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その他のタイトル	子宮体癌と卵巣癌において抗アポトーシスタンパクを制御する新規分子標的治療薬の探索
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論文の内容の要旨

Exploring novel molecularly-targeted therapies which modulate anti-apoptotic proteins in endometrial and ovarian cancers

（子宮体癌と卵巣癌において抗アポトーシスタンパクを制御する新規分子標的治療薬の探索）

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Abstract

Apoptosis, or programmed cell death, is a genetically-controlled process in which a cell undergoes self-destruction. It is characterized by definite morphological features which include cell shrinkage, blebbing of the membrane, condensation of the nucleus and DNA fragmentation. Apoptosis can be triggered by both intrinsic and extrinsic stimuli under both physiological and pathological conditions. Several anti- and pro-apoptotic proteins are involved in the control of this vital process. In most cancers, however, apoptosis is either aberrant or lacking which leads to the accumulation of cells with faulty DNA and altered cellular processes, consequently, resulting in relentless cellular proliferation. Circumventing apoptosis is a hallmark of most malignant tumors.

Endometrial cancer is the most frequent among malignant tumors affecting the female reproductive system in the western countries. Rising incidence is seen mostly among postmenopausal women, although younger women are becoming increasingly at risk due to the rising prevalence of nulliparity and metabolic diseases such as obesity and diabetes mellitus type 2. Nearly 80% of cases are of the endometrioid type referred to as type-I cancers and are strongly dependent on estrogens. Type I tumor is characterized by activation of the *PI3K* pathway, often due to mutations in *PTEN, PIK3CA, K-Ras*, and *AKT.* Although endometrial cancer is generally considered to have a favorable prognosis, patients with advanced stage or with recurrent disease respond poorly to standard treatment modalities, therefore, have a poor prognosis.

Survivin is a member of IAP (inhibitor of apoptosis) family proteins, which is upregulated almost exclusively in malignant tumors. Survivin is modulated by the genes in the *PI3K/mTOR* pathway. Survivin overexpression was detected in 83% of endometrial adenocarcinoma clinical samples. Estradiol is known to not only suppress the tumor suppressor gene-*PTEN*, but also induces upregulation of survivin through ERα in endometrial cancer. Survivin is can inhibit apoptosis through both caspase-dependent and independent mechanisms. Knockdown of ERα by siRNA resulted to suppression of survivin and subjected the cells to apoptosis in estrogen-responsive breast cancer, citing the role of ERα/survivin signaling. Nevertheless, the prognostic significance of survivin gene- *BIRC5* expression in endometrial carcinoma is unknown.

Ovarian cancer is the most mortal gynecologic tumor, despite advances in diagnostic and therapeutic approaches. A five-year survival rate is as low as just above 45% and the cure rate is only 30%. Treatment of ovarian cancer often involves surgery initially, but not all patients may be suitable candidates for PDS (primary debulking surgery).

Objective

This study was aimed to evaluate anti-apoptotic protein, survivin, its modulation as well as the prognostic significance of the expression of survivin encoding gene-*BIRC5* in endometrial cancer. It was also aimed to investigated whether survivin inhibitor-YM155 (sepantronium bromide) could inhibit endometrial cancer cell proliferation and induce cell death. Kaempferol, a bioflavonoid and celecoxib, a COX-2 inhibitor were also tested against endometrial and ovarian cancer cell lines respectively.

Materials and methods

Kaplan-Meier survival analysis was performed based on *BIRC5* gene expression in endometrial cancer by using RNA sequencing data from TCGA (The Cancer Genome Atlas). The data was available for 234 clinical samples of endometrial carcinoma. A panel of 16 endometrial cancer cell lines and ten ovarian cancer cell lines derived from varying histology were analyzed. Antitumor effect of survivin inhibitor-YM155, kaempferol and celecoxib were tested. Cell viability assay, FACS (fluorescence-activated cell sorting), annexin V-FITC/PI (fluorescein isothiocyanate/ propidium iodide), gene knockdown by siRNAs and western blotting analyses were employed in the study. Statistical analyses were carried out by using JMP v11 (SAS, Cary, NC, USA) and GraphPad Prism 6 (La Jolla, CA, USA). In all tests, differences were regarded as statistically significant at $p<0.05$.

Results

The results of this study showed that survivin is overexpressed in over 87% (14 of the 16) analyzed endometrial cancer cell lines as compared with endometrial immortalized cells (EIC).

High expression level of *BIRC5* gene in clinical samples was significantly associated with poor PFS (progression-free survival) (p=0.006) and showed a strong tendency toward poor OS (overall survival) (p=0.06). Univariate analysis showed that advanced stage (HR = 2.81, 95% CI =1.56–5.02, p<0.001), non-endometrioid histological subtype (HR =2.05, 95% CI =1.04–3.8, p= 0.04), and high expression of *BIRC5* (HR = 2.34, 95% CI = 1.28–4.53, p= 0.005) were significantly associated with poor prognosis. Also, this study found, for the very first time, that high expression level of *BIRC5* gene is an independent poor prognostic factor in endometrial carcinoma (HR= 1.97, 95% CI = 1.29–4.5, p= 0.045). Survivin inhibitor-YM155 successfully suppressed endometrial cancer cell viability at IC_{50} values ranging from 14nM to 150nM. YM155 also induced sub-G1 cell population and significantly (p=0.001) caused apoptotic cell death in all analyzed endometrial cancer cell lines. Kaempferol suppressed the expression of survivin and Bcl-2 and induced p53 and cleaved PARP. This is the first report to account for the ability of kaempferol to suppress survivin in endometrial cancer cells. The study also found that celecoxib successively inhibits proliferation of ovarian cancer cells and suppresses the expression of survivin and Bcl-2 in ovarian cancer cell lines.

Conclusions

The results of the current study suggest that high expression levels of survivin gene- *BIRC5* may be used as a poor prognostic marker in endometrial cancer. The study also suggests that survivin inhibitor- YM155 may serve as a novel molecularly-targeted therapy against endometrial carcinoma. Furthermore, the findings demonstrate that kaempferol and celecoxib may as well be considered as potential anticancer agents targeting survivin and Bcl-2 in endometrial and ovarian carcinomas respectively.