京都大学	博士（　医学　）	氏名	Nguyen Vu Hoang Trang
論文題目	Combination therapy with WEE1 inhibition and trifluridine/tipiracil against esophageal squamous cell carcinoma （食道扁平上皮癌に対する WEE1 阻害剤とトリフルリジン／チピラシル合剤の併用療法の開発）		

（論文内容の要旨）

This study explores a potential therapeutic strategy for esophageal squamous cell carcinoma (ESCC). Despite recent advances in cancer treatment, the prognosis for patients with ESCC remains poor and ESCC remains one of the deadliest cancers. The researchers investigate a novel therapeutic strategy based on synthetic lethality combining a drug called TAS-102, which consists of trifluridine (FTD) and tipiracil (TPI), together with a WEE1 inhibitor as a potential treatment for ESCC.

FTD/TPI is an orally administered drug formulated as a fixed combination (1:0.5) of FTD and TPI. FTD is incorporated into DNA as a thymidine analog that induces DNA damage, whereas the TPI component inhibits the degradation of FTD so that the blood concentration of FTD is maintained. In previous clinical research, FTD/TPI showed efficacy against refractory metastatic gastric cancer and colorectal cancer. However, this single treatment's antitumor effect against ESCC was not sufficient.

Synthetic lethality is a concept in genetics and cancer research where the simultaneous disruption or perturbation of two specific pathways or genes in a cell leads to cell death or vulnerability to DNA damage. This concept is often exploited in cancer therapy and drug development to target cancer cells while sparing normal cells. In ESCC, the *TP53* gene, a crucial component of the ATM-CHK2-p53 pathway governing DNA damage response, is frequently mutated. Consequently, ESCC heavily relies on the ATR-CHK1-WEE1 pathway to safeguard DNA integrity. Therefore, it is hypothesized that a combination therapy with WEE1 inhibitor for *TP53*-mutant ESCC may effectively induce synthetic lethality by inhibiting DNA damage response through perturbation of these two pathways, resulting in efficacy of ESCC treatment.

FTD induced single-strand DNA damage in ESCC cells, as evidenced by phosphorylation of replication protein 32, leading to the activation the ATR-CHK1-WEE1 pathway. Subsequently, p-CHK1 and p-CDK1, inactivated forms of CDK1, were induced, indicating cellular DNA damage response (DDR) activation. CDK1, a member of the cyclin-dependent kinases (CDK) family, can drive cells into mitosis by combining with cyclin B at the late G2 stage. Meanwhile, CDK1 inhibition is also reported to be associated with the DDR process. Indeed, CDK1 activity is reported to be regulated tightly by WEE1, a downstream substrate of DDR. Taken these together, it is suggested that deactivation of CDK1 causes a temporary pause of the cell cycle to allow for cellular DNA damage repair before proceeding to mitosis.

Next, a WEE1 inhibitor, MK1775, was shown to suppress CDK1 phosphorylation (Tyr15) and reactivated CDK1 in ESCC cells, indicating inhibition of DDR and allowing the cells to move into mitosis, indicated by the increase of phospho-histone H3-positive cells.
These results support the hypothesis that the combination of FTD and the WEE1 inhibitor, MK1775, induces an increase of mitotic cells without FTD-induced DNA repair leading to cell death.

Subsequently, the data showed that FTD combined with a WEE1 inhibitor increased FTD-mediated DNA damages, namely, DNA double-strand breaks, as indicated by increased γ-H2AX expression *in vitro*. The addition of MK1775 induced the sensitivity of FTD-treated ESCC cells and remarkedly decreased IC_{50} concentration of FTD compared with monotherapy. Moreover, the combination treatment with FTD/TPI and WEE1 inhibitor significantly suppressed tumor growth of ESCC-derived xenograft models. No significant adverse events were observed during the treatment. These results suggest an opportunity to apply this combination in a future clinical trial.

Through these experiments, it is revealed that the novel combination therapy between FTD/TPI and a WEE1 inhibitor is a robust candidate for a novel treatment strategy for ESCC.

（論文審査の結果の要旨）

本論文では、食道扁平上皮癌（ESCC）の新たな治療戦略を探索し、トリフルリジン（FTD）とチピラシル（TPI）の合剤である TAS-102 と、WEE1 阻害剤との併用療法を ESCC に対する新規治療法として検討している。
FTD はチミジン誘導体として DNA に取り込まれる。TPI は FTD の分解を阻害し、FTD の濃度を維持する。FTD/TPI は転移性胃癌や大腸癌に対して有効性が示され既に臨床で使用されているが、ESCC 症例に対する第 2 相試験では有効性が認められなかった。ESCC では、DNA 損傷応答を制御する ATM-CHK2-p53 経路の重要な構成要素である TP53 遺伝子が高頻度に変異しているため、他方の DNA 損傷応答経路である ATR-CHK1-WEE1 経路に依存している。以上の背景から、申請者は、TP53 変異頻度の高い ESCC における、FTD/TPI・WEE1 阻害剤併用療法の開発を研究目的とした。
FTD が ESCC 細胞において単鎖 DNA 損傷を誘導し、ATR-CHK1-WEE1 経路が活性化され CDK1 が不活性化されることが示された。WEE1 阻害剤が、CDK1 の抑制性のリン酸化（Tyr15）を抑制し、CDK1 を再活性化させることが示された。さらに、FTD と WEE1 阻害剤の併用が、γ-H2AX によって示される DNA 二本鎖切断を増加させることが示された。以上の結果は、FTD と WEE1 阻害剤の併用が、FTD による DNA 損傷が未修復のまま細胞分裂に進み細胞死を引き起こすことを示唆する。
さらに、FTD/TPI と WEE1 阻害剤の併用治療は、ESCC マウスモデルの腫瘍増殖を抑制した。この結果は、FTD/TPI と WEE1 阻害剤の併用療法を将来的な臨床試験に応用する可能性を示唆する。

以上の研究は食道扁平上皮癌の DNA 損傷への応答機序の解明に貢献し DNA 損傷修復機構を標的とする新規分子標的療法の開発に寄与するところが多い。

　　したがって、本論文は博士（　医学　）の学位論文として価値あるものと認める。

　　なお、本学位授与申請者は、令和 6 年　2 月　15 日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。