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

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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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［課程－２］

審査の結果の要旨

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This research work is about exploring novel molecularly- targeted therapies which modulate antiapoptotic proteins in endometrial and ovarian carcinomas. The focus of the research is “survivin”, an antiapoptotic protein which is required and expressed during embryogenesis, but almost undetectable in terminally differentiated cells, however, it is highly expressed in most of the human cancers.
To clarify the role of survivin in apoptosis-based therapies for endometrial and ovarian carcinomas, the prognostic significance of the expression of a survivin-encoding gene, *BIRC5* was evaluated in endometrial carcinoma clinical samples. Then, endometrial and ovarian carcinoma cells were treated with a survivin suppressant- sepantronium bromide, or a COX-2 (cyclooxygenase-2) inhibitor- celecoxib, or a natural dietary flavonoid- kaempferol, and antitumor effect were analyzed.

The following results were obtained;

1. Evaluation of a prognostic significance of the expression of survivin gene, *BIRC5* in endometrial cancer clinical samples by using Kaplan-Meier survival analysis and a log-rank test revealed that the higher expression levels of *BIRC5* were associated with poor progression-free survival (PFS) ($p$= 0.006) and showed a strong tendency towards poor overall survival (OS) ($p$= 0.06). A multivariate analysis picked high expression level of *BIRC5* gene as an independent poor prognostic factor in endometrial carcinoma (HR= 1.97, 95% CI = 1.29–4.5, $p$= 0.045).

2. Western blotting analysis of protein samples extracted from untreated cells revealed that survivin protein was highly upregulated in 87.5% of the analyzed cell lines (14 out of 16 cell lines).

3. The cell viability assay revealed that a survivin suppressant- sepantronium bromide, coded as “YM155” successfully suppressed the growth of all sixteen

analyzed endometrial carcinoma cell lines at $IC_{50}$ values (minimum inhibitory concentration) ranging from 14nM to 150nM. YM155 also significantly induced sub-G1 cell population ($p< 0.001$) and an apoptotic cell death as observed in annexin V-FITC/ PI assay ($p< 0.001$). Evaluation of protein samples from cells treated with YM155 by western blotting showed an induction of cleaved caspase-7 and cleaved PARP (poly (ADP-ribose) polymerase). Knockdown of *BIRC5* gene by siRNAs revealed in the similar results.

4. Evaluation of Ishikawa and HEC-265 endometrial carcinoma cell lines treated with a bioflavonoid-kaempferol demonstrated a significant growth suppression at $IC_{50}$ values of 83μM and 65μM respectively. Kaempferol also induced cell populations in sub-G1 and G2/M phases of a cell cycle ($p< 0.05$) and apoptosis in both cell lines ($p<0.001$). Western blot analysis revealed that kaempferol inhibited the expression of ERα (estrogen receptor-alpha), prevented estradiol-induced survivin expression, suppressed the expression of antiapoptotic protein-Bcl-2 and caused upregulation of p53 and induction of cleaved PARP.

5. Treatment of ovarian carcinoma cells with a COX-2 inhibitor-celecoxib suppressed cell growth at $IC_{50}$ values ranging from 17μM to 45μM, induced sub-G1 ($p< 0.01$) cells suppressed the expression of survivin and significantly induced an apoptotic cell death in all ten analyzed cell lines ($p< 0.01$).

This research work has become the first report to demonstrate the possibility for survivin gene-*BIRC5* which is located on chromosome 17q25.3 to be used as a biomarker for poor prognosis in endometrial carcinoma. It has also unearthed novel mechanisms for the antitumor effect of kaempferol and celecoxib through suppression of antiapoptotic protein-survivin in endometrial and ovarian carcinomas respectively. Unlike other apoptosis-based therapies, the survivin-targeting therapeutic approach may offer additional advantages by antagonizing multiple cellular signaling networks and providing more favorable toxicity profile since it does not affect normal cells or tissues.