Form 3

Abstract of Thesis

Name (高 剣文)	
Title	The Study of Low-dose Olaparib Inhibiting Ovarian Tumor Growth Based on the Mechanism of Cellular Senescence （細胞老化のメカニズムに基づいて卵巣腫瘍の成長を阻害する低用量オラパリブの研究）

Backgroud: Ovarian cancer is one of the most serious female cancers. According to the data collected by The Global Cancer Observatory, there are millions of new ovarian cancer cases and death cases every year. The incidence rate and mortality rates is 9.3 and 3.1 in Japan, 5.3 and 3.3 in China per 100,000 people. Due to the mild early symptoms and the lack of effective early diagnosis measures, 75% of patients are already in the advanced stage when discovered. Cytoreductive surgery plus platinum-based chemotherapy will bring a big damage to patients and poor prognosis. High dose chemotherapy may cause many side effects. Olaparib as a novel oral Poly (ADP-ribose) polymerase (PARP) inhibitor have been verified in many clinical trials to have good anti-tumor effects and are well tolerated. It is indicated in maintenance treatment of breast cancer susceptibility gene (BRCA) mutated advanced ovarian cancer or recurrent ovarian cancer. However, roles of olaparib in many aspects remain to be studied.

Objective: 1) The efficiency of olaparib on non-BRCA mutant ovarian cancer cells in short term. 2) The inhibitory effect of olaparib combined with cisplatin on non-BRAC mutant ovarian cancer cells in short term. 3) The effect and mechanism of continuous low-dose olaparib *in vivo* and *in vitro*. 4) After adding rosiglitazone, the inhibitory effect of olaparib on ovarian cancer cells and the improvement of side effect caused by cellular senescence.

Methods: Cell counting kit-8 (CCK-8) assay, crystal violet staining and fluorescence activated cell sorter (FACS) methods were used to detect the number and viability of ovarian cancer cells. The dose synergistic effect was analyzed by Chou's Compusyn Software. The ratio of side population (SP) cells, apoptosis and cell cycle were detected by FACS. Xenografted mice (n=6 or 12 per group) were treated with continuous low-dose (10 mg/kg/day) olaparib to study the changes of tumor volume and verify the effect of continuous low-dose olaparib combined with rosiglitazone (10 mg/kg/day). Senescence-associated ß-galactosidase (SA-ß-Gal) staining and senescence-associated heterochromatin aggregation (SAHF) were used to determine whether the cells were senescent. Senescence-associated secretory phenotype (SASP) was detected by RT-qPCR, and the main cell cycle signaling pathway was studied by Western bolt. Differences between multiple groups were analyzed by one-way ANOVA (Tukey). The student's t-test was adopted in the comparison of two groups. When $p< 0.05$, the difference was statistically significant.

Results: Through the results of Chapter Two and Three, we found that low-dose (0.25x IC_{50}) olaparib has an inhibitory effect on non-BRAC1/2 mutated ovarian cancer cells and when combined with cisplatin, it can significantly enhance its proliferation inhibitory effect with a strong synergistic effect ($p< 0.05$, $p< 0.01$). In Chapter Four, we have confirmed that ovarian cancer cell growth is inhibited, cell cycle (G0/G1 phase) is blocked, and SA-ß-Gal staining and SAHF positive cells significantly increased by the administration of low-dose (5μM) olaparib. At the same time, 5μM olaparib increased expression levels of P16/P53 and Rb protein in SKOV3 and A2780 ovarian cancer cells, which suggests that olaparib can depend on the P16-Rb /P53-Rb signing pathway to induce the ovarian cancer cell senescence, thereby inhibiting tumor cell proliferation. In Chapter Five, In vivo experiment, the final volume of ovarian tumors was significantly decreased in the olaparib + rosiglitazone group compared with the control group ($p<0.05$). In vitro experiment, the olaparib (5μM) + rosiglitazone (5μM) group showed a significant down-regulation of cell proliferation compared with the control group ($p<0.05$). According to the results of RT-PCR, rosiglitazone can down-regulate expression of SASP caused by olaparib-induced cellular senescence. Olaparib combined with rosiglitazone can promote the apoptosis of ovarian cancer cells. And its possible inhibitory mechanism is that the rosiglitazone inactivated olaparib-induced senescence of ovarian cancer cells through the SIRT1/P53 signaling pathway, decrease the SASP and promoted olaparib to inhibit tumor cell proliferation.

Conclusion: 1) low-dose olaparib has an inhibitory effect of on non-BRAC mutant ovarian cancer cells. 2) Olaparib combined with cisplatin can effectively enhance its inhibitory effect with a synergistic dose effect. 3) Continuous low-dose olaparib can induce cellular senescence under P16 or P53 dependent manner in ovarian cancer. 4) After rosiglitazone is added, continuous low-dose olaparib effectively improve its inhibitory effect on ovarian tumor and cells. Rosiglitazone ameliorates cellular senescence and promotes apoptosis in ovarian cancer induced by olaparib.

様式７

論文審査の結果の要旨及び担当者

氏　名　（　高　剣文　）			
論文審査担当者		（職）	氏　名
	主査	教授	大野ゆう子
	副査	教授	三善英知
	副査	教授	遠藤誠之

論文審査の結果の要旨

卵巣がんは、女性のがんの中でも予後の深刻ながんの一つである。初期にはほとんど自覚症状がなく検診方法も確立されていないことから、発見されたときにはすでに半数以上が進行期にあるとされている。オラパリブは、近年開発された経口のPARP; Poly（ADP-ribose）polymerase阻害剤であり、乳がん感受性遺伝子（BRCA）変異を有する進行卵巣がんや再発卵巣がんの維持療法に適応がある。しかし、オラパリブの役割についてはまだ解明されきれてはおらず、本論文では、in vitroにおける非BRCA変異卵巣癌細胞に対するオラパリブの低用量投与の短期的な効果、シスプラチンと低用量オラパリブの短期的な併用療法の効果、さらに持続的低用量オラパリブ投与のin vivoおよびin vitroにおける効果とメカニズムの検討、ロシグリタゾン投与後の卵巣がん細胞における持続的低用量オラパリブ投与の細胞増殖抑制効果と細胞老化による副作用の改善について検討した。

低用量(0.25x IC_{50})オラパリブを48時間、非BRCA変異卵巣がん細胞（A2780,OVCAR-3）に投与したところ増殖抑制効果を有し、パクリタキセルまたはシスプラチンと併用すると強い相乗効果で増殖抑制効果を有意に増強できることが示された（$p<0.05$、$p<0.01$）。さらに低用量（10mg/kg/day）オラパリブを2週間12匹/群のマウスに投与した結果、投与群は対照群より有意に腫瘍体積を減少させた($p<0.01$)。低用量（5μM）オラパリブを非BRCA変異卵巣がん細胞（A2780,SKOV3,OVCAR-3）に７日間投与した結果でも、増殖抑制効果が見られた。オラパリブは細胞周期（G0/G1期）を阻害すること、P16-Rb/P53-Rb 経路により癌細胞の老化を誘導し、腫瘍細胞の増殖を抑制していることが示唆された。最後にロシグリタゾンによるオラパリブ誘発細胞老化の副作用の緩和と卵巣癌のアポトーシスに関する実験を行った。ロシグリタゾン(10mg/kg/day)を投与後、低用量（10mg/kg/day）オラパリブを3週間6匹/群のマウスに投与した結果、ロシグリタゾンが抗腫瘍効果を強化する結果が得られた。非BRCA変異卵巣がん細胞（A2780,SKOV3）に7日間オラパリブ（5μM）＋ロシグリタゾン（5μM）を投与した群では対照群に比べて卵巣腫瘍体積が有意に減少した（$p<0.05$）。また、ロシグリタゾンはオラパリブ誘発細胞老化に起因するSASPの発現を低減させることが出来た。以上よりオラパリブとロシグリタゾンの併用で卵巣癌細胞のアポトーシスを促進する可能性があること、ロシグリタゾンはSIRT1/P53シグナル伝達経路を介してオラパリブ誘発性の卵巣癌細胞の老化を抑制し、SASPの発現を低下させ、オラパリブによる腫瘍細胞の増殖を抑制することが考えられた。以上のように、低用量オラパリブの非BRAC変異卵巣癌細胞に対する抑制効果、オラパリブとパクリタキセル、シスプラチンの併用の相乗効果、低用量オラパリブの継続的投与による非BRAC変異卵巣癌細胞におけるP16/P53経路依存的な細胞老化誘導効果、ロシグリタゾンを添加した後の継続的な低用量オラパリブ投与の効果について、新たな知見を得た。

これらは学術的にも臨床的にも重要な成果であり、博士（保健学）学位に値するものと評価した。