

기관고유연구사업 최종보고서

제 출 문

(과제번호 : 0910260)

난소암 맞춤치료를 위한 분자생물학적 지표연구 II

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이 보고서를 기관고유연구사업 “난소암 맞춤치료를 위한 분자생물학적 지표연구 II” 과제의 최종보고서로 제출합니다.

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※ 여러개의 세부과제로 과제가 구성된 경우 위 목차와 동일하게 세부과제별로 작성함  
 (I. 총괄과제, II. 제1세부과제, III. 제2세부과제.....)

|   |                             |   |      |                  |         |
|---|-----------------------------|---|------|------------------|---------|
| 연구분야(코드)  |                             |   |      | 과제번호             | 0910260 |
| 과제명   | 난소암 맞춤치료를 위한 분자생물학적 지표연구 II |   |      |                  |         |
| 연구기간/연구비<br>(천원)  | 합계                          | 2009년 1월 1일 ~ 2011년 12월 31일                             |      | 371,000          |         |
|   | 1차년도                        | 2009년 1월 1일 ~ 2009년 12월 31일                             |      | 90,000           |         |
|   | 2차년도                        | 2010년 1월 1일 ~ 2010년 12월 31일                             |      | 80,000           |         |
|   | 3차년도                        | 2011년 1월 1일 ~ 2011년 12월 31일                             |      | 201,000          |         |
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|   | 영문                          | ovarian cancer, biomolecular marker, prognosis, therapy |      |                  |         |
| <p><b>◆ 연구목표</b><br/>                 난소암 1차 치료 및 재발 예측을 위한 혈청 표지자(biomarker) 개발 및 임상 응용을 위한 모델 확립 및 유전체 정보 분석기법을 이용한 항암효과를 갖는 신규약제 발굴</p>  |                             |   |      |                  |         |
| <p><b>◆ 연구내용 및 방법</b></p> <p>1) 종양표지자를 이용한 난소암 맞춤치료를 위한 전략 수립<br/>                 가) 수술 결과 예측 모델: 종양감축술 예후인자 분석<br/>                 - CA125 (cut-off 500U/ml, positive likelihood ratio 1.87; odds ratio 3.7, strong association with suboptimal cytoreduction, 2010년 J Surg Oncol published)<br/>                 - 예후예측을 판별하는 Nadir CA125의 한국 여성에서의 표준치 조사 (2011 J Gynecol Oncol published)<br/>                 - HE4 (cut-off 100pM, strong association with suboptimal cytoreduction, odds ratio 2.5, p = 0.007, 2011년 SGO meeting 발표; 2011년 Annals of Surgical Oncology published)<br/>                 나) 맞춤치료 의사결정 모델: 환자에 따른 항암화학요법 개인화(individualized) 항암화학요법 주기(cycle) 설정<br/>                 - Subnormal CA125 level at remission state: retrospective cohort analysis - remission state의 serum CA125 level은 high risk factor이지만, chemotherapy의 individualization이 예후를 향상시키지 못함을 보고 (2011년 Gynecol Obstet Invest published)<br/>                 다) CA125 level에 따른 neoadjuvant chemotherapy 개인화(individualized) 적용<br/>                 - High serum CA125 level (&gt;2,000 U/ml) 환자에서 neoadjuvant chemotherapy가 환자 예후를 향상시킬 수 있음을 발표 (2010년 IGCS meeting 발표, 2011년 Gynecol Oncol published)</p> <p>2) 신규 종양 혈청표지자 개발을 위한 데이터베이스 확보</p> |                             |   |      |                  |         |

## Project Summary

|                           |  |
|---------------------------|--|
| <b>Title of Project</b>   | New biomarkers in ovarian cancer for individualized treatment (II) |
| <b>Key Words</b>          | ovarian cancer, biomolecular marker, prognosis, therapy            |
| <b>Project Leader</b>     | Sokbom Kang, M.D.  |
| <b>Associated Company</b> | N/A  |

1) Using in-silico gene signature based approach, we have demonstrated that thioridazine could inhibit phosphatidylinositol-3'-kinase (PI3K)/Akt pathway, and thus exert cytotoxicity in ovarian cancer cells. The current study indicated that in-silico approach, such as Connectivity Map, is a potentially useful method to identify the unknown cellular function among the drugs already in use in clinic. Owing to the property of Akt inhibition and additive cytotoxicity observed with the platinum compound, further research should be focused on this drug.

2) We measured HE4 in pretreatment serum samples from 80 women with epithelial ovarian cancer, using an enzyme-linked immunosorbent assay. The results were correlated with clinical data. Pretreatment serum HE4 seems to be an additional factor for predicting the outcome of patients with epithelial ovarian cancer. Due to its independence from established prognostic factors, serum HE4 may provide additional prognostic information.

3) Among patients who achieved remission after six cycles of carboplatin-paclitaxel chemotherapy, those with CA-125 of 10-35 U/ml at the time of remission were divided into two groups (group A: six cycles of standard chemotherapy vs. group B: two or more additional cycles) and were analyzed. The addition of two or more cycles of standard chemotherapy based on posttreatment CA-125 levels was not beneficial.

4) We retrospectively reviewed records of 314 patients with EOC including 94 patients who received NAC. After stratification by preoperative CA-125 levels, the progression-free survival (PFS) was compared between the NAC group and the primary debulking surgery (PDS) group. Our preliminary data suggests the possible interaction between CA-125 levels and survival benefit of NAC. The randomized trial data about NAC should be stratified by the reproducible and relevant criteria such as preoperative serum CA-125 level to elucidate true survival benefit of NAC in ovarian cancer.

|  |                           |   |    |                       |        |           |                       |             |      |                           |             |
|--|---------------------------|---|----|-----------------------|--------|-----------|-----------------------|-------------|------|---------------------------|-------------|
| <p>가) 정상대조군 및 난소암 환자군 간의 유망 신규 표지자 HE4를 이용한 조기 검진 모델의 한국 여성에서의 적용 가능성 확인 - 정상인 대 난소암 환자군 median level (111.7pM vs. 68.6pM). 한국 정상-난소암 대조군에서 HE4는 98% specificity에서 50.4%의 sensitivity를 보여서 CA125와 병용하였을 때 진단율의 상승을 기대하기 어려움을 발표 (2012 SGO meeting 발표예정)</p> <p>나) 혈청 데이터베이스의 확보: 2010년 10월 현재 160명의 난소암 환자에서 환자 임상경과에 따라 292개의 샘플을 확보하였음(목표치의 150% 달성)</p> <p>3) 분자표적치료제의 맞춤치료를 위한 표적치료관련 표현형 연구</p> <p>- Genomic database query를 통한 표현형 선택: gene-set enrichment analysis(GSEA) - Duke univ. DB 및 Australia DB의 분석을 통해 PI3K-AKT pathway가 난소암 예후와 연관된 중요인자임을 확인</p> <p>- Connectivity map을 이용하여 PI3K-AKT pathway를 억제할 수 있는 약제 발굴: thioridazine이 ovarian cancer cell에서 PI3K-AKT의 inhibition을 가져오며 cell cycle arrest를 통해 cytotoxicity를 가져오는 것을 발표 (2010 IGCS 발표, 2011 Gynecol Oncol published)</p> <p><b>3) 부인 암 세포주에서 Thioridazine 의 항암 활성 효과 검색 및 검증</b></p> <p>가. 암 세포주에 Thioridazine 처리 후 MTT assay를 이용한 농도별 생존력 관찰</p> <p>나. DAPI와 TUNEL 방법을 통한 세포내의 핵의 변화를 대조군과 비교하여 확인</p> <p>다. Flow cytometry를 이용하여 cell cycle 변화 및 cell division 분석</p> <p>라. 약물 처리 후 변화되는 단백질 (CDK, Cyclin D1, p21, p16, p-CDC25A etc.)을 분석</p> <p>마. Western blotting 을 통해 세포사멸 경로 확인</p> <p>바. Thioridazine을 처리한 세포주와 PI3K inhibitor인 LY294002 와 wortmannin 를 처리한 세포주를 가지 PI3K 활성 조사 및 비교 분석</p> <p>사. 농도별에 따른 Akt 인산화 변화 관찰 및 mTOR 억제제인 Rapamycin을 처리한 세포주와의 비교 분석 및 down-stream의 4E-BP1 단백질의 인산화 변화 관찰</p> <p>아. cisplatin 과 taxol 을 각각 동시에 처리했을 때 시너지를 볼 수 있는지 분석</p> |                           |   |    |                       |        |           |                       |             |      |                           |             |
| <p><b>◆ 연구성과</b></p> <p>-정량적 성과</p> <table border="1" style="width: 100%; border-collapse: collapse; text-align: center;"> <tr> <td style="width: 30%;">구분</td> <td style="width: 40%;">달성치/목표치<sup>1)</sup></td> <td style="width: 30%;">달성도(%)</td> </tr> <tr> <td>SCI 논문 편수</td> <td><b>총 21편 달성/8편 목표</b></td> <td><b>260%</b></td> </tr> <tr> <td>IF 합</td> <td><b>총 96.6점 달성//32점 목표</b></td> <td><b>300%</b></td> </tr> </table> <p>1) in submission 및 under revision 제외</p>  |                           |   | 구분 | 달성치/목표치 <sup>1)</sup> | 달성도(%) | SCI 논문 편수 | <b>총 21편 달성/8편 목표</b> | <b>260%</b> | IF 합 | <b>총 96.6점 달성//32점 목표</b> | <b>300%</b> |
| 구분   | 달성치/목표치 <sup>1)</sup>     | 달성도(%)  |    |                       |        |           |                       |             |      |                           |             |
| SCI 논문 편수  | <b>총 21편 달성/8편 목표</b>     | <b>260%</b>   |    |                       |        |           |                       |             |      |                           |             |
| IF 합   | <b>총 96.6점 달성//32점 목표</b> | <b>300%</b>   |    |                       |        |           |                       |             |      |                           |             |
| <b>◆ 참여연구원</b><br>(최종연도 참여인원)  | 성 명                       | <p>노승배, 공선영, 박상윤, 서상수, 임명철, 유종우,<br/>김주영, 정대철, 김석기, 박세현, 황종하, 이보영,<br/>서주희, 김보람, 동승명, 변현정</p> |    |                       |        |           |                       |             |      |                           |             |

## 1. 연구의 최종목표

난소암 1차 치료 및 재발 예측을 위한 혈청 표지자(biomarker)개발 및 임상 응용을 위한 모델 확립

- 다중 혈청 표지자를 이용 1차 치료 후 PFS 예측 모델 개발
- 혈청 표지자(serum biomarker)를 이용한 난소암 개인화 (individualized) 치료 모델 제시
- Genomic query를 이용하여, thioridazine을 비롯, 기존 임상에서 사용되고 있는 약제의 암 치료제로서의 이용 가능성 탐색

## 2. 연구의 내용 및 결과

### 1) 종양표지자를 이용한 난소암 맞춤치료를 위한 전략 수립

가) 수술 결과 예측 모델: 종양감축술 예후인자 분석

- CA125 (cut-off 500U/ml, positive likelihood ratio 1.87; odds ratio 3.7, strong association with suboptimal cytoreduction, 2010년 J Surg Oncol published)

- HE4 (cut-off 100pM, strong association with suboptimal cytoreduction, odds ratio 2.5, p = 0.007, 2011년 SGO meeting 발표)

나) 맞춤치료 의사결정 모델: 환자에 따른 항암화학요법 개인화(individualized) 항암화학요법 주기(cycle) 설정

- Subnormal CA125 level at remission state: retrospective cohort analysis - remission state의 serum CA125 level은 high risk factor이지만, chemotherapy의 individualization이 예후를 향상시키지 못함을 보고 (Gynecol Obstet Invest, published)

다) CA125 level에 따른 neoadjuvant chemotherapy 개인화(individualized) 적용

- High serum CA125 level (>2,000 U/ml) 환자에서 neoadjuvant chemotherapy가 환자 예후를 향상시킬 수 있음을 발표 (2010년 IGCS meeting 발표, Gynecol Oncol, published)

### 2) 신규종양혈청표지자 개발을 위한 데이터베이스 확보

가) 정상대조군 및 난소암 환자군 간의 유망 신규 표지자 HE4를 이용한 조기 검진 모델의 한국 여성에서의 적용 가능성 확인

- 정상인 대 난소암 환자군 median level (111.7pM vs. 68.6pM). 한국 정상-난소암 대조군에서 HE4는 98% specificity에서 50.4%의 sensitivity를 보여서 CA125와 병용하였을 때 진단

율의 상승을 기대하기 어려움을 발표 (2011 SGO meeting 발표)

나) 혈청 데이터베이스의 확보: 2010년 10월 현재 160명의 난소암 환자에서 환자 임상경과에 따라 292개의 샘플을 확보하였음(목표치의 150% 달성)

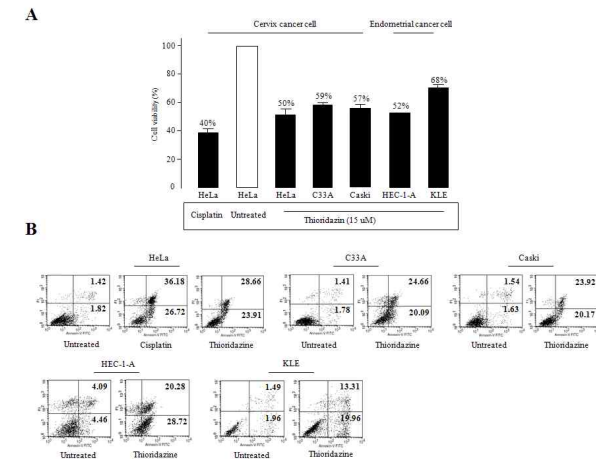
### 3) 분자표적치료제의 맞춤치료를 위한 표적치료관련 표현형 연구

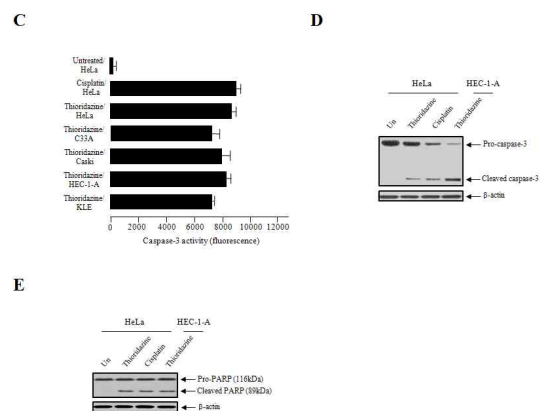
- Genomic database query를 통한 표현형 선택: gene-set enrichment analysis(GSEA) - Duke univ. DB 및 Australia DB의 분석을 통해 PI3K-AKT pathway가 난소암 예후와 연관된 중요인자임을 확인

- Connectivity map을 이용하여 PI3K-AKT pathway를 억제할 수 있는 약제 발굴: thioridazine이 ovarian cancer cell에서 PI3K-AKT의 inhibition을 가져오며 cell cycle arrest를 통해 cytotoxicity를 가져오는 것을 발표 (2011 IGCS 발표, Gynecol Oncol, published)

### 4) 자궁경부암 및 내막암에서의 thioridazine의 세포사멸 기능

난소암 뿐만아니라 자궁경부 및 내막암에서도 세포증식 억제효과를 나타내는지 알아보기 위하여 각각 2개씩 암 세포주를 이용하여 세포사멸 기능을 조사하였다 (그림 4).





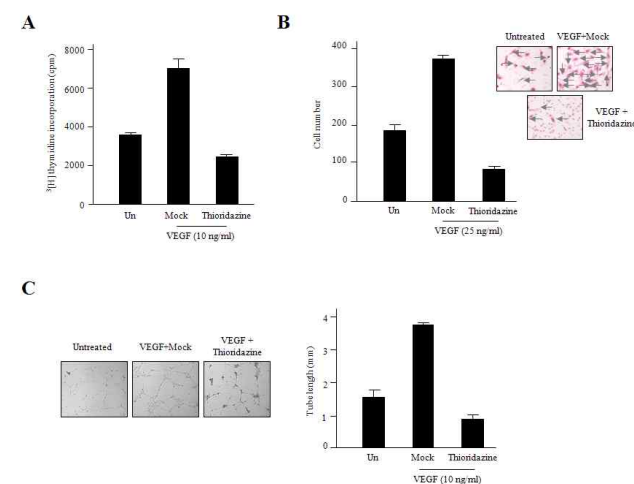
**Figure 4.** Treatment of thioridazine inhibits cellular proliferation and activates caspase-dependent pro-apoptotic activity. A. Effect of treatment with thioridazine (15  $\mu$ M) was analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assays. Inhibition of cellular proliferation by cisplatin (20  $\mu$ M) in HeLa cells was also illustrated for comparison. B. Early- and late-stage apoptosis induced by thioridazine was analyzed by fluorescein isothiocyanate (FITC)-labeled Annexin V assay. C. Caspase-3 enzymatic activity after treatment of thioridazine was determined using acetyl-DEVD-7-amino-4-trifluoromethyl coumarin as the substrate. D-E. Caspase-3 and PARP cleavages induced by cisplatin and thioridazine treatments. Soluble protein extracts were conducted by immunoblotting for cleaved caspase-3 and cleaved PARP.  $\beta$ -actin was used as an equal loading control.

그림에서 보시는 바와 같이 thioridazine을 자궁경부암 세포 주(HeLa의 경우)에 transfection 시킨 결과 early stage에서 26.72%의 자궁경부암 세포를 사멸시키는 효과를 보여 주고 있다. 그리고 growth를 조사한 결과 thioridazine을 처리했을 때 암 세포 주에서 성장이 일어나지 않았다. 동시에 caspase-3 activity와 PARP assay를 조사한 결과에서도 현저히 사멸하는 세포가 증가하는 것을 관찰하였다.

#### 4) Thioridazine의 신생혈관형성 억제 가능성 연구

신생혈관형성은 endothelial cell 의 proliferation, migration and invasion, vessel formation 에 의하여 이루어진다. 먼저 *in vitro* 에서의 thioridazine 의 신생혈관형성 억제 활성을 조사하기 위하여 HUVECs 에 thioridazine를 처리한 후 그 억제능을 분석하였다. 먼저 세포 증식효과를 알아보기 위하여 [ $^3$ H]thymidine incorporation assay를 실시하여 DNA 합성량을 조사하였다. 그 결과 VEGF 에 의하여 증가된 DNA 합성이 thioridazine 에 의하여 급격히 감소되는 현상이 나타났다 (그림 5A).

**Figure 5.** Thioridazine inhibits endothelial cell proliferation *in vitro*. (A) The angiogenic effects of



thioridazine on DNA synthesis in endothelial cells were followed with/without VEGF (10 ng/ml) treatment for 48 hours. cpm values of [ $^3$ H]thymidine were determined using a liquid scintillation counter. (B) Migration. (C) Tube formation in HUVECs.

배양한 인체 혈관내피세포에 thioridazine 처리한 후 HUVEC 세포의 침윤과, 이동, 및 관 형성을 대조군인 control Mock을 처리한 세포와 비교하였다. 보시는 바와 같이 VEGF에 의해 활성화 된 migration이 thioridazine에 의해 현저히 감소하는 현상을 볼 수 있었으며 (그림 5B), 동시에 Transwell tube formation을 실시한 결과에서도 VEGF에 의해 증가된 tube formation이 thioridazine에 의하여 완전히 저해되는 것을 볼 수 있었다 (그림 5C).

### 3. 연구결과 고찰 및 결론

본 연구의 결과 thioridazine은 세포내 세포사멸을 통해 PI3K/Akt 신호전달을 차단하였고 그 결과 암세포 신생혈관 형성을 유도하는 주 촉진인자인 VEGF의 발현 정도를 억제시킴으로 신생혈관을 일어나지 못하게 함으로서 암세포의 살상효과를 나타내었다. 동시에 정상세포에는 독성을 나타내지 않는 대단히 효과적이며 안전성이 높은 보조적 항암치료제로서의 가능성을 보여주었다. 또한 신생혈관을 저해하는 암 치료 연구 분야가 현재까지도 전 세계적으로 각광을 받고 있으나 보다 효과적이고 정상세포에는 독성을 나타내지 않는 치료제의 개발이 요구되고 있는 실정에 있다. 이러한 면에서 볼 때 결과적으로 본 연구는 PI3K/Akt를 targeting 할 수 있는 새로운 치료방법의 가능성을 제시하였으며 이를 이용한 새로운 보조적 항암치료제에 이용될 수 있을 것이다.

#### 4. 연구성과 및 목표달성도

##### (1) 연구성과

###### 가. 국내 및 국제 전문학술지 논문 게재 및 신청

| 논문명  | 저자<br>(저자구분 <sup>1)</sup> ) | 저널명(IF.)                      | Year;<br>Vol(No):Page | 구분 <sup>2)</sup> | 지원과제번호 <sup>3)</sup> |
|--|-----------------------------|-------------------------------|-----------------------|------------------|----------------------|
| A gene signature-based approach identifies thioridazine as an inhibitor of phosphatidylinositol-3'-kinase (PI3K)/AKT pathway in ovarian cancer cell  | 노승배<br>(제1)<br>강석범<br>(교신)  | Gynecol Oncol (3.73)          | 2011                  | 국외<br>SCI        | 0910260              |
| Interaction between Preoperative CA-125 Level and Survival Benefit of Neoadjuvant Chemotherapy in Advanced Epithelial Ovarian Cancer   | 강석범<br>(교신)                 | Gynecol Oncol (3.73)          | 2011                  | 국외<br>SCI        | 0910260              |
| Preoperative serum CA-125 levels and risk of suboptimal cytoreduction in ovarian cancer: a meta-analysis   | 강석범<br>(교신)                 | J Surg Oncol (2.5)            | 2010                  | 국외<br>SCI        | 0910260              |
| Multidetector CT predictors of incomplete resection in primary cytoreduction of patients with advanced ovarian cancer  | 강석범<br>(교신)                 | Eur Radiol (3,589)            | 2010                  | 국외<br>SCI        | 0910260              |
| Feasibility and Safety of Extensive Upper Abdominal Surgery in Elderly Patients with Advanced Epithelial Ovarian Cancer  | 강석범<br>(교신)                 | JKMS (0,838)                  | 2010                  | 국외<br>SCI        | 0910260              |
| Diagnostic value of (18)F-FDG PET for evaluation of paraaortic nodal metastasis in patients with cervical carcinoma: a metaanalysis  | 강석범<br>(교신)                 | JNM (6,424)                   | 2010                  | 국외<br>SCI        | 0910260              |
| Frequent promoter hypermethylation of TGFBI in epithelial ovarian cancer   | 강석범<br>(제1)                 | Gynecol Oncol (3.73)          | 2010                  | 국외<br>SCI        | 0910260              |
| Pelvic Lymph Node Status Assessed by 18f-Fluorodeoxyglucose Positron Emission Tomography Predicts Low-Risk Group for Distant Recurrence in Locally Advanced Cervical Cancer: A Prospective Study | 강석범<br>(교신)                 | IJROBP (4,592)                | 2010                  | 국외<br>SCI        | 0910260              |
| Identification of low risk group for parametrial invasion in early-stage cervical cancer   | 강석범<br>(교신)                 | Gynecol Oncol (3.73)          | 2010                  | 국외<br>SCI        | 0910260              |
| Preoperative prediction model of lymph node metastasis in endometrial cancer   | 강석범<br>(교신)                 | IJGC (2,179)                  | 2010                  | 국외<br>SCI        | 0910260              |
| Role of extended chemotherapy in advanced ovarian cancer patients with high posttreatment serum CA-125 levels.   | 강석범<br>(교신)                 | Gynecol Obstet Invest (1,031) | 2011                  | 국외<br>SCI        | 0910260              |
| Does Neoadjuvant Chemotherapy Increase Optimal Cytoreduction Rate in Advanced Ovarian Cancer? Meta-Analysis of 21 Studies  | 강석범<br>(교신)                 | Ann Surg Oncol (3,329)        | 2010                  | 국외<br>SCI        | 0910260              |
| Nadir CA-125 level is an independent   | 강석범                         | J Surg Oncol                  | 2009                  | 국외               | 0910260              |

|   |                        |                            |                             |             |         |
|---|------------------------|----------------------------|-----------------------------|-------------|---------|
| prognostic factor in advanced epithelial ovarian cancer   | (교신)                   | (2,183)                    |                             | SCI         |         |
| Epstein-Barr virus-transformation of B-cell lines in ovarian cancer patients: feasibility of genomic storage for unlimited use  | 강석범<br>(교신)            | J Gynecol Oncol            | 2009                        | 국내<br>SCI-E | 0910260 |
| Prediction of a high-risk group based on postoperative nadir CA-125 levels in patients with advanced epithelial ovarian cancer.   | 강석범<br>(교신)            | J Gynecol Oncol            | 2011                        | 국내<br>SCI-E | 0910260 |
| PREFACE 1.0: A Risk Assessment Tool for Distant Recurrence after Definitive Platinum-based Concurrent Chemoradiation in Patients with Locally Advanced Cervical Cancer: A Korean Gynecologic Oncology Group Study | 강석범<br>(제1)            | J Clin Oncol               | in press                    | 국외<br>SCI   | 0910260 |
| Preoperative Identification of a Low-Risk Group for Lymph Node Metastasis in Endometrial Cancer: A Korean Gynecologic Oncology Group Study  | 강석범<br>(제1)            | J Clin Oncol               | in press                    | 국외<br>SCI   | 0910260 |
| Programmed cell death 6 (PDCD6) inhibits angiogenesis through PI3K/mTOR/p70S6K pathway by interacting of VEGFR-2  | 노 승 배<br>(제 1 및<br>교신) | Cellular Signaling (4,243) | 2012;<br>24 (1):<br>131-139 | 국외<br>SCI   | 0910262 |
| CYR61 controls p53 and NF-κB expression through PI3K/Akt/mTOR pathways in carboplatin-induced ovarian cancer cells  | 노 승 배<br>(교신)          | Cancer Letters (4,864)     | 2012;<br>315 (1):<br>86-95  | 국외<br>SCI   | 0910262 |
| PDCD6 additively cooperates with anti-cancer drugs through activation of NF-κB pathways   | 노 승 배<br>(교신)          | Cellular Signaling (4,243) | in press                    | 국외<br>SCI   | 0910262 |

###### 나. 국내 및 국제 학술대회 논문 발표

| 논문명   | 저자  | 학술대회명                                    | 지역 <sup>1)</sup> | 지원과제번호  |
|---|-----|--|------------------|---------|
| A gene signature-based approach identifies thioridazine as an inhibitor of phosphatidylinositol-3'-kinase (PI3K)/AKT pathway in ovarian cancer cell | 강석범 | International Gynecologic Cancer Society | 국외               | 0910260 |
| Interaction between Preoperative CA-125 Level and Survival Benefit of Neoadjuvant Chemotherapy in Advanced Epithelial Ovarian Cancer                | 강석범 | International Gynecologic Cancer Society | 국외               | 0910260 |
| Identification of low risk group for parametrial invasion in early-stage cervical cancer  | 강석범 | Society of Gynecologic Oncologist        | 국외               | 0910260 |
| The Role of Extended Chemotherapy in Advanced Ovarian Cancer Patients with High Post-treatment Serum CA-125 Levels                                  | 강석범 | Society of Gynecologic Oncologis         | 국외               | 0910260 |
| Nadir CA-125 level is an independent prognostic factor in advanced epithelial ovarian cancer  | 강석범 | Annual Meeting of SGO                    | 미국               | 0910260 |
| Does Neoadjuvant Chemotherapy Increase Optimal Cytoreduction Rate in Advanced Ovarian Cancer? Meta-Analysis of 21 Studies                           | 강석범 | FIGO world congress                      | 남아공              | 0910260 |

다. 연구성과의 정부정책 기여

| 보고서명               | 정부정책    | 기여내용 |
|--------------------|---------|------|
| HE4(ELISA) 유용성/안전성 | 신의료기술평가 | 자문   |
| HE4(CMIA) 유용성/안전성  | 신의료기술평가 | 자문   |

(2) 목표달성도

가. 연구목표의 달성도

| 최종목표   | 연차별목표 |   | 달성내용   | 달성도(%) |       |
|--|-------|---|--|--------|-------|
|  | 연차    | 최종  |  | 연차     | 최종    |
| 난소암 1차 치료 및 재발 예측을 위한 혈청 표지자(biomarker) 개발 및 임상 응용을 위한 모델 확립 | 1차년도  | 다중 혈청 표지자를 이용 1차 치료 후 PFS 예측 모델 개발  | 다중 혈청 표지자를 이용하여 난소암 생존에 가장 중요한 인자인 1차 치료 후 PFS 예측 모델 개발  | 3      | 100 % |
|  | 2차년도  | 다중 혈청 표지자를 이용 1차 치료 후 PFS 예측 모델 개발  | HE4 및 CA125에 의한 suboptimal surgery risk 증가 및 PFS 연관 규명 및 CA125에 의한 neoadjuvant chemotherapy의 individualized treatment 가능성 제시   | 2      | 100 % |
|  | 3차년도  | 1) 혈청 표지자 (serum biomarker)를 이용한 난소암 개인화 치료 모델 제시<br>2) Genomic query를 이용하여, thioridazine을 비롯, 기존 임상에서 사용되고 있는 약제의 암 치료제로서의 이용 가능성 탐색 | 1) 난소암 신규 혈청 표지자 개발을 위한 검체 추가확보<br>2) 난소암 개인화 치료 모델 제시를 위한 다기관 연구 개시<br>3) Proteomics을 이용한 신규 혈청 표지자 개발 협력연구<br>4) Thioridazine의 혈관생성억제기능 규명<br>5) 기존 임상약제의 항암작용 가능성 추가 규명 | 3      | 100 % |

나. 평가의 착안점에 따른 목표달성도에 대한 자체평가

| 평가의 착안점                                 | 자체평가   |
|---|--|
| 혈청내 표지자를 이용한 1차 치료 후 PFS 예측 모델 개발 성공 여부 | HE4 및 CA125와 PFS, suboptimal surgery risk 증가를 확인하였으며 보다 다수의 cohort를 대상으로 validation 하는 작업이 향후 필요함              |
| 부가적 후보 혈청 표지자의 개발                       | 축적된 데이터베이스를 기반으로 프로테오믹스 기법 도입한 신규 표지자 개발 및 genomic query를 통한 신규 에후 연관 pathway targeting strategy를 적극 연구해야 할 것임 |

## 5. 연구결과와 활용계획

### (1) 연구종료 2년후 예상 연구성과

| 구분        | 건수 | 비고  |
|-----------|----|---|
| 학술지 논문 게재 | 5  | Journal of Clinical Oncology, Apoptosis, Angiogenesis 등 우수 저널에서 publish할 예정 |

### (2) 연구성과의 활용계획

- ◆ 부인의 새로운 보조적 화학요법에 대한 적합성 관련 작용 기전의 이해를 들 수 있으며, 최적의 약물을 환자에게 사용할 수 있는 기준을 위한 자료를 제공하고, 조절 기전에 따른 항암제에 저항성을 가지는 환자에게 다른 치료 방법을 제시함으로써 안전성과 암 치료 효과의 증진
- ◆ 작용 기전이 검증된 새로운 보조적 화학요법제의 선택적인 치료 방법은 특히 초기 예방 치료 및 재발방지를 위한 치료 요법으로 사용됨으로서 국민의 막대한 의료비 지출을 줄일 수 있음

## 6. 참고문헌

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Visintin I, Feng Z, Longton G, Ward DC, Alvero AB, Lai Y et al (2008). Diagnostic markers for early detection of ovarian cancer. *Clin Cancer Res* 14: 1065-72.



## 7. 첨부서류

- 각 세부과제 참조

## II. 제 1세부과제

세부과제명 : 난소암 맞춤치료를 위한 분자생물학적 지표연구 II  
세부과제책임자(성명/소속) : 강 석 범

# 제 출 문

국립암센터 원장 귀하

이 보고서를 기관고유연구사업 “**난소암 맞춤치료를 위한 분자생물학적 지표연구 II**” 과제의 최종보고서로 제출합니다.

2012. 1. 31

국립암센터

과제책임자: 강석범

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< 요약 문 >

|   |                             |   |         |                  |         |
|---|-----------------------------|---|---------|------------------|---------|
| 연구분야(코드)  |                             |   |         | 과제번호             | 0910260 |
| 과제명   | 난소암 맞춤치료를 위한 분자생물학적 지표연구 II |   |         |                  |         |
| 연구기간/연구비<br>(천원)  | 합계                          | 2009년 1월 1일 ~ 2011년 12월 31일                             | 251,000 |                  |         |
|   | 1차년도                        | 2009년 1월 1일 ~ 2009년 12월 31일                             | 90,000  |                  |         |
|   | 2차년도                        | 2010년 1월 1일 ~ 2010년 12월 31일                             | 80,000  |                  |         |
|   | 3차년도                        | 2011년 1월 1일 ~ 2011년 12월 31일                             | 81,000  |                  |         |
| 과제책임자   | 성명                          | 강석범   | 소속      | 부인암연구과           |         |
|   | 전화번호                        | 031-920-2382  | 전자우편    | sokbom@ncc.re.kr |         |
| 색인단어  | 국문                          | 난소암, 맞춤치료, 분자생물학, 종양표지자                                 |         |                  |         |
|   | 영문                          | ovarian cancer, biomolecular marker, prognosis, therapy |         |                  |         |
| <p><b>◆ 연구목표</b><br/>난소암 1차 치료 및 재발 예측을 위한 혈청 표지자(biomarker) 개발 및 임상 응용을 위한 모델 확립</p>   |                             |   |         |                  |         |
| <p><b>◆ 연구내용 및 방법</b></p> <p>1) 종양표지자를 이용한 난소암 맞춤치료를 위한 전략 수립</p> <p>가) 수술 결과 예측 모델: 종양감축술 예후인자 분석</p> <ul style="list-style-type: none"> <li>- CA125 (cut-off 500U/ml, positive likelihood ratio 1.87; odds ratio 3.7, strong association with suboptimal cytoreduction, 2010년 J Surg Oncol published)</li> <li>- 예후예측을 판별하는 Nadir CA125의 한국 여성에서의 표준치 조사 (2011 J Gynecol Oncol published)</li> <li>- HE4 (cut-off 100pM, strong association with suboptimal cytoreduction, odds ratio 2.5, p = 0.007, 2011년 SGO meeting 발표; 2011년 Annals of Surgical Oncology published)</li> </ul> <p>나) 맞춤치료 의사결정 모델: 환자에 따른 항암화학요법 개인화(individualized) 항암화학요법 주기(cycle) 설정</p> <ul style="list-style-type: none"> <li>- Subnormal CA125 level at remission state: retrospective cohort analysis - remission state의 serum CA125 level은 high risk factor이지만, chemotherapy의 individualization이 예후를 향상시키지 못함을 보고 (2011년 Gynecol Obstet Invest published)</li> <li>다) CA125 level에 따른 neoadjuvant chemotherapy 개인화(individualized) 적용             <ul style="list-style-type: none"> <li>- High serum CA125 level (&gt;2,000 U/ml) 환자에서 neoadjuvant chemotherapy가 환자 예후를 향상시킬 수 있음을 발표 (2010년 IGCS meeting 발표, 2011년 Gynecol Oncol published)</li> </ul> </li> </ul> <p>2) 신규 종양 혈청표지자 개발을 위한 데이터베이스 확보</p> |                             |   |         |                  |         |

| <p>가) 정상대조군 및 난소암 환자군 간의 유망 신규 표지자 HE4를 이용한 조기 검진 모델의 한국 여성에서의 적용 가능성 확인 - 정상인 대 난소암 환자군 median level (111.7pM vs. 68.6pM). 한국 정상-난소암 대조군에서 HE4는 98% specificity에서 50.4%의 sensitivity를 보여서 CA125와 병용하였을 때 진단율의 상승을 기대하기 어려움을 발표 (2012 SGO meeting 발표예정)</p> <p>나) 혈청 데이터베이스의 확보: 2010년 10월 현재 160명의 난소암 환자에서 환자 임상경과에 따라 292개의 샘플을 확보하였음(목표치의 150% 달성)</p> <p>3) 분자표적치료제의 맞춤치료를 위한 표적치료관련 표현형 연구</p> <ul style="list-style-type: none"> <li>- Genomic database query를 통한 표현형 선택: gene-set enrichment analysis(GSEA) - Duke Univ. DB 및 Australia DB의 분석을 통해 PI3K-AKT pathway가 난소암 예후와 연관된 중요인자임을 확인</li> <li>- Connectivity map을 이용하여 PI3K-AKT pathway를 억제할 수 있는 약제 발굴: thioridazine이 ovarian cancer cell에서 PI3K-AKT의 inhibition을 가져오며 cell cycle arrest를 통해 cytotoxicity를 가져오는 것을 발표 (2010 IGCS 발표, 2011 Gynecol Oncol published)</li> </ul> |                       |   |    |                       |        |           |     |      |          |          |          |                |  |  |      |          |      |          |          |                    |
|--|-----------------------|---|----|-----------------------|--------|-----------|-----|------|----------|----------|----------|----------------|--|--|------|----------|------|----------|----------|--------------------|
| <p><b>◆ 연구성과</b></p> <p>-정량적 성과</p> <table border="1"> <thead> <tr> <th>구분</th> <th>달성치/목표치<sup>1)</sup></th> <th>달성도(%)</th> </tr> </thead> <tbody> <tr> <td rowspan="4">SCI 논문 편수</td> <td>목표치</td> <td rowspan="4">300%</td> </tr> <tr> <td>2009년 2편</td> </tr> <tr> <td>2010년 2편</td> </tr> <tr> <td>2011년 2편</td> </tr> <tr> <td colspan="2">총 18편 달성/6편 목표</td> <td></td> </tr> <tr> <td rowspan="4">IF 합</td> <td>2009년 8점</td> <td rowspan="4">347%</td> </tr> <tr> <td>2010년 8점</td> </tr> <tr> <td>2011년 8점</td> </tr> <tr> <td>총 83.3점 달성 /24점 목표</td> </tr> </tbody> </table>  |                       |   | 구분 | 달성치/목표치 <sup>1)</sup> | 달성도(%) | SCI 논문 편수 | 목표치 | 300% | 2009년 2편 | 2010년 2편 | 2011년 2편 | 총 18편 달성/6편 목표 |  |  | IF 합 | 2009년 8점 | 347% | 2010년 8점 | 2011년 8점 | 총 83.3점 달성 /24점 목표 |
| 구분   | 달성치/목표치 <sup>1)</sup> | 달성도(%)  |    |                       |        |           |     |      |          |          |          |                |  |  |      |          |      |          |          |                    |
| SCI 논문 편수  | 목표치                   | 300%  |    |                       |        |           |     |      |          |          |          |                |  |  |      |          |      |          |          |                    |
|  | 2009년 2편              |   |    |                       |        |           |     |      |          |          |          |                |  |  |      |          |      |          |          |                    |
|  | 2010년 2편              |   |    |                       |        |           |     |      |          |          |          |                |  |  |      |          |      |          |          |                    |
|  | 2011년 2편              |   |    |                       |        |           |     |      |          |          |          |                |  |  |      |          |      |          |          |                    |
| 총 18편 달성/6편 목표   |                       |   |    |                       |        |           |     |      |          |          |          |                |  |  |      |          |      |          |          |                    |
| IF 합   | 2009년 8점              | 347%  |    |                       |        |           |     |      |          |          |          |                |  |  |      |          |      |          |          |                    |
|  | 2010년 8점              |   |    |                       |        |           |     |      |          |          |          |                |  |  |      |          |      |          |          |                    |
|  | 2011년 8점              |   |    |                       |        |           |     |      |          |          |          |                |  |  |      |          |      |          |          |                    |
|  | 총 83.3점 달성 /24점 목표    |   |    |                       |        |           |     |      |          |          |          |                |  |  |      |          |      |          |          |                    |
| <p><b>◆ 참여연구원<br/>(최종연도 참여인원)</b></p>  | <p>성명</p>             | <p>노승배, 공선영, 박상윤, 서상수, 임명철, 유종우, 김주영, 정대철, 김석기, 박세현, 황종하, 이보영, 서주희, 김보람, 동승명, 변현정</p> |    |                       |        |           |     |      |          |          |          |                |  |  |      |          |      |          |          |                    |

## Project Summary

|                           |  |
|---------------------------|--|
| <b>Title of Project</b>   | New biomarkers in ovarian cancer for individualized treatment (II) |
| <b>Key Words</b>          | ovarian cancer, biomolecular marker, prognosis, therapy            |
| <b>Project Leader</b>     | Sokbom Kang, M.D.  |
| <b>Associated Company</b> | N/A  |

1) Using in-silico gene signature based approach, we have demonstrated that thioridazine could inhibit phosphatidylinositol-3'-kinase (PI3K)/Akt pathway, and thus exert cytotoxicity in ovarian cancer cells. The current study indicated that in-silico approach, such as Connectivity Map, is a potentially useful method to identify the unknown cellular function among the drugs already in use in clinic. Owing to the property of Akt inhibition and additive cytotoxicity observed with the platinum compound, further research should be focused on this drug.

2) We measured HE4 in pretreatment serum samples from 80 women with epithelial ovarian cancer, using an enzyme-linked immunosorbent assay. The results were correlated with clinical data. Pretreatment serum HE4 seems to be an additional factor for predicting the outcome of patients with epithelial ovarian cancer. Due to its independence from established prognostic factors, serum HE4 may provide additional prognostic information.

3) Among patients who achieved remission after six cycles of carboplatin-paclitaxel chemotherapy, those with CA-125 of 10-35 U/ml at the time of remission were divided into two groups (group A: six cycles of standard chemotherapy vs. group B: two or more additional cycles) and were analyzed. The addition of two or more cycles of standard chemotherapy based on posttreatment CA-125 levels was not beneficial.

4) We retrospectively reviewed records of 314 patients with EOC including 94 patients who received NAC. After stratification by preoperative CA-125 levels, the progression-free survival (PFS) was compared between the NAC group and the primary debulking surgery (PDS) group. Our preliminary data suggests the possible interaction between CA-125 levels and survival benefit of NAC. The randomized trial data about NAC should be stratified by the reproducible and relevant criteria such as preoperative serum CA-125 level to elucidate true survival benefit of NAC in ovarian cancer.

### 1. 연구의 최종목표

- 난소암 1차 치료 및 재발 예측을 위한 혈청 표지자(biomarker) 개발 및 임상 응용을 위한 모델 확립
- 다중 혈청 표지자를 이용 1차 치료 후 PFS 예측 모델 개발
- 혈청 표지자(serum biomarker)를 이용한 난소암 개인화 (individualized) 치료 모델 제시
- Genomic query를 이용하여, thioridazine을 비롯, 기존 임상에서 사용되고 있는 약제의 암 치료제로서의 이용 가능성 탐색

### 2. 연구의 내용 및 결과

#### 1) 종양표지자를 이용한 난소암 맞춤치료를 위한 전략 수립

- 가) 수술 결과 예측 모델: 종양감축술 예후인자 분석
  - CA125 (cut-off 500U/ml, positive likelihood ratio 1.87; odds ratio 3.7, strong association with suboptimal cytoreduction, 2010년 J Surg Oncol published)
  - HE4 (cut-off 100pM, strong association with suboptimal cytoreduction, odds ratio 2.5, p = 0.007, 2011년 SGO meeting 발표)
- 나) 맞춤치료 의사결정 모델: 환자에 따른 항암화학요법 개인화(individualized) 항암화학요법 주기(cycle) 설정
  - Subnormal CA125 level at remission state: retrospective cohort analysis - remission state의 serum CA125 level은 high risk factor이지만, chemotherapy의 individualization이 예후를 향상시키지 못함을 보고 (Gynecol Obstet Invest, published)
  - 다) CA125 level에 따른 neoadjuvant chemotherapy 개인화(individualized) 적용
    - High serum CA125 level (>2,000 U/ml) 환자에서 neoadjuvant chemotherapy가 환자 예후를 향상시킬 수 있음을 발표 (2010년 IGCS meeting 발표, Gynecol Oncol, published)

#### 2) 신규종양혈청표지자 개발을 위한 데이터베이스 확보

- 가) 정상대조군 및 난소암 환자군 간의 유망 신규 표지자 HE4를 이용한 조기 검진 모델의 한국 여성에서의 적용 가능성 확인
  - 정상인 대 난소암 환자군 median level (111.7pM vs. 68.6pM). 한국 정상-난소암 대조

군에서 HE4는 98% specificity에서 50.4%의 sensitivity를 보여서 CA125와 병용하였을 때 진단율의 상승을 기대하기 어려움을 발표 (2011 SGO meeting 발표)

나) 혈청 데이터베이스의 확보: 2010년 10월 현재 160명의 난소암 환자에서 환자 임상경과에 따라 292개의 샘플을 확보하였음(목표치의 150% 달성)

### 3) 분자표적치료제의 맞춤치료를 위한 표적치료관련 표현형 연구

- Genomic database query를 통한 표현형 선택: gene-set enrichment analysis(GSEA) - Duke Univ. DB 및 Australia DB의 분석을 통해 PI3K-AKT pathway가 난소암 예후와 연관된 중요인자임을 확인

- Connectivity map을 이용하여 PI3K-AKT pathway를 억제할 수 있는 약제 발굴: thioridazine이 ovarian cancer cell에서 PI3K-AKT의 inhibition을 가져오며 cell cycle arrest를 통해 cytotoxicity를 가져오는 것을 발표 (2011 IGCS 발표, Gynecol Oncol, published)

### 3. 연구결과 고찰 및 결론

- 본 연구 결과에 따라서 난소암 1차 치료 종료 시 추가적 공고요법 또는 표적치료에 대한 새로운 임상시험을 trigger할 수 있을 것으로 기대됨. 현재 부인암 분야에서는 KGOG가 Gynecologic Cancer Intergroup (GCI), JGOG (Japanese Gynecologic Oncology Group) 및 Gynecologic Oncology Group (GOG)와 연계한 임상시험 기반이 구축되어 이에 따른 다국적 임상시험까지 노력할 수 있을 것으로 기대됨

- 재발 예측 모델에 의하여 새로운 biochemical relapse 진단 기준을 수립할 수 있을 것으로 기대됨. 단, 재발암의 조기 진단이 효과적 후행 치료에 의해 생존률 향상으로 연결될 수 있을지는 미지수이며, 이는 추가적 임상시험이 필요함

- 현재 multiple molecular panel에 의한 분자표지자 연구는 걸음마 단계이며 추후 multi-dimensional omics study의 결과에 따른 다중 표지자 연구 결과가 우후죽순 격으로 쏟아질 것으로 예상됨. 이에 따라, 쏟아지는 결과를 효과적으로 validation 할 수 있는 검체 database가 요구되며 본 연구는 이러한 결과를 만족시킬 수 있을 것으로 판단됨.

- 난소암의 1차 치료 이후 예후 모델링에 따른 예후불량군에 대한 효과적인 대책이 있어야 모델링의 의의가 있음. 따라서, 표적치료 연구의 다음 단계는 예후불량군에 대한 clustering analysis 및 각 subgroup에 대한 효과적인 맞춤 치료를 제시하는 것이 될 것임.

### 4. 연구성과 및 목표달성도

#### (1) 연구성과

가. 국내 및 국제 전문학술지 논문 게재 및 신청

| 논문명  | 저자<br>(저자구분 <sup>1)</sup> ) | 저널명(IF.)                      | Year;<br>Vol(No):Page | 구분 <sup>2)</sup> | 지원과제번호 <sup>3)</sup> |
|--|-----------------------------|-------------------------------|-----------------------|------------------|----------------------|
| A gene signature-based approach identifies thioridazine as an inhibitor of phosphatidylinositol-3'-kinase (PI3K)/AKT pathway in ovarian cancer cell  | 강석범<br>(교신)                 | Gynecol Oncol (3.73)          | 2011                  | 국외 SCI           | 0910260              |
| Interaction between Preoperative CA-125 Level and Survival Benefit of Neoadjuvant Chemotherapy in Advanced Epithelial Ovarian Cancer   | 강석범<br>(교신)                 | Gynecol Oncol (3.73)          | 2011                  | 국외 SCI           | 0910260              |
| Preoperative serum CA-125 levels and risk of suboptimal cytoreduction in ovarian cancer: a meta-analysis   | 강석범<br>(교신)                 | J Surg Oncol (2.5)            | 2010                  | 국외 SCI           | 0910260              |
| Multidetector CT predictors of incomplete resection in primary cytoreduction of patients with advanced ovarian cancer  | 강석범<br>(교신)                 | Eur Radiol (3,589)            | 2010                  | 국외 SCI           | 0910260              |
| Feasibility and Safety of Extensive Upper Abdominal Surgery in Elderly Patients with Advanced Epithelial Ovarian Cancer  | 강석범<br>(교신)                 | JKMS (0.838)                  | 2010                  | 국외 SCI           | 0910260              |
| Diagnostic value of (18)F-FDG PET for evaluation of paraaortic nodal metastasis in patients with cervical carcinoma: a metaanalysis  | 강석범<br>(교신)                 | JNM (6.424)                   | 2010                  | 국외 SCI           | 0910260              |
| Frequent promoter hypermethylation of TGFBI in epithelial ovarian cancer   | 강석범<br>(제1)                 | Gynecol Oncol (3.73)          | 2010                  | 국외 SCI           | 0910260              |
| Pelvic Lymph Node Status Assessed by 18f-Fluorodeoxyglucose Positron Emission Tomography Predicts Low-Risk Group for Distant Recurrence in Locally Advanced Cervical Cancer: A Prospective Study | 강석범<br>(교신)                 | IJROBP (4.592)                | 2010                  | 국외 SCI           | 0910260              |
| Identification of low risk group for parametrial invasion in early-stage cervical cancer   | 강석범<br>(교신)                 | Gynecol Oncol (3.73)          | 2010                  | 국외 SCI           | 0910260              |
| Preoperative prediction model of lymph node metastasis in endometrial cancer   | 강석범<br>(교신)                 | IJGC (2.179)                  | 2010                  | 국외 SCI           | 0910260              |
| Role of extended chemotherapy in advanced ovarian cancer patients with high posttreatment serum CA-125 levels.   | 강석범<br>(교신)                 | Gynecol Obstet Invest (1.031) | 2011                  | 국외 SCI           | 0910260              |
| Does Neoadjuvant Chemotherapy Increase Optimal Cytoreduction Rate in Advanced Ovarian Cancer? Meta-Analysis of 21 Studies  | 강석범<br>(교신)                 | Ann Surg Oncol (3.329)        | 2010                  | 국외 SCI           | 0910260              |
| Nadir CA-125 level is an independent   | 강석범                         | J Surg Oncol                  | 2009                  | 국외               | 0910260              |

|   |          |                      |          |          |         |
|---|----------|----------------------|----------|----------|---------|
| prognostic factor in advanced epithelial ovarian cancer   | (교신)     | (2,183)              |          | SCI      |         |
| Epstein-Barr virus-transformation of B-cell lines in ovarian cancer patients: feasibility of genomic storage for unlimited use  | 강석범 (교신) | J Gynecol Oncol      | 2009     | 국내 SCI-E | 0910260 |
| Prediction of a high-risk group based on postoperative nadir CA-125 levels in patients with advanced epithelial ovarian cancer.   | 강석범 (교신) | J Gynecol Oncol      | 2011     | 국내 SCI-E | 0910260 |
| Sentinel lymph node biopsy in endometrial cancer: meta-analysis of 26 studies.  | 강석범 (교신) | Gynecol Oncol (3.73) | 2010     | 국외 SCI   | 0910260 |
| PREFACE 1.0: A Risk Assessment Tool for Distant Recurrence after Definitive Platinum-based Concurrent Chemoradiation in Patients with Locally Advanced Cervical Cancer: A Korean Gynecologic Oncology Group Study | 강석범 (제1) | J Clin Oncol (18.97) | in press | 국외 SCI   | 0910260 |
| Preoperative Identification of a Low-Risk Group for Lymph Node Metastasis in Endometrial Cancer: A Korean Gynecologic Oncology Group Study  | 강석범 (제1) | J Clin Oncol (18.97) | in press | 국외 SCI   | 0910260 |

다. 연구성과의 정부정책 기여

| 보고서명               | 정부정책    | 기여내용 |
|--------------------|---------|------|
| HE4(ELISA) 유용성/안전성 | 신의료기술평가 | 자문   |
| HE4(CMIA) 유용성/안전성  | 신의료기술평가 | 자문   |

나. 국내 및 국제 학술대회 논문 발표

| 논문명   | 저자  | 학술대회명                                    | 지역 <sup>1)</sup> | 지원과제번호  |
|---|-----|--|------------------|---------|
| A gene signature-based approach identifies thioridazine as an inhibitor of phosphatidylinositol-3'-kinase (PI3K)/AKT pathway in ovarian cancer cell | 강석범 | International Gynecologic Cancer Society | 국외               | 0910260 |
| Interaction between Preoperative CA-125 Level and Survival Benefit of Neoadjuvant Chemotherapy in Advanced Epithelial Ovarian Cancer                | 강석범 | International Gynecologic Cancer Society | 국외               | 0910260 |
| Identification of low risk group for parametrial invasion in early-stage cervical cancer  | 강석범 | Society of Gynecologic Oncologist        | 국외               | 0910260 |
| The Role of Extended Chemotherapy in Advanced Ovarian Cancer Patients with High Post-treatment Serum CA-125 Levels                                  | 강석범 | Society of Gynecologic Oncologis         | 국외               | 0910260 |
| Nadir CA-125 level is an independent prognostic factor in advanced epithelial ovarian cancer  | 강석범 | Annual Meeting of SGO                    | 미국               | 0910260 |
| Does Neoadjuvant Chemotherapy Increase Optimal Cytoreduction Rate in Advanced Ovarian Cancer? Meta-Analysis of 21 Studies                           | 강석범 | FIGO world congress                      | 남아공              | 0910260 |

(2) 목표달성도

가. 연구목표의 달성도

| 최종목표   | 연차별목표 |   | 달성내용   | 달성도(%) |      |
|--|-------|---|--|--------|------|
|  | 연차    | 최종  |  | 연차     | 최종   |
| 난소암 1차 치료 및 재발 예측을 위한 혈청 표지자(biomarker) 개발 및 임상 응용을 위한 모델 확립 | 1차년도  | 다중 혈청 표지자를 이용한 1차 치료 후 PFS 예측 모델 개발   | 다중 혈청 표지자를 이용하여 난소암 생존에 가장 중요한 인자인 1차 치료 후 PFS 예측 모델 개발  | 3      | 100% |
|  | 2차년도  | 다중 혈청 표지자를 이용한 1차 치료 후 PFS 예측 모델 개발   | HE4 및 CA125에 의한 suboptimal surgery risk 증가 및 PFS 연관 규명 및 CA125에 의한 neoadjuvant chemotherapy의 individualized treatment 가능성 제시   | 2      | 100% |
|  | 3차년도  | 1) 혈청 표지자 (serum biomarker)를 이용한 난소암 개인화 치료 모델 제시<br>2) Genomic query를 이용하여, thioridazine을 비롯, 기존 임상에서 사용되고 있는 약제의 암 치료제로서의 이용 가능성 탐색 | 1) 난소암 신규 혈청 표지자 개발을 위한 검체 추가확보<br>2) 난소암 개인화 치료 모델 제시를 위한 다기관 연구 개시<br>3) Proteomics을 이용한 신규 혈청 표지자 개발 협력연구<br>4) Thioridazine의 혈관생성억제기능 규명<br>5) 기존 임상약제의 항암작용 가능성 추가 규명 | 3      | 100% |

나. 평가의 착안점에 따른 목표달성도에 대한 자체평가

| 평가의 착안점                                 | 자체평가   |
|---|--|
| 혈청내 표지자를 이용한 1차 치료 후 PFS 예측 모델 개발 성공 여부 | HE4 및 CA125와 PFS, suboptimal surgery risk 증가를 확인하였으며 보다 다수의 cohort를 대상으로 validation 하는 작업이 향후 필요함              |
| 부가적 후보 혈청 표지자의 개발                       | 축적된 데이터베이스를 기반으로 프로테오믹스 기법 도입한 신규 표지자 개발 및 genomic query를 통한 신규 예후 연관 pathway targeting strategy를 적극 연구해야 할 것임 |

5. 연구결과의 활용계획

(1) 연구종료 2년후 예상 연구성과

| 구분        | 건수 | 비고                                |
|-----------|----|-----------------------------------|
| 학술지 논문 게재 | 5  | Journal of Clinical Oncology 2편 외 |

6. 참고문헌

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7. 첨부서류

## Prediction of a high-risk group based on postoperative nadir CA-125 levels in patients with advanced epithelial ovarian cancer

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**Objective:** We aimed to determine the ideal cut-off of nadir serum CA-125 level for prediction of progression free survival. **Methods:** Among 267 patients who achieved complete remission after chemotherapy, the correlation between nadir CA-125 and progression free survival were compared among the subgroups classified according to the distribution of CA-125. The diagnostic odds ratio and area under the receiver operator characteristics curve were compared at various cut-off points.

**Results:** The nadir CA-125 levels did not have prognostic value under 12 U/mL (to 75 percentile). In contrast, they were significantly correlated with progression free survival only when the CA-125 level was greater than 12, which was 75 percentile ( $p=0.034$ ). In predicting progression free survival <6 and 12 months, the cut-off value of 18 (90 percentile) showed superior diagnostic performance over 10 or 12 U/mL. Compared with patients who showed nadir levels between 0 and 12 U/mL (0 to 75 percentile), those with nadir >18 U/mL showed a hazard ratio of 2.85 (95% confidence interval, 1.70 to 4.76;  $p<0.001$ ); patients with nadir levels between 18 and 12 U/mL showed a hazard ratio of 1.68 (95% confidence interval, 1.11 to 2.56;  $p=0.015$ ) compared with those whose nadir levels were under 12 U/mL.

**Conclusion:** The predictive power of the traditional cut-off of 10 U/mL to classify a risk group or to identify high risk patients was unsatisfactory. The optimal diagnostic performance was observed at the cut-off of 18 U/mL and this can be proposed to dichotomize cut-off values to predict outcomes among individual patients.

**Keywords:** Biomarker, CA-125, Ovarian cancer, Prognosis, Progression free survival, Risk factor

### INTRODUCTION

Epithelial ovarian cancer (EOC) is the leading cause of gynecologic cancer death in Western countries [1]. Despite

standardized treatment consisting of cytoreductive surgery followed by platinum-based systemic chemotherapy, the prognosis of patients with advanced ovarian cancer is poor and the majority of them experience recurrences [2,3]. As a result, there has been considerable interest in developing predictive markers to assess the risk of a relapse in advanced EOC patients. The most famous biomarker in EOC is CA-125, which has been used in both diagnosis of the disease and monitoring of treatment [4-6]. Recent evidence indicates that the nadir CA-125 level after completion of primary treatment was associated with prognosis [7-11]. Though the use of prog-

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[www.ejgo.org](http://www.ejgo.org)

## Serum HE4 Level is an Independent Prognostic Factor in Epithelial Ovarian Cancer

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### ABSTRACT

**Background.** This study was designed to determine whether serum HE4 is an independent prognostic factor in ovarian cancer patients.

**Methods.** We measured HE4 in pretreatment serum samples from 80 women with epithelial ovarian cancer, using an enzyme-linked immunosorbent assay. The results were correlated with clinical data.

**Results.** The median serum HE4 level in ovarian cancer patients was 98.7 (range, 80.3–222.8) pg/ml. Elevated serum HE4 levels before therapy significantly correlated with a poorer progression free survival (log-rank test,  $P = 0.017$ ). Multivariate analysis revealed serum HE4 to be an independent prognostic factor for progression-free survival ( $P = 0.036$ ). In multivariate regression analysis, high serum HE4 levels significantly correlated with high tumor grade and serous histology ( $P = 0.004$  and  $0.017$ ). In addition, high serum HE4 levels were significantly associated with residual tumor size and operative time ( $P = 0.003$  and  $0.033$ ).

**Conclusions.** Pretreatment serum HE4 seems to be an additional factor for predicting the outcome of patients with epithelial ovarian cancer. Due to its independence

from established prognostic factors, serum HE4 may provide additional prognostic information.

Ovarian cancer is the leading cause of mortality among women with gynecologic malignancies.<sup>1,2</sup> Therefore, the development of novel biomarkers that can sufficiently contribute to predicting the prognosis of this disease process is paramount in identifying specific patients who may benefit from aggressive therapies.<sup>3</sup>

HE4 (WFDC2) is composed of two whey acidic protein (WAP) domains and a four-disulfide core and has been shown to be overexpressed by epithelial ovarian cancer tumors.<sup>4-6</sup> HE4 is expressed primarily in the reproductive tract and upper airways and can be detected in the sera of patients.<sup>4,7,8,10-12</sup> Recent evidence has shown that the serum HE4 (human epididymal secretory protein E4; WAP four-disulfide core domain protein 2, WFDC2) level is a novel biomarker for the early detection of ovarian cancer.<sup>8,11-17</sup>

Although many studies have suggested that serum HE4 is useful for the detection of ovarian cancer, its prognostic role has not been determined. The present study investigated the prognostic significance of serum HE4 levels in 80 patients with primary ovarian cancer. The correlation between serum HE4 levels and other established prognostic factors also was investigated.

### MATERIALS AND METHODS

#### Patients

Blood samples were obtained from patients with primary epithelial ovarian cancer before surgery between

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## Is Neo-adjuvant Chemotherapy a “Waiver” of Extensive Upper Abdominal Surgery in Advanced Epithelial Ovarian Cancer?

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### ABSTRACT

**Background.** The goal of this study was to explore the necessity of extensive surgical procedures in patients who received neoadjuvant chemotherapy (NAC).

**Methods.** We analyzed the surgical outcomes and frequency of extensive procedures required for maximal cytoreductive surgery after NAC and primary debulking surgery (PDS) in 256 women with advanced epithelial ovarian cancer.

**Results.** NAC was performed in 116 of 256 women (45.3%). In NAC group, complete cytoreduction rate and optimal cytoreduction rate were 60.3 and 92.2%, respectively. Although the NAC group comprised patients with higher risk of suboptimal cytoreduction, complete cytoreduction rate was similar to that of PDS group (57.9%,  $P = .69$ ). Moreover, blood loss and surgical complexity significantly reduced in NAC group ( $P = .011$  and  $.017$ ). Extensive upper abdominal surgery (EUAS) was performed in 70 of 116 patients (60.3%) in the NAC group. The frequency of EUAS was similar between NAC and PDS group ( $P = .60$ ). Among NAC group, gross upper abdominal metastasis requiring EUAS was found in 51 patients (44%, 95% confidence interval = 35.3–53.1%).

**Conclusions.** A significant proportion of patients who received NAC still have gross metastatic tumors requiring EUAS. Gynecologic oncologists should be familiar with EUAS and be ready to perform any required procedures together with multidisciplinary teams, even in the patients who have received NAC.

Ovarian cancer is the leading cause of gynecologic cancer death in Western countries.<sup>1</sup> The standard treatment for advanced epithelial ovarian cancer (EOC) is maximal cytoreductive surgery followed by chemotherapy with a platinum and taxane regimen.<sup>2–4</sup> Although optimal cytoreductive surgery is the most important prognostic factor, gynecologic oncologists are often frustrated because optimal cytoreductive surgery cannot be achieved in some proportion of patients with advanced EOC.<sup>2,5</sup> Therefore, many gynecologic oncologists administer neoadjuvant chemotherapy (NAC) in patients with advanced EOC when they anticipate that optimal cytoreductive surgery may not be possible.<sup>6,7</sup> However, despite the results of a recent randomized trial, the true benefit of NAC is still unclear.<sup>7</sup>

Although a variety of benefits of NAC for the management of EOC have been suggested, many of the proposed benefits have yet to be validated.<sup>8–10</sup> One of the suggested benefits is that NAC enables less extensive surgery to be performed.<sup>11–15</sup> Indeed, it was claimed that, with NAC, optimal surgery requires less extensive procedures, such as a significantly reduced need for bowel resections and splenectomies.<sup>13</sup>

In the retrospective and the prospective observational cohort, we aimed to explore the need for extensive surgical procedures in patients who received NAC. To that end, we analyzed how often extensive procedures were performed to achieve maximal cytoreduction in patients who received NAC before surgery.

### MATERIAL AND METHODS

#### Patient Selection

We identified 256 patients who had undergone surgery for stage III or IV EOC between January 2001 and June 2010, from the institutional registry. All patients with EOC treated at our institution during that period were included in this analysis, and the patients with borderline tumors were

## III. 제 2세부과제

세부과제명 : 부인 암에서의 Thioridazine을 이용한 항암효과 및 신생 혈관형성 억제 기전 연구(Anti-cancer effect and molecular mechanism of angiogenesis inhibitor by thioridazine)

세부과제책임자(성명/소속) : 노 승 배

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|   |  |   |         |                 |         |
|---|--|---|---------|-----------------|---------|
| 연구분야(코드)  |  |   |         | 과제번호            | 0910260 |
| 과제명   | 부인 암에서의 thioridazine을 이용한 항암효과 및 신생혈관형성 억제 기전 연구 |   |         |                 |         |
| 연구기간/연구비<br>(천원)  | 합계   | 2011년 01월01일 ~ 2011년 12월31일                       | 120,000 |                 |         |
|   | 1차년도   | 2011년 01월01일 ~ 2011년 12월31일                       | 120,000 |                 |         |
| 과제책임자   | 성명   | 노승배   |         |                 |         |
|   | 전화번호   | 031-920-2383                                      | 전자우편    | sbrho@ncc.re.kr |         |
| 책임단어  | 국문   | 세포사멸, 인테그린, 신생혈관형성, 신호전달                          |         |                 |         |
|   | 영문   | apoptosis, integrin, angiogenesis, signal pathway |         |                 |         |
| <p><b>◆ 연구목표</b></p> <p>&lt;최종목표&gt;</p> <p>암화과정에서 중요한 역할 수행을 하는 integrin 활성을 저해하는 세포내 신호전달체계를 규명하고 항암 분자 기전을 통하여 이행성 연구에 있어 질한 치료법의 개발을 위한 중요한 모델을 제공</p> <ul style="list-style-type: none"> <li>· 부인 암 세포주에서 Thioridazine이 갖는 종양형성 억제 및 세포사멸 기능 연구</li> <li>· 종양신생혈관형성 억제제로서 기존 항암제와의 병합치료 효과 검증</li> <li>· Thioridazine이 갖는 혈관생성 억제 활성을 통한 세포내 신호전달체계 확립 및 (진)임상 연구를 위한 자료를 제공</li> </ul> <p>&lt;당해연도목표&gt;</p> <p>가) 부인 암 세포주 (자궁경부암, 난소암, 자궁내막암 - 6종류)에서 Thioridazine 의 항암 활성 효과 검색 및 검증</p> <p>나) Thioridazine 의 세포사멸 기전 경로 확인</p> <p>다) 기존 항암 화학 요법과 병합효과 기능성 검증</p> |  |   |         |                 |         |
| <p><b>◆ 연구내용 및 방법</b></p> <p>가. 암 세포주에 Thioridazine 처리 후 MTT assay를 이용한 농도별 생존력 관찰</p> <p>나. DAPI와 TUNEL 방법을 통한 세포내의 핵의 변화를 대조군과 비교하여 확인</p> <p>다. Flow cytometry을 이용하여 cell cycle 변화 및 cell division 분석</p>  |  |   |         |                 |         |

## Project Summary

|   |  |
|---|--|
| <b>Title of Project</b>   | Anti-cancer effect and molecular mechanism of angiogenesis inhibitor by thioridazine |
| <b>Key Words</b>  | apoptosis, integrin, angiogenesis, signal pathway                                    |
| <b>Project Leader</b>   | Seung Bae Rho  |
| <b>Associated Company</b>   |  |
| <p>Thioridazine, a derivative of phenothiazine, has been reported to have antiproliferative activity on tumor cells. However, the mechanism has not been well defined. Using in-silico gene signature based approach, we have demonstrated that thioridazine could inhibit phosphatidylinositol-3'-kinase (PI3K)/Akt pathway, and thus exert cytotoxicity in ovarian cancer cells. The Connectivity Map indicated that thioridazine induces gene signature similar to that of Akt inhibition. Moreover, preexisting inhibitors of PI3K/Akt pathway were also found to reveal similar signature. In SKOV-3 cells, immunoblot using p85 antibody showed that thioridazine could inhibit PI3K signal. In addition, thioridazine was found to inhibit p-Akt (Ser 473) in a dose-dependent manner. Furthermore, thioridazine was found to decrease cell viability and induce apoptosis. Exposure to thioridazine induced G0/G1 arrest and down-regulated the cell cycle regulator, Cyclin D1 and CDK4, and up-regulated p21, p16, and p-CDC25A. Finally, additive cytotoxicity was observed when cisplatin and thioridazine were treated simultaneously. The current study indicated that in-silico approach, such as Connectivity Map, is a potentially useful method to identify the unknown cellular function among the drugs already in use in clinic. Owing to the property of Akt inhibition and additive cytotoxicity observed with the platinum compound, further research should be focused on this drug.</p> |  |

라. 약물 처리 후 변화되는 단백질 (CDK, Cyclin D1, p21, p16, p-CDC25A etc.)을 관찰 및 분석

마. Western blotting 을 통해 세포사멸 경로 확인 [Caspase-3 (z-DEVD-fmk) and 9 (z-LEHD-fmk) inhibitor 처리]

바. Thioridazine을 처리한 세포주와 PI3K inhibitor인 LY294002 와 wortmannin 를 처리한 세포주를 가지 PI3K 활성 조사 및 비교 분석

사. 농도별에 따른 Akt 인산화 변화 관찰 및 mTOR 억제제인 Rapamycin을 처리한 세포주와의 비교 분석 및 down-stream의 4E-BP1 단백질의 인산화 변화 관찰

아. 기존의 항암효과를 나타내는 cisplatin 과 taxol 을 각각 동시에 처리했을 때 시너지 항암 활성 효과를 볼 수 있는지를 MTT assay 및 caspase activity assay 방법을 통해 비교 분석

### ◆ 연구성과

- 정량적 성과

| 구분        | 달성치/목표치  | 달성도(%) |
|-----------|--|--------|
| SCI 논문 편수 | 3/3  | 100 %  |
| IF 합      | 12.87/11   | 117 %  |
| 기타 성과     | 현재 논문 1편 in press, 1편 revision중이며,<br>1편 submitted |        |

- 정성적 성과

1. Thioridazine는 난소암에 있어 세포사멸을 유도함으로써 종양 억제 효과를 보임.
2. 이러한 현상은 PI3K/Akt 신호전달 경로를 차단함으로써 세포주기를 arrest 시키고, 기존 항암제와의 병합효과 결과에서도 탁월한 효과를 보임.
3. 신생혈관 형성 억제 효과에도 관여

### ◆ 참여연구원 (최종연도 참여인원)

성 명

박상운, 강석범, 변현정, 김보람

## 1. 연구의 최종목표

암화과정에서 중요한 역할 수행을 하는 integrin 활성을 저해하는 세포내 신호전달체계를 규명하고, 항암 분자 기전을 통하여 이행성 연구에 있어 질환 치료법의 개발을 위한 중요한 모델을 제공

## 2. 연구의 내용 및 결과

### (1) 2011년도 (2011년 1월 - 12월) 연구내용

#### 1) 부인암 세포주에서 thioridazine의 항암 활성 효과 검색 및 검증

- 가. 암 세포주에 thioridazine 처리 후 MTT assay를 이용한 농도별 생존력 관찰
- 나. DAPI와 TUNEL 방법을 통한 세포내의 핵의 변화를 대조군과 비교하여 확인
- 다. Flow cytometry을 이용하여 cell cycle 변화 및 cell division 분석
- 라. 약물 처리 후 변화되는 단백질 (CDK, Cyclin D1, p21, p16, p-CDC25A etc.)을 관찰 및 분석

#### 2) Thioridazine의 세포사멸 기전 경로 확인

- 가. Western blotting 을 통해 세포사멸 경로 확인 [Caspase-3 (z-DEVD-fmk) and 9 (z-LEHD-fmk) inhibitor 처리]
- 나. Thioridazine을 처리한 세포주와 PI3K inhibitor인 LY294002 와 wortmannin를 처리한 세포주를 가지고 PI3K 활성 조사 및 비교 분석
- 다. 농도별에 따른 Akt 인산화 변화 관찰 및 mTOR 억제제인 Rapamycin을 처리한 세포주와의 비교 분석 및 down-stream의 4E-BP1 단백질의 인산화 변화 관찰

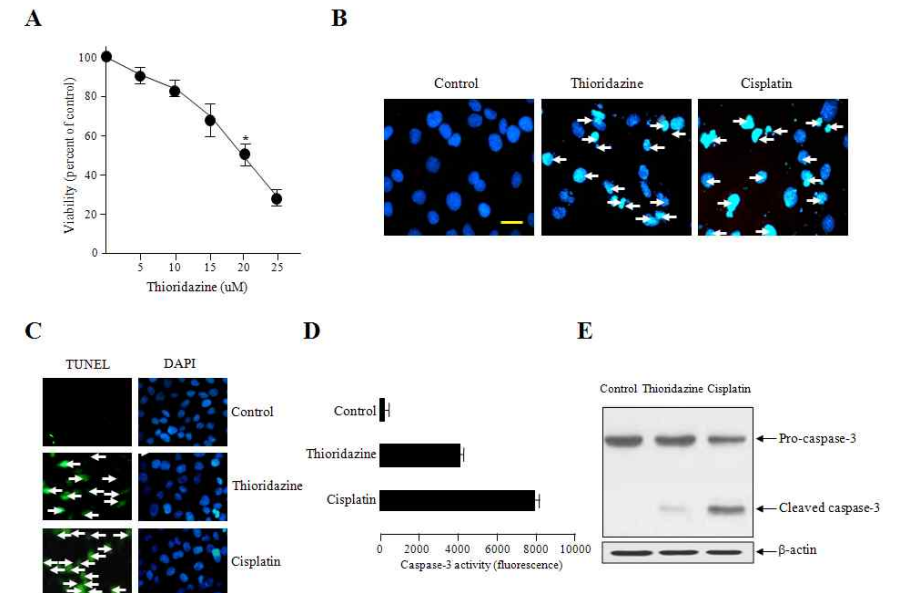
#### 3) 기존 항암 화학 요법과 병합효과 가증성 검증

- 가. 기존의 항암효과를 나타내는 cisplatin과 taxol 을 각각 동시에 처리 했을때 시너지 항암 활성 효과를 볼 수 있는지를 MTT assay 및 caspase activity assay 방법을 통해 비교 분석

## (2) 연구결과

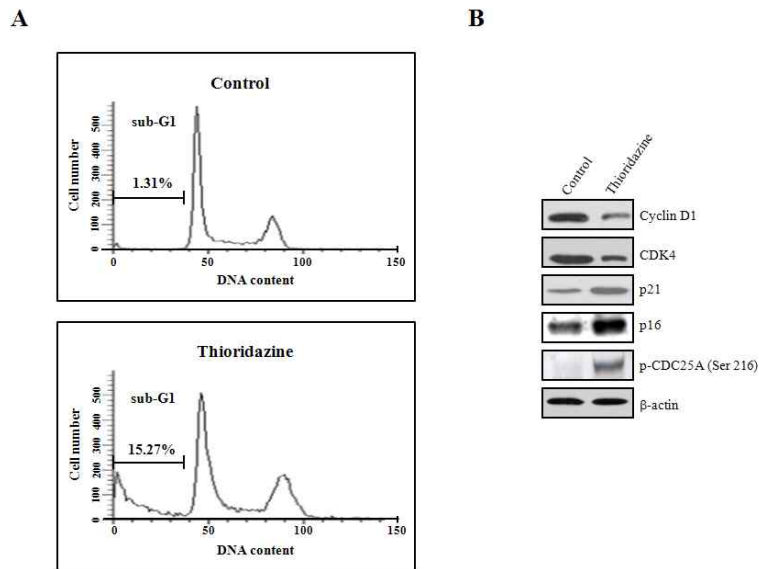
### 1) 난소암 세포주에서 thioridazine의 세포내 기능

본 연구진은 먼저 여성생식기 암 중 난소암에서 Thioridazine의 특성을 알아보기 위하여 난소암 세포주인 SKOV-3 세포에 농도별로 처리한 후 세포 생존력을 관찰하였다. 그림에서 보시는 바와 같이 (그림 1) 농도에 따라 점진적으로 세포의 생존 능력이 감소하는 경향을 볼 수 있으며 20  $\mu$ M의 농도를 처리했을 때 50% 가까이 세포 사멸을 유도하는 것을 알 수 있었다 (Thioridazine의 약물 농도를 20  $\mu$ M로 모든 실험에 사용). 이러한 현상을 좀 더 세밀하게 조사하기 위하여 다양한 세포사멸 방법들을 도입하여 재 확인해 본 결과 기존의 항암제인 Cisplatin과 비교 분석 했을 때 Cisplatin 보다는 약간 사멸 능력이 떨어지거나 세포사멸을 유도하는 것을 볼 수가 있었다. 따라서 현재까지 Thioridazine이 가지고 있는 정확한 특성 및 기능에 대해서 전혀 알려진 바가 없는 상태이므로 여성생식기암과의 관계를 좀 더 자세히 연구할 필요가 있다고 사료되었다.



**Figure 1.** The effect of thioridazine on cell growth. A) Various concentration of thioridazine used for the assays (5~25  $\mu$ M). SKOV-3 cells were treated with or without thioridazine. 24 hour after treated,

cell viability was measured using MTT colorimetric assay. B) MTT assay was performed on SKOV-3 cancer cells treated for 24 hours with 20  $\mu$ M drug. The relative rate of cell viability/proliferation was determined using the MTT assays. The amount of MTT-formazan was measured as absorbance at 540 nm. Absorbance of sample was converted as the relative rate of proliferation. C) Apoptosis examined by DNA fragmentations. Cells were stained with DAPI and visualized by fluorescence microscopy. The *arrow* indicates DNA fragmentations. Size bar, 20  $\mu$ m. D) Cultured SKOV-3 cells were treated with and without thioridazine for 24 hours, cells were harvested, cell lysates prepared. Cell lysates were incubated with substrate, and absorbance at 405 nM (Molecular Devices, Sunnyvale, USA) was measured to determine caspase-3 activity. E) SKOV-3 cells were treated for 24 hours with/without 20  $\mu$ M thioridazine. Cell lysates were immunoblotted with anti-caspase-3 (left), anti-caspase-9 (right), and with  $\beta$ -actin for internal control.



**Figure 2.** Effects of thioridazine of cell cycle progression of human SKOV-3 ovarian cancer cells. A) SKOV-3 cells were exposed with and without drug for 24 hours. Cellular DNA was stained with propidium iodide(PI) and cell cycle distributions was determined by flow cytometry (FACScalibur, Becton Dickinson). When treated to only thioridazine, the cell proportion in  $G_1$  phase increased, indicating that thioridazine arrested the cell cycle at the  $G_0$ - $G_1$  phase. B: Subsequently, the effect of thioridazine on downstream expression profile of proteins associated with cell cycle arrest was tested. Protein expression was visualized by immunoblot analysis using the appropriate specific antibodies.

세포사멸을 유도한 세포들이 정확히 어느 단계에서 일어나는지를 확인하기 위하여 cell division 분석 방법 중 하나인 flow cytometry (FACS) 방법을 통하여 세포들의 분포도를 관찰하

였다. 그 결과 그림에서 보시는 바와 같이 (그림 2) sub-G1 에 세포들이 많이 분포되어 있는 것을 알 수 있었는데 이것을 다시 말하면  $G_0$ - $G_1$  phase에서 세포주기를 arrest 한다는 것을 의미를 갖고 있다. 이처럼 Thioridazine이 정확하게 세포주기를 arrest 한다면, 이로 인한 세포주기에 관계된 단백질들의 발현은 감소하거나 또는 증가하는 현상이 뚜렷이 나타날 것으로 생각되어 지며 그 결과 역시 증가하거나 감소하는 경향들을 볼 수 있었다 (그림 2B).

## 2) 난소암 세포주에서 thioridazine의 병합처리 효과

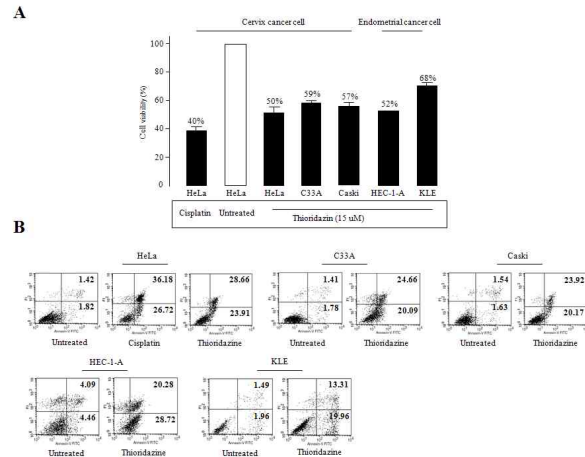
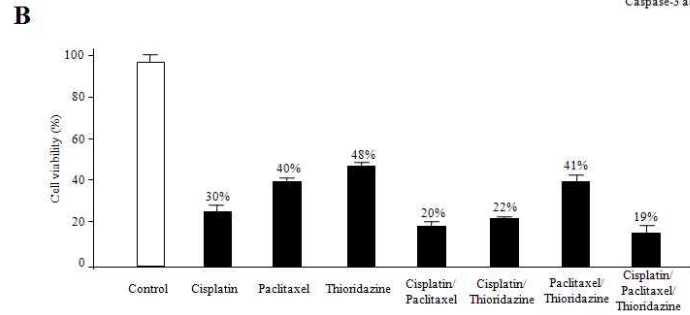
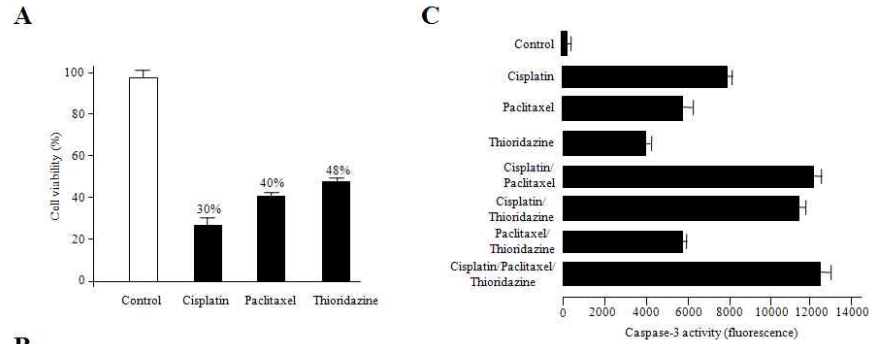
기존 항암제와의 병합 투여 효과를 알아보기 위하여 본 연구에 Cisplatin 과 Paclitaxel을 사용하였다. 그림에서 보시는 바와 같이 (그림 3) Cisplatin 과의 병합 투여 효과만이 증가하는 현상을 보였으며, Paclitaxel 과의 병합 투여에서는 반응을 보이지 않았다. 이는 크게 두 가지 생각해 볼 수가 있는데 하나는 약물들이 서로 세포내에서 인지하고 억제하고자 하는 리셉터나 단백질들이 다르거나, 즉 다시 말하면 신호전달체계에 다를 수 있기 때문이다. 또 하나는 약물을 처리하는데 있어 순서도 중요하리라 생각이 든다. 현재까지의 결과는 Cisplatin 과 Paclitaxel 을 각각 먼저 처리한 후 Thioridazine을 처리한 결과만을 보여 주고 있다. 따라서 Thioridazine 을 먼저 처리한 후 그 다음 Cisplatin 과 Paclitaxel을 각각 처리한 후의 결과를 얻는다면 이와 같이 병합 투여 효과에서 나타나는 결과들을 좀 더 정확히 해석되어지리라 생각되어 진다.

**Figure 3.** The additive effects of cell cycle arrest by thioridazine and two conventional chemotherapeutic agents. Cells were then incubated with each drug-agent for 24 hours. Cell viability was then determined using a viable cell assay. Apoptosis was assessed by fluorescence microscopy. Triplicate samples were determined for every data point and SDs indicated as *error bars*. The experiments were repeated four times with similar results.

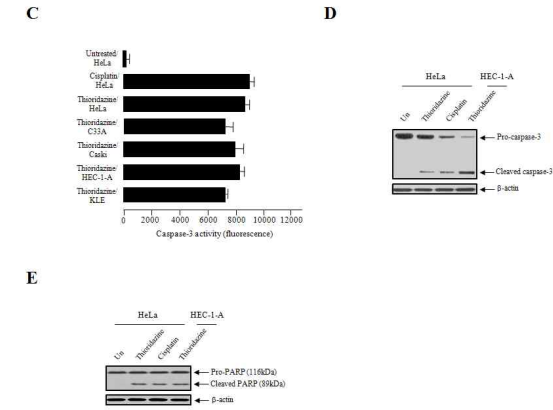
## 3) 자궁경부암 및 내막암에서의 thioridazine의 세포사멸 기능

난소암 뿐만아니라 자궁경부 및 내막암에서도 세포증식 억제효과를 나타내는지를 알아보기 위하여 각각 2개씩 암 세포주를 이용하여 세포사멸 기능을 조사하였다 (그림 4).

**Figure 4.** Treatment of thioridazine inhibits cellular proliferation and activates caspase-dependent pro-apoptotic activity. A. Effect of treatment with thioridazine (15  $\mu$ M) was analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assays. Inhibition of cellular proliferation by cisplatin (20  $\mu$ M) in HeLa cells was also illustrated for comparison. B. Early- and late-stage apoptosis induced by thioridazine was analyzed by fluorescein isothiocyanate (FITC)-labeled



Annexin V assay. C. Caspase-3 enzymatic activity after treatment of thioridazine was determined using acetyl-DEVD-7-amino-4-trifluoromethyl coumarin as the substrate. D-E. Caspase-3 and PARP cleavages induced by cisplatin and thioridazine treatments. Soluble protein extracts were conducted by immunoblotting for cleaved caspase-3 and cleaved PARP.  $\beta$ -actin was used as an equal loading control.



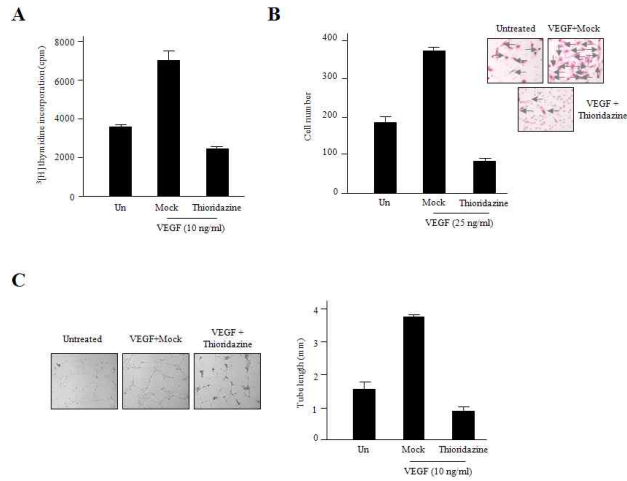
그림에서 보시는 바와 같이 thioridazine을 자궁경부암 세포 주(HeLa의 경우)에 transfection 시킨 결과 early stage에서 26.72%의 자궁경부암 세포를 사멸시키는 효과를 보여 주고 있다. 그리고 growth를 조사한 결과 thioridazine을 처리했을 때 암 세포 주에서 성장이 일어나지 않았다. 동시에 caspase-3 activity와 PARP assay를 조사한 결과에서도 현저히 사멸하는 세포가 증가하는 것을 관찰하였다.

#### 4) Thioridazine의 신생혈관형성 억제 가능성 연구

신생혈관형성은 endothelial cell 의 proliferation, migration and invasion, vessel formation 에 의하여 이루어진다. 먼저 *in vitro* 에서의 thioridazine 의 신생혈관형성 억제 활성을 조사하기 위하여 HUVECs 에 thioridazine를 처리한 후 그 억제능을 분석하였다. 먼저 세포 증식효과를 알아보기 위하여 [ $^3H$ ]thymidine incorporation assay를 실시하여 DNA 합성량을 조사하였다. 그 결과 VEGF 에 의하여 증가된 DNA 합성이 thioridazine 에 의하여 급격히 감소되는 현상이 나타났다 (그림 5A).

**Figure 5.** Thioridazine inhibits endothelial cell proliferation *in vitro*. (A) The angiogenic effects of thioridazine on DNA synthesis in endothelial cells were followed with/without VEGF (10 ng/ml) treatment for 48 hours. cpm values of [ $^3H$ ]thymidine were determined using a liquid scintillation counter. (B) Migration. (C) Tube formation in HUVECs.

배양한 인체 혈관내피세포에 thioridazine 처리한 후 HUVEC 세포의 침윤과, 이동, 및 관 형성을 대조군인 control Mock을 처리한 세포와 비교하였다. 보시는 바와 같이 VEGF에 의해 활성화 된 migration이 thioridazine에 의해 현저히 감소하는 현상을 볼 수 있었으며 (그림 5B), 동시에 Transwell tube formation을 실시한 결과에서도 VEGF에 의해 증가된 tube formation이 thioridazine에 의하여 완전히 저해되는 것을 볼 수 있었다 (그림 5C).



### 3. 연구결과 고찰 및 결론

본 연구의 결과 thionidazine은 세포내 세포사멸을 통해 PI3K/Akt 신호전달을 차단하였고 그 결과 암세포 신생혈관 형성을 유도하는 주 촉진인자인 VEGF의 발현 정도를 억제시킴으로 신생혈관을 일어나지 못하게 함으로서 암세포의 살상효과를 나타내었다. 동시에 정상세포에는 독성을 나타내지 않는 대단히 효과적이며 안전성이 높은 보조적 항암치료제로서의 가능성을 보여주었다. 또한 신생혈관을 저해하는 암 치료 연구 분야가 현재까지도 전 세계적으로 각광을 받고 있으나 보다 효과적이고 정상세포에는 독성을 나타내지 않는 치료제의 개발이 요구되고 있는 실정에 있다. 이러한 면에서 볼 때 결과적으로 본 연구는 PI3K/Akt를 targeting 할 수 있는 새로운 치료방법의 가능성을 제시하였으며 이를 이용한 새로운 보조적 항암치료제에 이용될 수 있을 것이다.

현 연구 과제를 수행하면서 그동안 어려웠던 점은 1년이란 짧은 기간 동안 많은 성과물을 도출해 내었지만 지속적인 연구수행 기간과 연구비용이 절실히 필요한 부분이 있다.

### 4. 연구성과 및 목표달성도

#### (1) 연구성과

가. 국내 및 국제 전문학술지 논문 게재 및 신청

| 논문명  | 저자 (저자구분)     | 지널명 (I.F.)                  | Year; Vol(No):Page     | 구분     | 지원과제번호    |
|--|---------------|-----------------------------|------------------------|--------|-----------|
| A gene signature-based approach identifies thionidazine as an inhibitor of phosphatidylinositol-3'-kinase (PI3K)/AKT pathway | 노승배 (제1)      | Gynecologic Oncology (3.76) | 2011; 120 (1): 121-127 | 국외 SCI | 0910260,2 |
| Programmed cell death 6 (PDCD6) inhibits angiogenesis through PI3K/mTOR/p70S6K pathway by interacting of VEGFR-2             | 노승배 (제1 및 교신) | Cellular Signaling (4.243)  | 2012; 24 (1): 131-139  | 국외 SCI | 0910262   |
| CYR61 controls p53 and NF-κB expression through PI3K/Akt/mTOR pathways in carboplatin-induced ovarian cancer cells           | 노승배 (교신)      | Cancer Letters (4.864)      | 2012; 315 (1): 86-95   | 국외 SCI | 0910262   |
| PDCD6 additively cooperates with anti-cancer drugs through activation of NF-κB pathways                                      | 노승배 (교신)      | Cellular Signaling (4.243)  | <i>in press</i>        | 국외 SCI | 0910262   |
| Thionidazine induces apoptosis by targeting the PI3K/Akt/mTOR pathway in cervical and endometrial cancer cells               | 노승배 (교신)      | Apoptosis (4.397)           | <i>under revision</i>  | 국외 SCI | 0910262   |
| Thionidazine regulates VEGF-induced angiogenesis in HUVEC  | 노승배 (교신)      | Angiogenesis (6.188)        | <i>submitted</i>       | 국외 SCI | 0910262   |

#### (2) 목표달성도

가. 연구목표의 달성

| 최종목표  | 연차별목표            | 달성내용   | 달성도(%) |      |
|---|------------------|--|--------|------|
|   |                  |  | 연차     | 최종   |
| 암화과정에서 중요한 역할 수행을 하는 integrin 활성을 저해하는 세포 내 신호전달체계를 규명하고 항암 분자 기전을 통하여 이행성 연구에 있어 질환 치료법의 개 | 2011년도           | 암 세포주에 thionidazine 처리 후 MTT assay를 이용한 농도별 생존력 관찰과 flow cytometry을 이용하여 cell cycle 변화 및 cell division 분석 함으로서 항암 활성 효과를 검증하였고, 약물 처리 후 변화되는 단백질 (CDK, Cyclin D1, p21, p16, p-CDC25A etc.)을 관찰 및 자료 확보 | 100%   | 100% |
|   | Thionidazine 의 세 | Western blotting 을 통해 세포사멸   |        |      |

|                  |                         |  |
|------------------|-------------------------|--|
| 발을 위한 중요한 모델을 제공 | 포사별 기전 경로 확인            | 경로확인 [Caspase-3 (z-DEVD-fmk) and 9(z-LEHD-fmk) inhibitor 처리] 하였고 thioridazine을 처리한 세포주와 PI3K inhibitor인 LY294002 와 wortmannin를 처리한 세포주를 가지고 PI3K 활성 조사 및 비교 분석함으로써 PI3K/Akt 신호전달을 targeting 할 수 있다는 가능한 정보를 얻음 |
|                  | 기존 항암 화학요법과 병합효과 가능성 검증 | 기존의 항암효과를 나타내는 cisplatin 과 taxol 을 각각 동시에 처리 했을때 시너지 항암 활성 효과를 볼 수 있는지를 MTT assay 및 caspase activity assay 방법을 통해 비교 분석함으로써 thioridazine의 병합효과 가능성을 제시 할 수 있는 자료를 확보   |

나. 평가의 착안점에 따른 목표달성도에 대한 자체평가

| 평가의 착안점        | 자 체 평 가   |
|----------------|---|
| 연구의 창의 및 독창성   | 기존의 비 항암화학약제를 이용하여 부인 암에서 이전에 그 정확한 신호전달체계 기능이 규명되지 않은 암 억제 화학약제를 발굴하여, 임상 응용도가 높은 암 세포사멸 뿐만 아니라 고형 암의 경우에 있어 새로운 신생혈관 형성이 무엇보다도 중요한 요소로 작용하는바 이를 차단하는 신호전달체계를 확립함과 동시에 기존의 항암효과를 나타내는 항암제와 병행함으로써 제 발암 환자에서 큰 문제점으로 대두되고 있는 내성 문제를 해결하고 분석함으로써 암 발생기전 연구 및 치료 기술 개발 연구에 중요한 기초 자료를 제공함과 동시에 향후 고형암 이행성 연구의 중요 모델이 될 수 있을 것으로 판단됨. 또한 혈관신생과 연관이 있는 여러 질환의 새로운 병리 기전을 이해하는데 중요한 자료로 제시 |
| 연구결과의 응용도 및 효과 |   |

5. 연구결과의 활용계획

(1) 연구종료 후 예상 연구성과

| 구 분        | 건 수 | 비 고                                       |
|------------|-----|---|
| 학술지 논문 게재  | 2   | Apoptosis (4.397)<br>Angiogenesis (6.188) |
| 산업재산권 등록   |     |   |
| 기 타 (특허출원) | 1   |   |

(2) 연구성과의 활용계획

- ◆ 부인의 새로운 보조적 화학요법에 대한 적합성 관련 작용 기전의 이해를 들 수 있으며, 최적의 약물을 환자에게 사용할 수 있는 기준을 위한 자료를 제공하고, 조절 기전에 따른 항암제에 저항성을 가지는 환자에게 다른 치료 방법을 제시함으로써 안전성과 암 치료 효과의 증진
- ◆ 작용 기전이 검증된 새로운 보조적 화학요법제의 선택적인 치료 방법은 특히 초기 예방 치료 및 재발방지를 위한 치료 요법으로 사용됨으로서 국민의 막대한 의료비 지출을 줄일 수 있음
- ◆ Thioridazine의 약효를 증진 시킬 수 있는 target 단백질의 발굴을 통하여 환자의 개인별 맞춤

6. 참고문헌

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## 7. 첨부서류

- 연구 성과 대표 논문 초록
- 특허 출원 사본



## A gene signature-based approach identifies thioridazine as an inhibitor of phosphatidylinositol-3'-kinase (PI3K)/AKT pathway in ovarian cancer cells

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### ABSTRACT

**Objective.** Thioridazine, a derivative of phenothiazine, has been reported to have antiproliferative activity on tumor cells. However, the mechanism has not been well defined.

**Methods.** Using in-silico gene signature based approach, we have demonstrated that thioridazine could inhibit phosphatidylinositol-3'-kinase (PI3K)/Akt pathway, and thus exert cytotoxicity in ovarian cancer cells. **Results.** The Connectivity Map indicated that thioridazine induces gene signature similar to that of Akt inhibition. Moreover, preexisting inhibitors of PI3K/Akt pathway were also found to reveal similar signature. In SKOV-3 cells, immunoblot using p85 antibody showed that thioridazine could inhibit PI3K signal. In addition, thioridazine was found to inhibit p-Akt (Ser 473) in a dose-dependent manner. Furthermore, thioridazine was found to decrease cell viability and induce apoptosis. Exposure to thioridazine induced G<sub>0</sub>/G<sub>1</sub> arrest and down-regulated the cell cycle regulator, cyclin D1 and CDK4, and up-regulated p21, p16, and p-CDK2A. Finally, additive cytotoxicity was observed when cisplatin and thioridazine were treated simultaneously.

**Conclusions.** The current study indicated that in-silico approach, such as Connectivity Map, is a potentially useful method to identify the unknown cellular function among the drugs already in use in clinic. Owing to the property of Akt inhibition and additive cytotoxicity observed with the platinum compound, further research should be focused on this drug.

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### Introduction

Ovarian cancer is the leading cause of gynecologic cancer death in Western countries [1]. At least two-thirds of the women with ovarian cancer have advanced disease at the time of their diagnosis. Despite recent advances in treatment strategy, the prognosis of patients with advanced ovarian cancer is poor. The major limitation of standard treatment with platinum and taxane is the development of chemoresistance [2]. Suppression of apoptosis is generally accepted as one of the major contributing factors to chemoresistance.

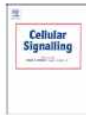
Phosphatidylinositol 3-kinase (PI3K)/Akt signal transduction plays a critical role in cell survival through suppression of apoptosis in various types of human cancers including ovarian cancer [3–6]. Activation of Akt also promotes tumor invasion and metastasis, antagonizes cell-cycle arrest, and phosphorylates mTOR (mammalian target of rapamycin) protein kinase. The phosphorylation of Akt is mediated by phosphatidylinositol-3,4-bisphosphate and phosphatidylinositol-3,4,5-triphosphate that are generated by (PI3K) [7]. PI3K is

an activator of Akt, which consists of catalytic subunits (110 kDa) and regulatory subunits (85 kDa) [8]. This gene has been proposed as a putative oncogene in ovarian cancer and its amplification was found to give rise to the activation of Akt [9–12]. In ovarian cancer, activation of Akt pathway has been associated with aggressiveness of the tumor behavior and decreased survival [13,14].

Thioridazine (10-[2-[1-methyl-2-piperidyl] ethyl]-2-methylthio-phenothiazine), a phenothiazine derivative, is a piperidine antipsychotic drug belonging to the phenothiazine drug group. In the management of advanced cancer, the drug has been used to treat cancer-related sweating [15,16] and depression [17]. However, there have been several reports that the drug shows cytotoxic effects and reversal of drug resistance [18–22]. Although several mechanisms including DNA damage [23], inhibition of P-glycoprotein [24], or antioxidant activity [25] have been proposed to explain the anticancer effect of the drug, the true mechanism of anticancer effect of thioridazine is unclear. Recently, a group of researchers observed that the antiproliferative effect of chlorpromazine, another phenothiazine derivative, can be blocked by wortmannin, a selective PI3K inhibitor [26].

Therefore, we have hypothesized the presence of possible connection between the anticancer effect of thioridazine and PI3K/Akt pathway. Given that thioridazine is a drug used in clinical practice

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## Programmed cell death 6 (PDCD6) inhibits angiogenesis through PI3K/mTOR/p70S6K pathway by interacting of VEGFR-2

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### ABSTRACT

Programmed cell death 6 (PDCD6) was originally found as a pro-apoptotic protein, but its molecular mechanism is not well understood. In this study, we have attempted to investigate the effects of PDCD6 on the inhibition of angiogenesis-mediated cell growth as a novel anti-angiogenic protein. Purified recombinant human PDCD6 inhibited cell migration in a concentration-time-dependent manner. We also found that over-expressed PDCD6 suppressed vascular endothelial growth factor (VEGF)-induced proliferation, invasion, and capillary-like structure tube formation in vitro. PDCD6 suppressed phosphorylation of signaling regulators downstream from PI3K, including Akt, mammalian target of rapamycin (mTOR), glycogen synthase kinase-3β (GSK-3β), ribosomal protein S6 kinase (p70S6K), and also decreased cyclin D1 expression. We found binding of PDCD6 to VEGFR-2, a key player in the PI3K/mTOR/p70S6K signaling pathway. Taken together, these data suggest that PDCD6 plays a significant role in modulating cellular angiogenesis.

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### 1. Introduction

The 22-kDa calcium-binding protein programmed cell death 6 (PDCD6) was first identified as a pro-apoptotic protein in a genetic screen. PDCD6 contains five EF-hand motifs and an open reading frame encoding 191 amino acids [1]. During the formation of an organism, the balance between cell growth, differentiation, and apoptosis is controlled by a number of regulatory genes. Alterations in this balance are found in diseases such as cancer [2]. The calcium-binding domain of PDCD6 plays a significant role in homodimerization and in the structural changes required for binding to various intracellular protein partners, including Alix [3,4], Fas [5], annexin XI [6], DAPk1 [7], TSG101 [8], Sec31A [9], and PLSCR3 [10].

Several cancer studies have analyzed the mRNA and protein expression of PDCD6 in tissues and cell lines. PDCD6 is ubiquitously expressed in adult mouse tissues but is over-expressed in rat liver hepatoma cells compared to normal liver tissues [11]. PDCD6 expression also appears to be up-regulated in lung cancer patients [11]. In contrast, reduced PDCD6 expression was recently observed in gastric cancer [12]. Hence, we examined whether PDCD6 is generally up-

regulated or down-regulated in ovarian tumors. In some cancers, the physiological conditions (e.g., endogenous mRNA and protein levels) in the cancer cells differ from those in normal cells. Our major focus is the regulatory mechanisms of human umbilical vein endothelial cell (HUVEC) and cancer cells. However, we did not investigate the functional role of PDCD6 in angiogenesis further.

In this study, we investigated the detailed functions of PDCD6 during angiogenesis using an in vitro HUVEC system and purified PDCD6. Specifically, we characterized the molecular mechanism of PDCD6 as a novel anti-angiogenic protein. PDCD6 inhibited HUVEC migration in a concentration- and time-dependent manner. In addition, PDCD6 suppressed phosphorylation of signaling components downstream from PI3K, such as Akt, mammalian target of rapamycin (mTOR), glycogen synthase kinase-3β (GSK-3β), ribosomal protein S6 kinase (p70S6K) through direct interactions with VEGFR-2. These interactions significantly control potent anti-angiogenic and anti-tumor activity. Therefore, our results strongly suggest that PDCD6 plays a significant role in modulating cellular angiogenesis.

### 2. Materials and methods

#### 2.1. Cell lines, tissue samples, cell culture, and antibodies

Primary HUVECs (#CC-2519; Clonetics, San Diego, CA) were cultured on 0.3% gelatin-coated dishes (Sigma, St. Louis, MO) using EGM-2 BulletKit medium (Clonetics). OVCAR-3 ovarian cancer cells were maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS). All

Abbreviations: mTOR, mammalian target of rapamycin; S6K1, ribosomal p70 S6 kinase; MTI, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-4H-tetrazolium bromide; HUVECs, human umbilical vein endothelial cells; siRNA, small interfering RNA; PDCD6, programmed cell death 6.

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## CYR61 controls p53 and NF- $\kappa$ B expression through PI3K/Akt/mTOR pathways in carboplatin-induced ovarian cancer cells

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### ABSTRACT

CYR61 over-expression promotes cell proliferation by inhibiting carboplatin-induced apoptosis, decreasing Bax expression, and increasing Bcl-xL, Mcl-1, and Bcl-2. At the same time, down-regulating p53 expression, while up-regulated NF- $\kappa$ B expression. Additionally, p21 and p53 promoter activities were reduced, while NF- $\kappa$ B and Bcl-2 activities increased. In parallel, CYR61-expressing cells, during carboplatin-induced apoptosis, resulted in an increase of Akt phosphorylation, while rapamycin-treated cells were not affected. Carboplatin effectively inhibited the activation of mTOR signaling cascade, which includes mTOR, 4E-BP1, p70S6K, HIF-1 $\alpha$ , and VEGF. These results provide evidence that CYR61 promotes cell proliferation and inhibits apoptosis.

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### 1. Introduction

Cysteine-rich 61 (CYR61), one of the tissue growth factors in the CCN family (CYR61/CTGF/NOV), is highly expressed in various cancer tissues and cell lines, including breast cancer, endometrial cancer, MCF-7 cells, SKOV-3 cells, gastric cancer cells, benign prostatic hyperplasia, gliomas, and melanomas [1–7]. In contrast, resting cells were down-regulated, but various agents including growth factor are involved in regulating the resting cells [8]. The various agents are also responsible for regulating multiple cellular functions, such as cell proliferation, inflammation,

cell adhesion, migration, embryogenesis, and wound healing [6,9–12]. Interestingly, the over-expression of CYR61 inhibits apoptosis, which is mediated by paclitaxel and cisplatin, and may also contribute to cancer cell viability [3,13,14]. In addition, CYR61 protein promotes angiogenesis and migration, as well as adhesive signaling by interacting with  $\alpha$ v $\beta$ 3 and  $\alpha$ 6 $\beta$ 1 [15,16].

Carboplatin (cis-diammine-1,1-cyclobutanedicarboxylate-platinum II) is widely used clinically on various human carcinomas, including ovarian, lung, head, and neck carcinomas [17–19]. Carboplatin, an analog to cisplatin, has fewer adverse toxic effects, unlike cisplatin, which generally involves serious side effects, such as nephrotoxicity, neurotoxicity, myelosuppression, nausea, and vomiting [20,21]. As such, carboplatin has been suggested as a means to suppress cell growth in cancer cells through the death receptor [22,23].

Apoptosis plays an important role by which cells undergo death to control cell proliferation. Generally, regulation

**Abbreviations:** CYR61, cysteine-rich 61; Carboplatin, cis-diammine-1,1-cyclobutanedicarboxylate-platinum II; DAPI, 4',6'-diamidino-2-phenylindole; mTOR, the mammalian target of rapamycin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-*N*-tetrazolium bromide; FITC, fluorescein isothiocyanate; PI, propidium iodide.

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## PDCD6 additively cooperates with anti-cancer drugs through activation of NF- $\kappa$ B pathways

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### ABSTRACT

The expression of programmed cell death 6 (PDCD6) is known to be down-regulated in cancer cell lines and ovarian cancer tissues compared to normal cells and tissues. In the current study, we characterized the specific function of PDCD6 as a novel pro-apoptotic protein. To define the roles of PDCD6 and cisplatin in tumorigenesis, we either over-expressed PDCD6 or treated it with cisplatin in SKOV-3 ovarian cancer cells. Both PDCD6 and cisplatin respectively inhibited cancer cell proliferation in a dose-dependent manner. The combined treatment of PDCD6 and cisplatin was more effective at suppressing cell growth than with either drug treatment alone, but had no effect with the treatment of caspase-3 and caspase-9 inhibitors. Cleavages of caspase-3, -8, -9, and poly (ADP-ribose) polymerase (PARP) in PDCD6-overexpressing cells were significantly increased after cisplatin treatment. Cell cycle analysis highly correlated with down-regulation of cyclin D1 and CDK4, and the induction of p16 and p27 as a cyclin-dependent kinase inhibitor. Additionally, PDCD6 also suppressed the phosphorylation of signaling regulators downstream of PI3K, including p90K1 and Akt. PDCD6 promotes TNF $\alpha$ -dependent apoptosis through the activation of NF- $\kappa$ B signaling pathways, increasing Bax, p53, and p21 expression, while also down-regulating Bcl-2 and Bcl-xL expression. The p21 and p53 promoter luciferase activities were enhanced by PDCD6, while there was no effect in p53<sup>-/-</sup> and p21<sup>-/-</sup>. At the same time, p53 activity was confirmed by UV irradiation and siPDCD6. Taken together, these results provide evidence that PDCD6 can mediate the pro-apoptotic activity of cisplatin or TNF $\alpha$  through the down-regulation of NF- $\kappa$ B expression.

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### 1. Introduction

PDCD6 (programmed cell death 6), is a calcium-binding modulator protein associated with cell proliferation and death. It is one of the prototype members of the penta EF-hand protein family, and as a 22 kDa protein, contains an open reading frame encoding 191 amino acids [1,2]. PDCD6, also well-known as ALG-2 (apoptosis-linked gene-2), was first described as a pro-apoptotic protein in a functional screen of T-cell hybridoma cells [3]. However, studies showed that ALG-2-deficient mice exhibited normal T-cell development and function [4]. Nonetheless, potential physiological functions of PDCD6 in modulation of endoplasmic reticulum (ER)-stress-stimulated cell death [5], neuronal apoptosis during organ formation [6], and cancer diseases [7,8], have been reported. During the pathological development of an organism,

**Abbreviations:** PDCD6, programmed cell death 6; ALG-2, apoptosis-linked gene-2; Cisplatin, (SP-4-2)-diamminedichloroplatinum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-*N*-tetrazolium bromide; PARP, poly (ADP-ribose) polymerase.  
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the balance between cell differentiation, proliferation, and apoptosis is controlled by certain types of regulatory genes. Alterations in this balance are found in a variety of diseases, such as cancer [9].

Several independent studies reported mRNA and protein expression of PDCD6 in various tumor types. PDCD6 has been shown to be highly expressed in rat liver hepatoma cells and in lung cancer patients when compared to normal tissues [7]. On the other hand, down-regulation of PDCD6 expression was recently observed in gastric cancer and HeLa cells [10,11]. In some type of tumors, the cellular physiological states (e.g., endogenous mRNA protein expression levels, and interacting partners) in the tumor cells differ from those in normal cells. This difference could possibly be due to the type of human tumor.

Cisplatin [(SP-4-2)-diamminedichloroplatinum] is a well-known chemotherapeutic agent, which acts as an inducer of apoptosis and mediator of DNA replication [12,13]. It has been used in the first-line of therapy for several malignancies, such as ovarian, cervical, head and neck, bladder, colorectal, gastric, pancreatic, testicular, small-cell lung, and esophageal tumors either alone or in combination with other anti-cancer agents [14–20]. Unfortunately the use of this compound is generally limited by its side effects because cytotoxic