主論文の要旨

Downregulating vaccinia-related kinase 1 by luteolin suppresses ovarian cancer cell proliferation by activating the p53 signaling pathway

（ルテオリンによるVRK1のダウンレギュレーションは、
p53シグナル経路を活性化することで
卵巣がん細胞の増殖を抑制する）

名古屋大学大学院医学系研究科　総合医学専攻
発育・加齢医学講座　産婦人科学分野
（指導：梶山 広明　教授）
常 续博雅

【Introduction】

Ovarian cancer constitutes one of the most common causes of cancer-related deaths, and preventing chemotherapy resistance and recurrence in patients with ovarian cancer remains a challenge. Luteolin, a flavonoid, has been studied for its health benefits, such as preventing type 2 diabetes mellitus and various cancers, including gastric cancer, colorectal carcinoma, and bladder cancer. However, it has not yet been used to treat human patients with cancer. Inhibiting vaccinia-related kinase (VRK1) is an underlying mechanism of the antitumor effects of luteolin. p53 is a critical tumor suppressor protein and target of VRK1 that controls cell cycle, DNA repair, and uncontrolled cell division during tumor growth. Herein, we aimed to identify the effect of luteolin, a novel therapeutic agent targeting vaccinia-related kinase 1 (VRK1), on high-grade serous ovarian cancer (HGSOC).

【Methods】

Phosphokinase array, RNA sequencing, western blotting, and cell cycle and apoptosis assays were conducted to determine the underlying mechanism of the effect of luteolin on HGSOC cells. The anticancer effects of oral and intraperitoneal luteolin administration were assessed in patient-derived xenograft models via several methods, including the assessment of tumor size and immunohistochemistry of phospho-p53, phosphor-HistoneH3 and cleaved caspase 3.

【Results】

Effects of luteolin-induced VRK1 downregulation on A2780 and ES2 cell viability

Western blot analysis revealed that luteolin significantly decreased VRK1 expression (Fig. 1A). Cell proliferation was decreased by siVRK1 (Fig. 1B). We further determined the inhibitory effects of luteolin on A2780 and ES2 ovarian cancer cell viability. Luteolin dose- and time-dependent decreased HGSOC cell viability (Fig. 1C). To determine the mechanism through which luteolin inhibits cell viability, apoptosis(Fig. 1C) and cell cycle assays were conducted. Fig. 1C shows that luteolin increased apoptosis in A2780 and ES2 cells. The cell cycle assays revealed that both luteolin and siVRK1 induced G2/M arrest in A2780 and ES2 cells.

We investigated whether luteolin, which inhibits VRK1 expression, could circumvent chemotherapeutic drug resistance. We assessed the effects of luteolin and cisplatin combination therapy. The cells treated for 72 h with different combinations of luteolin and cisplatin exhibited a significant decrease in proliferation compared with the cells treated with luteolin or cisplatin alone.

Luteolin activates the p53 signaling pathway

The phosphorylation assay revealed that luteolin inhibits cell proliferation by decreasing CREB phosphorylation and increasing p53 phosphorylation at ser15 and ser46 (Fig. 2A).

Furthermore, RNA-seq analysis showed that p53 was activated following luteolin and siVRK1 treatments(Fig. 2B). This indicates that luteolin inhibits the CREB phosphorylation, cell cycle, and apoptosis by inhibiting VRK1 expression. Western blot analysis (Fig. 2C) confirmed these findings and further revealed that both luteolin and siVRK1 increased p53 phosphorylation at ser15 and ser 46 and decreased MDM2 expression compared to controls. These findings indicate that luteolin decreases cell proliferation by targeting VRK1.

Luteolin suppresses tumor growth in vivo

The tumor volume in the luteolin-treated group was lesser than that in the control group (Figure 3A). The growth of the tumor size in the luteolin plus cisplatin-treated group was slower than that in the cisplatin only-treated group. Tumor volume and weight in the luteolin plus cisplatin-treated group were lesser than that in the cisplatin only-treated group.

Furthermore, immunohistochemistry was employed to analyze protein expression in the tumor tissue samples (Fig. 3B). Histone H3 phosphorylation was decreased while p53 phosphorylation was increased in tumors in the luteolin-treated group than in the control group.

Oral luteolin therapy suppresses tumor growth in vivo

After the tumors were formed, nude mice were divided into two groups (n = 6/group) and fed a normal diet or a diet including luteolin (50 ppm) for 4 weeks. Tumor growth in mice administered with oral luteolin therapy was slower than in controls(Figure 4A). Furthermore, immunohistochemistry revealed that histone H3 phosphorylation decreased and p53 phosphorylation increased in the tumors of the mice administered with luteolin therapy (Figure 4B).

【Discussion】

To the best of our knowledge, this study is the first to evaluate the effects of luteolin in a PDX model. Animal experiments demonstrated the synergistic effects of luteolin and cisplatin in vivo. The effect of the intraperitoneal injection of luteolin in combination with cisplatin is better than that of cisplatin alone. The effects of the oral administration of luteolin on HGSOC have also been demonstrated.

Most HGSOC harbor TP53 mutations and the mutated TP53 might decrease the effect of the p53 signaling pathway. However, luteolin can decrease cell proliferation in all cell lines with or without TP53 mutation. Moreover, the human phosphokinase array result shows that luteolin can decrease the phosphorylation of CREB, especially in ES2 which has a TP53 mutation. These results indicate that in TP53 mutation cell lines, luteolin inhibits the p53 signaling pathway by downregulating VRK1. However, decreasing the phosphorylation of CREB by downregulating VRK1 might be more effective on cell proliferation. This assumption might be addressed in our future studies.

【Conclusions】

The findings of this study show that luteolin, a natural flavonoid, significantly inhibits the proliferation of HGSOC cells both in vitro and in vivo (Fig. 3C). Luteolin suppresses HGSOC cell proliferation by decreasing VRK1 expression, thereby rendering the cells susceptible to apoptosis and cell cycle arrest through the upregulation of the p53 signaling pathway. We confirmed the tumor-suppressing effects of luteolin both through intraperitoneal injection and oral administration. Luteolin can also increase the effect of cisplatin on tumor inhibition. Specifically, as the oral administration of luteolin also suppresses HGSOC progress in mice, this may be a more convenient route of administration than intraperitoneal injection in maintenance treatment.