論 文 の 内 容 の 要 旨

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論 文 題 目

SIRT1 regulates the chemoresistance, and invasiveness of ovarian carcinoma cells

（SIRT1 は卵巣癌細胞の抗がん剤耐性および浸潤能を調節する）

（論文の内容の要旨）

The incidence of epithelial ovarian carcinoma (OvCa) has markedly increased in Japan. Current treatment approaches for advanced OvCa including debulking surgery and adjuvant platinum-based chemotherapy have offered minimal survival benefits because of frequent recurrences and increasing drug resistance leading to treatment failures. Therefore, it is urgent to discover the new therapeutic targets overcoming the resistance to anticancer drugs.

Sirtuin 1 (SIRT1) is an NAD-dependent protein deacetylase involved in many cellular processes including the regulation of cell cycle, apoptosis, senescence, metabolism, DNA repair etc., through the suppression by deacetylation of histones and other target proteins such as p53. **SIRT1 is also known as a longevity gene** because it suppresses cell death and prolongs the survival under severe conditions such as low nutrition. SIRT1 was originally considered to function as a tumor suppressor gene. However, it seems to be a "double-edged sword" because its functions may **act as an advantage for the survival of cancer cells** as well as normal cells. We previously demonstrated that SIRT1 enhanced the chemoresistance and aggressiveness of endometrial carcinoma cells. In addition, we recently reported that **the overexpression of SIRT1 in OvCa tissue was a poor prognostic factor in OvCa patients** (Mvunta DH et al. Appl Immunohistochem Mol Morphol. 2016) **The present study aimed to clarify the mechanistic insights into the expression and function of SIRT1 in OvCa cells.**

First, we demonstrated that the expressions of SIRT1 in a panel of human OvCa cell lines was stronger than that of immortalized ovarian surface epithelium cell line (OSE7E) by the quantitative real-time RT-PCR (qRT-PCR) and Western blotting (WB). We then examined the effect of SIRT1 on cell proliferation and chemoresistance in 3 CCC cell lines (RMG1, TOV21G, and ES2) and one cisplatin-resistant EC cell line (A2780CDDP) by SIRT1 overexpression (-OEX) or knockdown (-KD). The WST1 assay revealed that **SIRT1-KD decreased the proliferation ($P<0.05$) and chemoresistance ($P<0.05$).** The treatment with selective SIRT1 inhibitor, **EX527**, also attenuated the chemoresistance of RMG1 cells ($P<0.05$). In contrast, **SIRT1-OEX enhanced chemoresistance** ($P<0.05$). **Apoptotic cells under CDDP were increased by SIRT1-KD** ($P<0.05$) **and decreased by SIRT1-OEX** ($P<0.05$). To clarify the underlying mechanism of these effects, the expression of apoptosis-related proteins such as BAX, BCL-2 was examined; however, no difference was observed by SIRT1-OEX and -KD.

We next focused on the effect of SIRT1 on the "stemness". Soft-agar colony formation assay, which evaluates the stemness, revealed that the number of ES2 colonies was increased by SIRT1-OEX ($P<0.05$) and decreased by the treatment with EX527 ($P<0.05$). The mRNA expression of stem cell markers (Nanog, Lin28, Sox2, Smo, and Bmi-1) was increased by SIRT1-OEX ($P<0.05$). These findings suggest that SIRT1 may act to keep the stemness, resulting in chemoresistance.

Since resistance to oxidative stress is an important mechanism of chemoresistance in cancer stem cells (CSCs), we investigated the effect of SIRT1 on oxidative stress. The dichlorodihydrofluorescein diacetate (DCF-DA) assay which detects the production of reactive oxygen species (ROS) revealed that **SIRT1-KD increased ROS production ($P<0.05$), whereas SIRT1-OEX decreased ROS production ($P<0.05$).** To elucidate the underlying mechanism, we then examined the involvement of antioxidant molecules. Glutathione, one of the important endogenous antioxidant, was decreased by SIRT1-KD in TOV21G cells ($P<0.05$). The expression of antioxidative enzymes, such as heme oxygenase-1 (HO-1) and thioredoxin (TRDX), was decreased by SIRT1-KD in TOV21G, ES2, and A2780CDDP, and increased by SIRT1-OEX in ES2 cells. The expressions of CD44v, known as one of the major CSC marker which acts with xCT to decrease ROS, was decreased by SIRT1-KD and increased by SIRT1-OEX.

We then examined the effect of SIRT1 on cell migration and invasion. In ES2 cells, **SIRT1-KD decreased the migration and invasive abilities, whereas SIRT1-OEX enhanced these abilities.**

In conclusion, these results suggest that SIRT1 enriches the CSC pool and confer the chemoresistance to OvCa by counteracting oxidative stress. The targeting of SIRT1 may be a novel therapeutic strategy against CSCs, ultimately reducing the chemoresistance burden of OvCa.