromatin regulation (DNA damage response) and cell survival and growth, in response to different stimuli in the microenviroenment in both normal conditions and in tumorigenesis. These findings provided a basis for the potential novel cancer therapy.

【最終試験の結果 Result】
The final examination committee conducted a meeting as a final examination on 15th September, 2017. 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】
Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Human Biology.