Active and passive smoking and risk of ovarian cancer

Julie Baker,[1] Olufunmilayo Odunuga,[2] Kerry Rodabaugh,[3] Mary Reid,[3] Ravi Menezes,[1] Kirsten Moysich[3]

[1] University at Buffalo, Roswell Park Cancer Institute
[2] University at Buffalo
[3] Roswell Park Cancer Institute

Introduction: It is unclear whether smoking is a risk factor for epithelial ovarian cancer, although some studies have suggested it may be associated with increased risk of mucinous tumors. **Methods:** The current study investigated the effects of smoking and environmental tobacco smoke (ETS) on ovarian cancer risk among 434 women with primary epithelial ovarian, peritoneal, or fallopian cancer and 868 age- and region-matched hospital controls with non-neoplastic conditions. All participants completed a comprehensive epidemiologic questionnaire. **Results:** Results indicate that decreased risk of ovarian cancer was associated with being a non-smoker exposed to ETS [adjusted odds ratio (aOR) 0.68, 95% confidence interval (CI) 0.46-0.99), a former smoker (aOR 0.76, 95% CI 0.53-1.10), or a current smoker (aOR 0.53, 95% CI 0.32-0.88). A similar protective effect was noted for smokers with moderate or high exposure based on years of smoking, cigarettes smoked per day, or pack-years of smoking. Results did not differ substantially by histologic subtype. **Conclusion:** Although prevailing theories of ovarian cancer etiology implicate incessant ovulation, characteristics of the study population suggest that anovulation was not the protective mechanism in this study. Immunosuppression by nicotine or upregulation of enzymes that metabolize carcinogens may be responsible for the effects observed.

Characteristics related to the intrauterine and early life environment and risk of epithelial ovarian cancer

MaryAnne Rossing,[1] Mei-Tzu C. Tang,[1] Linda K. Weiss,[2] Elaine W. Flagg,[3] Kristine G. Wicklund[1]

[1] Fred Hutchinson Cancer Research Center
[2] US National Cancer Institute
[3] Emory University

Introduction: While the possible contribution of the intrauterine and early life environment to risk of breast, prostate, and other hormonally-mediated cancers has been examined in multiple studies, almost no data are available regarding the relation of these factors to risk of ovarian cancer. In a population-based, multi-center case-control study, we assessed the relation of prenatal and early life characteristics with risk of epithelial ovarian cancer in a study of women aged 35-54 years. **Methods:** In-person interviews were sought with female residents of metropolitan Atlanta, Seattle or Detroit diagnosed with ovarian cancer during 1994-1998 and with controls sampled from these areas. Information was provided by 355 women with borderline or invasive epithelial ovarian tumors and 1637 controls, and was analyzed using unconditional logistic regression. **Results:** Cancer risk was not associated with maternal age or smoking during pregnancy, or with the birth weight or birth order of the study participant. We observed a tendency towards decreasing risk with increasing number of siblings: relative to women with one sibling, the risk among women with five or more siblings was 0.65 (95% confidence interval (CI) 0.41-1.01). A somewhat stronger association was seen with number of full sisters: among women with at least one sibling, the risk among women with four or more sisters relative to women with no sisters was 0.54 (95% CI 0.29-0.99), whereas no association was observed with number of brothers. **Conclusions:** While we noted little evidence that characteristics reflective of the intrauterine environment are associated with risk of epithelial ovarian cancer, some unidentified aspect of the early life environment related to or mediated by a woman's number of siblings or sisters may influence risk among women diagnosed with this disease at a relatively young age. This finding is consistent with a "hygiene hypothesis" for ovarian cancer, whereby the patterns of exposure to infectious agents during early life or related immunologic responses may influence risk.

Obesity and risk of ovarian cancer

Manveen Sekhon,[1] Gregory P Beehler,[2] Julie Baker,[2] Barbara E Teter,[3] Susan E McCann,[2] Kirsten Moysich[2]

[1] Department of Family Medicine, University at Buffalo
[2] Department of Epidemiology, Roswell Park Cancer Institute
[3] Department of Social and Preventive Medicine, University at Buffalo

Introduction: Obesity has been linked to increased risk of a number of malignancies, but the role of obesity in the etiology of ovarian cancer remains unclear. We conducted a hospital-based case-control study to further investigate the association between this modifiable risk factors and risk of ovarian cancer. **Methods:** Participants included 427 women with primary, incident ovarian cancer and 854 age and residence matched controls without benign or malignant neoplasms. All participants received medical services at Roswell Park Cancer Institute in Buffalo, NY between 1982 and 1999 and completed a comprehensive epidemiological questionnaire. The instrument included questions regarding height and usual weight prior to Body mass indices (BMI) were computed for all participants, as defined by weight (kg)/height (m)2. Participants were classified as normal or underweight (BMI 18.5-25 kg/m2), overweight (BMI 25-30 kg/m2), or obese (BMI >30 kg/m2). Normal or underweight women served as the referent group. We used unconditional logistic regression analyses to compute crude and adjusted odds ratios (ORs) with corresponding 95 % confidence intervals (CIs). **Results:** Results indicated that overall being overweight (adjusted OR=1.02; 95% CI 0.77-1.36) or obese (adjusted OR=1.17; 95% CI 0.84-1.65) was not significantly associated with elevated risk of ovarian cancer. After stratification by age, results were not substantially different for older women (> 50 years). However, while being overweight was not associated with risk among younger women (< 50 years), we observed a significant risk increase among obese women (adjusted OR=2.19; 95% CI 1.19-4.04) when compared to normal or underweight women. **Conclusion:** Our findings suggest that obesity, but not overweight, might be associated with greater risk of ovarian cancer in younger women. In light of the fact that obesity is a modifiable risk factor, further investigation on this topic is warranted.

Consumption of black tea or coffee and risk of ovarian cancer

Julie Baker,[1] Kwabena Boakye,[2] Susan E McCann,[1] Gregory P. Beehler,[1] Kerry J Rodabaugh,[3] Jeannine A Villella,[3] Kirsten Moysich[1]

[1] Department of Epidemiology, Roswell Park Cancer Institute
[2] Ross University School of Medicine
[3] Department of Gynecologic Oncology, Roswell Park Cancer Institute

Introduction: Tea and coffee consumption may be related to risk of epithelial cancer at a variety of sites, including ovaries. Both beverages contain catechins and flavonoids, which exhibit anti-carcinogenic properties. However, caffeine in these beverages may elevate cancer risk through a variety of mechanisms. **Methods:** Using a hospital-based case-control design, we investigated the associations between ovarian cancer risk and usual consumption of black tea, regular coffee, or decaffeinated coffee. Participants included 414 women with primary epithelial ovarian, fallopian, or peritoneal cancer and 868 age- and region-matched women with non-neoplastic conditions. All participants completed a comprehensive epidemiologic questionnaire. **Results:** Results indicate that black tea consumption was associated with a linear decline in ovarian cancer risk (p for trend 0.03), with individuals consuming 2 or more cups daily experiencing a 30% decline in risk [adjusted odds ratio (aOR) 0.70, 95% confidence interval (CI) 0.51-0.97). Similar declines were noted among individuals consuming 2 or more cups of decaffeinated coffee daily (aOR 0.71, 95% CI 0.51-0.99; p for trend 0.002). However, no association was noted between any level of regular coffee consumption and risk of ovarian cancer. **Conclusion:** These results suggest that the chemoprotective effects of phytochemicals in black tea and decaffeinated coffee may be important, although the effects of phytochemicals in regular coffee may be overshadowed by the elevated risk associated with its higher caffeine content.

Use of cosmetic talc on contraceptive diaphragms and risk of ovarian cancer: A meta-analysis of nine observational studies

Michael Huncharek,[1] Joshua Muscat[2]

[1] Department of Clinical Oncology, Marshfield Clinic Cancer Center
[2] Department of Health Evaluation Sciences, Pennsylvania State College of Medicine

Objective: Prior work suggests an association between perineal use of cosmetic talc and increased ovarian cancer risk. A meta-analysis was performed to examine this hypothesis by evaluating ovarian cancer risk associated with direct exposure of the female genital tract to talc via dusting of contraceptive diaphragms. **Methods:** Data were pooled from epidemiological studies using a general variance-based meta-analytic method that employs confidence intervals. The outcome of interest was a summary relative risk (RRs) reflecting the risk of ovarian cancer development associated with the use of cosmetic talc on contraceptive diaphragms. Sensitivity analyses were performed to explain any observed statistical heterogeneity and to explore the influence of specific study characteristics on the summary estimate of effect. **Results:** Initially combining homogeneous data from nine case-control studies yielded a non-statistically significant summary relative risk of 1.03 (0.80-1.37), suggesting no association between talc dusted diaphragms and ovarian cancer development. Sensitivity analyses were performed to evaluate the robustness of this finding. All resultant summary relative risks were not statistically significant. **Conclusions:** The available epidemiological data do not support a causal association between the use of cosmetic talc dusted diaphragms and ovarian cancer development.

Intake of folate and related nutrients in relation to risk of epithelial ovarian cancer

Shelley S. Tworoger,[1,2] Jonathan L.Hecht,[3] Edward Giovannucci,[1,2,4] Susan E. Hankinson[1,2]

[1] Channing Laboratory, Department of Medicine, Brigham and Women.s Hospital and Harvard Medical School, Boston, Massachusetts
[2] Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts
[3] Department of Pathology, Beth-Israel Deaconess Medical Center, Boston, Massachusetts
[4] Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts

Folate and related nutrients in the one-carbon metabolism pathway have been associated with a reduced risk of breast and colon cancers, but results for ovarian cancer are inconsistent. Therefore, we examined the association of dietary and supplemental folate, methionine, and vitamin B6 intakes, prospectively, with ovarian cancer risk among 80,254 Nurses' Health Study participants. Women completed biennial questionnaires assessing information about known and suspected ovarian cancer risk factors beginning in 1976, with food frequency questionnaires every 2-4 years starting in 1980. Newly reported cases of ovarian cancer were confirmed by medical record review. During 22 years of follow-up (1980-2002), we confirmed 481 incident cases of epithelial ovarian cancer who had no previous report of a cancer diagnosis. Overall, there were no associations between total folate (RR, top vs. bottom quintile: 1.21, 95%CI: 0.92, 1.60), methionine (RR: 1.00, 95%CI: 0.76, 1.33), dietary vitamin B6 (RR: 1.09, 95%CI: 0.81, 1.47), or total vitamin B6 intakes (RR: 1.13, 95%CI: 0.85, 1.51) and ovarian cancer risk. Higher dietary folate was associated with a modestly decreased risk after excluding the first four years of follow-up (RR: 0.66, 95% CI: 0.43, 1.03) or for the serous subtype (RR: 0.51, 95% CI: 0.31, 0.84). Results did not vary by alcohol intake, multivitamin use, menopausal status, or oral contraceptive use. There was little evidence that folate, methionine, and vitamin B6 intakes were associated with ovarian cancer risk, although dietary folate was inversely associated with risk in some analyses, suggesting that a further detailed assessment is warranted.

Tissue Microarray Analysis of Biomarkers p53, BRCA1, Annexin I, and Gelsolin Supports the Two-Pathway Theory of Ovarian Serous Carcinoma

Peggy Soung,[1] Jessica Chan,[1] David Seligson,[1] Jonathan Berek,[2] JianYu Rao[1]

[1] UCLA Dept of Pathology
[2] UCLA Dept of Obstetrics and Gynecology

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Background: Recently, data from clinical and molecular studies support the hypothesis that there may be two-types of ovarian serous neoplasms: one type (Type I) that follows a slow progression from a borderline lesion and another type (Type II) that is highly aggressive, arising de novo from surface epithelium. The Type I tumor is usually associated with K-ras mutations whereas the Type II tumor is not (Kurman, Int J Gynecol Pathol. 2003). The goal of our study is to perform tissue microarray analysis on tumor suppressor proteins including p53, BRCA1, Annexin I, and Gelsolin, in an attempt to further characterize the molecular pathogenesis of Type I and Type II tumors. Annexin I and Gelsolin are novel tumor suppressor proteins associated with cytoskeletal actin remodeling. **Methods:** 29 cases of ovarian serous carcinoma (23 Type I and 6 Type II), 23 borderline serous tumors, and 27 serous cystadenomas were retrieved from the archival files. Tissue cores from formalin-fixed, paraffin-embedded blocks (3 cores per block) were constructed into a tissue microarray of 0.6 mm cores. Sections were stained with monoclonal antibodies against p53, BRCA1, Gelsolin and Annexin I. For each marker, the maximum staining intensity (Max), the percentage of positive staining (Pos), and the product of both Max and Pos (MaxPos) were analyzed. **Results:** Overall, there was a gradual decrease in actin remodeling proteins (Gelsolin and Annexin I) with progression of cancer, but the greatest decrease occurred from cystadenoma to borderline, which suggests loss of actin remodeling proteins is an early event. Expression of p53 showed a gradual increase with progression of cancer. Of the carcinomas that develop through a slow progression (Type I) and those that develop de novo (Type II), the Type II carcinomas showed increased p53, and Type I carcinomas showed decreased BRCA1 expression. No significant difference in the expression of actin-remodeling proteins (Gelsolin and Annexin I) was observed between Type I and Type II serous carcinomas. **Conclusions:** Our data of p53 and BRCA1 expression in Type I and Type II tumors further supports the two pathway theory of ovarian serous carcinoma. However, the loss of actin remodeling proteins probably occurs as an early event in both types of tumors.

Anchorage-independent activation of and nuclear signaling by MAP Kinase in SKOV-3 ovarian cancer cells

Scott Eblen,[1] Adel Tarcsafalvi,[1] Michael Weber[1]

[1] University of Virginia

Introduction: MAP Kinases are key regulators of many cellular processes including proliferation, migration, and survival. Normal epithelial cells and fibroblasts require cellular adhesion to a substratum for MAP Kinase activation and nuclear translocation in response to extracellular mitogens and mutational activation of upstream members of the pathway. The ability of some cancer cells to overcome the adhesion requirement for MAP Kinase activation and nuclear signaling may provide them with an increased ability to survive and proliferate during the process of metastasis. The importance of the MAP Kinase pathway in ovarian cancer is being increasingly recognized, but its role in the disease is poorly understood. **Methods:** Cells growing on tissue culture dishes were trypsinized, treated with trypsin inhibitor, spun down, resuspended in serum-free media and incubated in 6 well dishes coated with 1 % bactoagar to prevent attachment. **Results:** We demonstrate that while activation of the MAP Kinase ERK2 by growth factors was transient in adherent SKOV-3 cells, loss of adherence to a substratum resulted in sustained activation of ERK2 in the absence of serum or growth factors. Activation of ERK was not observed in suspended IOSE 398 cells, a benign immortalized ovarian cell line. The anchorage independent activation of ERK in suspended SKOV-3 cells was not due to a deficiency in cellular phosphatase activity, as inhibition of upstream signaling to ERK with the MEK inhibitor U0126 resulted in the rapid loss of active ERK. Replating suspended SKOV-3 cells onto fibronectin coated dishes resulted in a reduction in ERK activation compared to cells that remained in suspension, demonstrating a role for cellular adhesion in the proper maintenance of the duration of ERK activation. We further show that signaling by ERK to the nucleus is not impaired in suspended cells. Using a luciferase reporter system under the control of the ERK substrate Elk1, we show that basal signaling as well as mutationally activated forms of Raf or MEK induce comparable ERK signaling to the nucleus in adherent and suspended cells. **Conclusions:** Collectively, these results suggest that MAP Kinase activation may contribute to the process of ovarian cell metastasis under anchorage independent conditions.

Review of the clinical course and pedigree analysis of a rare case of Malignant Peripheral Nerve Sheath Tumor (MPNST) of the ovary

Robert Enck,[1] Gloria Morris,[1] Kulbir Sidhu,[1] Norman Rosenblum,[1] David Silver,[1] Pamela Edmonds,[1] Megan Harlan,[1] John Young Jun,[1] Mary Cunnane[1]

[1] Thomas Jefferson University

Introduction: Ovarian sarcomas such as carcinosarcoma account for less than 1% of ovarian malignancies and occur predominantly in postmenopausal women. Malignant peripheral nerve sheath tumor (MPNST) of the ovary is distinctly unusual. **Methods:** Review of the clinical course and family history analysis. **Results:** A 54 year old female presented with a three month history of weight gain and enlarging abdominal girth. An ultrasound of the abdomen and pelvis identified a 20 cm left ovarian complex mass. Exploratory surgery revealed a tumor extending from the left ovary into the uterus and retroperitoneal space. Pathologically, this tumor was classified as a malignant peripheral nerve sheath tumor. Immunopathology included positive smooth muscle actin, desmin, vimentin, S100, and CD117. Treatment with aggressive multidrug chemotherapy failed to produce any significant response and the patient expired some 6 months after diagnosis. Due to the rarity of this tumor, young age of the patient and psychological distress of the patient and family regarding potential inheritance of MPNST, a detailed family history was obtained. Although the pedigree identified a strong family history of cancer, there was no suggestion that inheritance played a role in the occurrence of this ovarian sarcoma. **Conclusions:** This case presented the unique opportunity to study the clinical course and family history of a patient with an exceedingly rare ovarian sarcoma. By pedigree analysis, no inherited link was identified. Ovarian MPNST can occur in young women, is resistant to modern day sarcoma chemotherapy and is associated with a short survival.

Genome-wide expression analysis identifies cholesterol homeostasis genes as downstream targets of progesterone in ovarian surface epithelial cells

Bora Baysal,[1,2] Julie DeLoia,[1] Joan Willett-Brozick,[1] Jeanine Patterson,[1] Lih-Ching Hsu[1]

[1] Magee-Women's Research Institute
[2] University of Pittsburgh School of Medicine

Background: Ovarian cancer most often derives from ovarian surface epithelial cells. Several lines of evidence strongly suggest that progesterone exposure protects against ovarian cancer. However, the underlying mechanisms of this protection are incompletely understood. **Methods:** Here, we established short term in vitro cultures of non-neoplastic ovarian surface epithelial cells from six subjects, exposed the cells to progesterone (10-6 M) for five days and performed transcriptional profiling with oligonucleotide microarrays containing over 22,000 transcripts. **Results:** We found that, in three of the six cultures, transcripts encoding 14 cholesterol biosynthesis enzymes, insulin-induced gene 1, low density lipoprotein receptor, ABCG1, endothelial lipase, stearoyl- CoA and fatty acid desaturases, long-chain fatty-acyl elongase, and MAC30 were upregulated; steroidogenic acute regulatory protein and ABCC6 were downregulated by progesterone. We confirmed the microarray results for a subset of the genes by quantitative RT-PCR analysis, and in one of three additional ovarian surface epithelial cell cultures. The cultures derived from a BRCA1 mutation carrier and another subject with early-onset breast cancer showed no evidence of transcriptional response to progesterone. **Conclusions:** These findings indicate that progesterone regulates a very broad network of genes involved in cholesterol homeostasis in certain ovarian surface epithelial cells and provide new insights for understanding the protective role of progesterone against ovarian cancer.

Investigating the Binding of Mesothelin and MUC16 (CA125), Two Markers that are Elevated in Patients With Epithelial Ovarian Cancer

Jennifer Arens,[1] Jennifer Hebda,[1] Masanori Onda,[2] Claudine Rancourt,[3] Mitchell H,[2] Tapan Bera,[2] Ira Pastan,[2] Manish Patankar[1]

[1] University of Wisconsin-Madison
[2] Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health
[3] Université de Sherbrooke

Introduction: Mesothelin, a GPI anchored glycoprotein is overexpressed by ovarian tumors, mesotheliomas and other cancers. Combined monitoring of serum levels of mesothelin and MUC16- a well established ovarian tumor marker previously designated CA125- may provide greater benefit for early detection of epithelial ovarian cancer (EOC). The physiological roles of both mesothelin and MUC16 are not clear. Mesothelin binds to a recombinant epitope of MUC16 (Rump et al, J. Biol. Chem., 279, 9190-9198). Here we further investigate the molecular basis of this interaction. Our initial data suggests that MUC16 oligosaccharides play an important role in mediating interaction with mesothelin. **Methods:** MUC16 from OVCAR-3 and ascites was isolated by serial size exclusion and affinity chromatography. Recombinant mesothelin-Fc chimera was produced and isolated from 293T cells. Binding of this chimera with MUC16 was studied by Western blotting. The terminal sialic acids on MUC16 were either removed by treatment with neuraminidase from Clostridium Perfringens or oxidized with 1 mM sodium metaperiodate. Oxidation of the entire MUC16 oligosaccharide chains was carried out by treatment with 10 mM sodium metaperiodate. The N-linked glycans of MUC16 were released by treatment with PNGaseF. OVCAR-3 cells not expressing MUC16 on their cell surface were produced by stably transfecting them with the endoplasmic reticulum localized scFv fragment of the anti-MUC16 antibody VK-8. Mesothelin-Fc binding to the cell surface was determined by flow cytometry. **Results:** Using Western blotting, we have shown that a recombinant form of mesothelin binds to native MUC16 from the OVCAR-3 cell line and patient ascites. Mesothelin-Fc was also shown to bind to OVCAR-3 cell surface by flow cytometry. The scFv expressing clones of OVCAR-3 that do not express MUC16 show no binding to mesothein-Fc. Very mild periodate oxidation of MUC16 oligosaccharides under conditions that only affect the terminal sialic acid residues or enzymatic removal of this monosaccharide has no effect on binding to mesothelin-Fc. However, upon oxidation of the entire oligosaccharide chains of MUC16, mesothelin-Fc was unable to recognize the mucin. Removal of the MUC16 N-linked oligosaccharides with PNGaseF also significantly depleted the interaction of the mucin with mesothelin-Fc. **Conclusions:** We have conclusively shown that mesothelin is a binding partner of MUC16. The N-linked oligosaccharides of MUC16 likely play an important role in mediating interaction with mesothelin. Our on-going studies will focus on completely characterizing the molecular and physiological basis of the interaction between these two ovarian tumor markers.

Influence of Skepticism on health seeking behavior

Rhonda Johnson,[1] Joan Walker,[1] Scott McMeekin,[1] Cheryl Crichley[1]

[1] University of Oklahoma Health Sciences Center

Introduction: This study sought to investigate ways in which medical skepticism, health locus of control, and mood were associated with treatment seeking behavior in women. It also compares these variables between groups of women who are seeking treatment for symptoms common to ovarian cancer to women in the general population who also endorse symptoms suggestive of possible ovarian cancer and who are not seeking care. **Method:** Women targeted by the survey were those who reported symptoms suggestive of ovarian cancer and who have presented for either a gynecologic or gastrointestinal examination due to those symptoms. These symptoms included bloating, fatigue, and abdominal pain with a severity of at least 2 out of a possible 0-5 on a scale of severity. Other women were those surveyed in the general population who also endorsed similar symptoms but who were not seeking health care. Correlations between anxiety and depression, locus of control, medical skepticism and likelihood of seeking health care were examined. **Results:** Medial skepticism correlated positively with an internal locus of control ($r=0.18$, $p=0.03$) and negatively with a power locus of control ($r=-0.21$, $p=0.01$). Anxiety was negatively correlated with an internal locus of control ($r=-0.22$, $p=<0.01$). There was not a statistically significant correlation between depression or anxiety and medical skepticism. Further analysis examined differences between the two groups of women on measures of locus of control, mood and medical skepticism. There were no statistical differences between the groups on measures of locus of control, mood, or medical skepticism. **Discussion:** Our findings indicate that for women there is a positive correlation between medical skepticism and an internal locus of control. In other words, those women who believe they have good ability to influence their own medical outcome also have increased medical skepticism. For those women then it is likely that they would want to understand and work more collaboratively with health care workers, On the other hand, those who believe a powerful other is more likely to influence their outcome are also less likely to be skeptical about their medical care. Likewise, it seems logical these women are best served by a health care provider who provides more information to the patient.

United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): Design and Characteristics of the Study Population

United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) Group

Introduction: While it is well known that screening can detect ovarian cancer, it is not yet known whether this will lead to a mortality benefit. UKCTOCS is a randomised control trial whose primary aim is to assess impact of screening on ovarian cancer mortality in the general population. The study also addresses the issues of target population, compliance, health economics and physical and psychological morbidity of screening. The objective of this report is to describe the trial design and the characteristics of the study population. **Methods:** Women aged 50-74 years were randomly chosen from participating Health Authority registers and send postal invitations. Those who agreed to participate were invited for a recruitment interview where they completed a datasheet and gave written consent. Eligible women were randomised to annual screening for 6 years by serum CA125 or ultrasound or to a control arm in a 1:1:2 ratio. In the serum CA125 arm, results are interpreted using a novel 'Risk of Ovarian Cancer' algorithm with ultrasound as a second line test. All women are followed up 3.5 and 7 years after randomisation using a postal questionnaire. In addition, all women are 'flagged' through the Office of National Statistics in the UK who provide data on cancer registration and death. A parallel quality of life study assesses psychological impact of screening in women who are recalled due to abnormalities detected on first and second line screening and surgery. **Results:** 200,000 women were randomised between 2001 and 2005. At recruitment, the median age of the women was 59 years. All were postmenopausal. 19% had undergone a hysterectomy. 58% had used the oral contraceptive pill in the past. The median number of pregnancies over 6 months was 2 (IQ Range 2-3). 3.5 % had been diagnosed with breast cancer in the past, 22% reported a history of breast cancer in first and second degree relatives and 4.5% reported a history of ovarian cancer in first and second degree relatives. **Conclusions:** The characteristics of the women recruited to UKCTOCS are similar to those of the UK Million Women Study and do not differ substantially from women of a similar age in the general population.

1H-NMR-Based Metabonomics for the Detection of Epithelial Ovarian Cancer

Kunle Odunsi,[1] Robert Wollman,[1] Christine Ambrosone,[1] Alan Hutson,[2] Susan McCann,[1] Jonathan Tammela,[1] John Geisler,[3] Gregory Miller,[3] Thomas Sellers,[4] William Cliby,[5] Feng Qian,[1] Shashikant Lele,[1] James Alderfer[1]

[1] Roswell Park Cancer Institute
[2] State University of New York at Buffalo
[3] St. Vincent's Hospital
[4] H. Lee Moffitt Cancer Center & Research Institute
[5] Mayo Clinic

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Hypothesis: Metabonomics, the study of metabolic processes in biological systems, is based on the use of 1H-NMR spectroscopy and multivariate statistics for biochemical data generation and interpretation. 1H-NMR spectrum of body fluids allows the detection and quantification of thousands of proton-containing, low-molecular weight species, resulting in the generation of an endogenous profile that may provide a characteristic 'fingerprint' in disease. We hypothesized that the analysis of a global view of metabolites in serum could discriminate sera from women with epithelial ovarian cancer (EOC) from healthy controls. **Methods:** Conventional 1H-NMR spectroscopic analysis was performed on pre-operative serum specimens obtained from 38 patients with EOC (stages I to IV) and 53 healthy women, using Bruker AMX-600 spectrometer. Each 1H-NMR spectrum was corrected for phase and baseline distortions using NutsPro and reduced to 200-250 integral segments of equal width of delta 0.04. Subsequently, we applied unsupervised Principal Component Analysis (PCA) and supervised Soft Independent Modeling of Class Analogy (SIMCA) for pattern recognition. The sensitivity and specificity trade-offs were summarized for each variable using the area under the receiver-operating characteristic (ROC) curve. In addition, the regions of NMR spectra that most strongly influence separation of sera of EOC patients from healthy controls were identified. **Results:** PCA analysis allowed correct separation of all serum specimens from 38 patients with EOC (100%) from all of the 21 pre-menopausal normal samples (100%). In addition, it was possible to correctly separate 37 of 38 (97.4%) cancer specimens from 31 of 32 (97%) postmenopausal control serum specimens. SIMCA analysis using the Cooman's plot demonstrated that the EOC sera class and the postmenopausal control sera class did not share multivariate space, providing validation for the class separation. ROC analysis indicated that the sera from patients with and without disease could be identified with 100% sensitivity and specificity at the 1H-NMR regions 2.77ppm and 2.04ppm from the origin (AUC of ROC curve=1.0). In addition, the regression coefficients most influential for the EOC samples compared with postmenopausal controls lie around delta 3.7ppm (due mainly to sugar hydrogens) while the loadings most influential for the EOC samples compared with pre-menopausal controls lie around delta 2.25ppm (due to acetoacetate). **Conclusions:** These findings indicate that 1H-NMR metabonomic analysis of serum achieves complete separation of EOC patients from healthy controls and may prove useful as a novel strategy for the early detection of epithelial ovarian cancer.

B7-H4 (DD-O110) is over-expressed in tissue lysates of early-stage ovarian cancer patients

Iris Simon,[1] Eleftherios Diamandis,[2] Dionyssios Katsaros,[3] Nam Kim,[1] Robert Wolfert[1]

[1] diaDexus, Inc
[2] Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto
[3] Department of Gynecology, University of Turin, Turin

Introduction: B7-H4 (DD-O110) is a novel membrane protein that functions as a negative regulator of T-cell response. We have previously shown that B7-H4 is over-expressed in ovarian and breast cancer with little or no expression in normal tissues. We have also developed a sensitive ELISA for B7-H4 and shown that the B7-H4 level in serum is elevated in ovarian cancer patients when compared to normal controls and patients with benign gynecological diseases. The aim of the current study was to compare the levels of B7-H4 and CA-125 protein in tissue lysates from ovarian cancers of various histological types and stages. **Methods:** Ovarian tissue lysates from 256 patients with ovarian carcinoma were assessed for the levels of B7-H4 and CA-125. Eighty-seven patients were diagnosed with early stage cancer, 169 patients had late stage cancer. For comparison, ovarian tissue from patients with benign diseases (n=43) and from normal control patients (n=32) were tested. The concentrations of B7-H4 and CA-125 in ovarian tissue extracts were correlated with clinicopathological variables documented at the time of surgical excision and with patient outcome. **Results:** The 95th percentile of B7-H4 concentration in the control group was selected to categorize patients as B7-H4 positive or B7-H4 negative. Using this cut-off value, B7-H4 was over-expressed in 73% of undifferentiated cancers, 67% of serous adenocarcinomas, 63% of endometrioid cancer, 45% of mucinous cancer and 24% of non-epithelial cancers. In early stage cancers, 48% of patients with stage I cancer, 57% of patients with stage II cancer, and 67% of patients with late stage cancer had B7-H4 values higher than normal controls or benign diseases. B7-H4 was elevated in 46 patients with early stage cancer, CA125 was elevated in 26 patients; combined, 58 patients (65%) with early stage disease were positive for B7-H4 and/ or CA125. The survival analysis showed that neither B7-H4 nor CA125 were markers for the prognosis of relapse or survival of patients when the data were analyzed for stage subgroups. The multivariate Cox regression analysis confirmed that the risk of poor outcome increased with stage but not with expression of either B7-H4 or CA125. **Conclusions:** More than 50% of early stage and 67% of late stage cancers over-expressed B7-H4 in tissue lysate. The data support the biological rationale for proposing B7-H4 will be elevated in serum of women with early stage disease and suggest B7-H4 as a promising new diagnostic marker and therapeutic target.

Identifying Associations Between SNPs In Cell Cycle Control Genes And Susceptibility To Ovarian Cancer

Simon Gayther,[1] Honglin Song,[2] Susan Ramus,[1] Lydia Quaye,[1] Richard DiCioccio,[3] Estrid Hogdall,[4] Claus Hogdall,[4] Ian Jacobs,[1] Alice Whittemore,[5] Bruce Ponder,[2] Susanne Kruger Kjaer,[4] Paul Pharoah[2]

[1] University College London, UK
[2] University of Cambridge
[3] Roswell Park Cancer Institute
[4] Danish Cancer Society, Denmark
[5] Stanford University School of Medicine, USA

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Introduction: BRCA1 and BRCA2 are responsible for the majority of families containing multiple cases of epithelial ovarian cancer. However, high-risk susceptibility genes are responsible for less than 30% of the excess familial ovarian cancer risks, which suggests other ovarian cancer susceptibility genes exist. The remaining risk may due to combinations of several common alleles of moderate/low risk, rather than other rare high-risk genes. We have used a SNP tagging candidate gene approach to look for associations between single nucleotide polymorphisms (SNPs) in three ovarian cancer case-control studies from Denmark, the UK and USA. These studies comprise ~1,500 cases and 2,500 controls. We chose to look for disease associations in genes involved in cell cycle control because there is extensive evidence for a ubiquitous role of cell cycle genes (e.g. p53, RB, p16) in cancer development generally and ovarian cancer specifically. **Results:** So far we have genotyped 51 SNPs in 13 genes (CCND1, CCND2, CCND3, CCNE1, CDK2, CDK4, CDK6, CDKN1A, CDKN1B, CDKN2A, CDKN2B, CDKN2C, RB1, p53). Genotype distributions were close to those expected under Hardy-Weinberg equilibrium. Genotype frequencies in cases and controls, stratified by study, were compared using a likelihood ratio test in a logistic regression model. For 46 SNPs, we detected no significant differences in genotype frequency between cases and controls. Two associations with $P < 0.05$ and three associations with $P < 0.01$ were seen for SNPs in CCND1, CDK6 and CDKN1B. **Conclusions:** We have found evidence that common variants in cell-cycle control genes are associated with increased ovarian cancer risk. In order to validate the role of these genes in ovarian cancer, it will first be necessary to confirm these results in even larger series of ovarian cancer cases and to identify the causative functional variant(s)

The contribution of BRCA1 and BRCA2 mutations to inherited ovarian cancer

Susan Ramus,[1] Patricia Harrington,[2] Richard DiCioccio,[3] Bruce Werness,[4] Kim Garlinghouse-Jones,[5] Ingrid Oakley-Girvan,[5] Carole Pye,[2] Ian Jacobs,[1] Alice Whittemore,[5] Bruce Ponder,[2] Paul Pharoah,[2] M. Steven Piver,[3] Simon Gayther[1]

[1] University College London, UK
[2] University of Cambridge, UK
[3] Roswell Park Cancer Institute
[4] Inova Fair Oaks Hospital
[5] Stanford University School of Medicine, USA

Introduction: Three hundred families, from the UKCCCR and Gilda Radner ovarian cancer registries, containing at least two confirmed cases of epithelial ovarian cancer (EOC) in first-degree relatives were screened for coding mutations in the BRCA1 and BRCA2 genes and genomic deletion/rearrangement mutations in BRCA1. **Method:** An affected individual from each family was first screened by SSCP/HA analysis. Abnormal variants were sequenced to identify the causative nucleotide change. Families in which no deleterious mutation was identified (174 families) were further analysed for genomic rearrangements of BRCA1 using MLPA. **Results:** Functional BRCA1 mutations were identified in 111 families (37%), of which 10 were detected by MLPA; thus 9% of all BRCA1 mutations were large rearrangements. Of these, 5 were the same exon 13 amplification (a UK founder mutation). The remaining 5 rearrangements were previously unreported deletions of exon 2, exons 3-16, exons 8-13, exons 15-20, and exons 21-24. Functional BRCA2 mutations were identified in 25 families (8%). BRCA1/2 mutation prevalence correlated with the extent of ovarian and breast cancer in families. Of families with >2 EOC cases and at least one breast cancer case under 60 years, 86%; had a BRCA1/2 mutation. Mutation prevelence was significantly less in families containing no breast cancer. Of 156 families containing 2 ovarian cancer cases, only 28% had an identifiable mutation compared to 64% of 58 families containing 3 or more EOC cases. **Conclusion:** These data indicate that BRCA1 and BRCA2 are the major EOC susceptibility genes; but other susceptibility genes may also exist.

Indoleamine 2, 3-dioxygenase serves as a marker of poor prognosis in gene expression profiles of serous ovarian cancer cells

Aikou Okamoto,[1] Takashi Nikaido,[2] Kazunori Ochiai,[1] Satoshi Takakura,[1] Kyosuke Yamada,[1] Miho Takao,[1] Nozomu Yanaihara,[1] Kazu Ueda,[1] Hirokuni Takano,[1] Mitsuyoshi Urashima,[3] Tadao Tanaka[1]

[1] Dept OB/GYN, Jikei University School of Medicine
[2] Dept Pathology, Jikei University School of Medicine
[3] Division of Clinical Research and Development, Jikei University School of Medicine

Purpose: Although ovarian cancer is considered highly responsive to combination therapy with paclitaxel (PTX) and carboplatin (CBDCA), cancer recurs rapidly in more than 50% of responsive patients, and in many cases, the recurring cancer cells develop chemoresistance. Therefore, countering chemoresistance is essential for ovarian cancer management. We aimed to find key molecules associated with chemoresistance using gene expression profiling as a screening tool. **Experimental Design:** Using 2 newly established PTX-resistant ovarian cancer cell lines from an original PTX-sensitive cell line and 4 super-sensitive and 4 refractory surgical ovarian cancer specimens from PTX-based chemotherapy, molecules associated with chemoresistance were screened with gene expression profiling arrays containing 39,000 genes. We further analyzed 44 genes that showed significantly different expressions between PTX-sensitive samples and PTX-resistant samples with permutation tests, which were common in cell lines and patients' tumors. **Results:** Eight of these genes showed reproducible results with the real time reverse transcriptase polymerase chain reaction, of which indoleamine 2, 3-dioxygenase (IDO) gene expression was the most prominent and consistent. Moreover, by immunohistochemical analysis using a total of 24 serous type ovarian cancer surgical specimens (stage III: n=21, stage IV: n=7) excluding samples used for GeneChip analysis, the Kaplan-Meier survival curve showed a clear relationship between IDO staining patterns and overall survival (log-rank test: $P = 0.0001$). All patients classified as negative survived without relapse. The 50% survival of patients classified as sporadic, focal and diffuse was 41, 17 and 11 months, respectively. **Conclusion:** The IDO screened with the GeneChip was positively associated with PTX resistance and with impaired survival in patients with serous type ovarian cancer.

Epigenetic Silencing of HSulf-1 in Ovarian Cancer

Julie Staub,[1] Jeremy Chien,[1] Yunqina Pan,[1] Melissa Scheerer,[1] Julian Molina,[1] Viji Shridhar[1]

[1] Mayo College of Medicine

Introduction: Gene silencing by hypermethylation of certain tumor suppressor genes in cancer cells has gained intense attention in recent years. CpG island methylation in cancer can be used as a marker for tumor behavior, as a predictor of response to treatment and reactivation of silenced genes in an epigenetic therapy of cancer. In this study, we report on the epigenetic regulation involving both methylation and histone acetylation of the newly cloned HSulf-1 gene in ovarian cancer cell lines and primary ovarian tumors. Induction of HSulf-1 transcription following 5-aza-Cdr or TSA treatment sensitized these cells to cisplatin and siRNA mediated downregulation of endogenous HSulf-1 expression in OV202, abrogated the chemoresponse. **Methods:** We analyzed the methylation status of the putative CpG island of the recently cloned gene, HSulf-1 that is downregulated in a substantial portion of ovarian cancer by genomic sequencing of bisulfite modified DNA from ovarian cell lines and primary ovarian tumors. In addition, Chromatin Immunoprecipitation (ChIP) assays were performed on cell lines with and without HSulf-1 expression to determine if HSulf-1 methylation was associated with loss of histone acetylation. Using siRNA mediated downregulation of endogenous HSulf-1 expression in OV202, we determined the effectiveness of cisplatin to induce apoptosis. **Results:** Sequence analysis of bisulfite modified genomic DNA from ovarian and cancer cell lines and primary ovarian tumors with loss of HSulf-1 expression revealed that there was an increase in the frequency of methylation of 12 CpG sites in exon 1A that bound to a CpG binding column. Chromatin immunoprecipitation (ChIP) assays showed that HSulf-1 methylation was associated with loss of histone acetylation in cell lines with loss of HSulf-1 expression and increased methylation compared to cell lines with HSulf-1 expression. Finally, reexpression of HSulf-1 by 5-aza-2'-dC and or TSA followed by cisplatin treatment resulted in increased rate of apoptosis in these cells compared to untreated cells. siRNA mediated downregulation of endogenous HSulf-1 expression in OV202 lead to an attenuation of cisplatin induced cytotoxicity specifically implicating HSulf-1 in this process. **Conclusion:** Collectively these results implicate HSulf-1 in modulating the chemoresponse to the commonly used chemotherapeutic agents and provide the first evidence of methylation in combination with chromatin histone modification as a mechanism of HSulf-1 inactivation.

Expression of Spermatogenesis-Associated Retrogenes in Ovarian Cancer

Jan Rohozinski,[1] Matthew L. Anderson,[1] Colin E. Bishop[1]

[1] Dept of Obstetrics and Gynecology, Baylor College of Medicine

During spermatogenesis many retrogenes that originated from the X chromosome are expressed in a stage-specific manner to compensate for transcriptional inactivation of the sex chromosomes during meiosis. In man transcription from these retrogenes is generally limited to the testis and in some cases a few somatic tissues as well. Our group has been studying one such retrogene, UTP14c, with respect to male infertility. We have found that besides the testis UTP14c is also expressed in the epithelium lining the surface of the normal human ovary and a panel of established ovarian cancer cell lines (8/8). UTP14c expression was also observed in 93% of papillary serous ovarian cancers tested (56/60) as well as ovarian cancers with other histologies, including endometroid cancers (2/5), immature teratomas (2/2) and cancers with mixed histology (12/17). To determine whether the expression of spermatogenesis specific retrogenes in ovarian cancer is a general phenomenon we tested for the expression of the X-derived retrogenes BIRC8, RPL10L and RPL39L. All were found to be expressed in a large number of ovarian cancer samples and a panel of established ovarian cancer cell lines. The X chromosome progenitors for each of these genes (UTP14a, BIRC4, RPL10 and RPL39) are expressed in all tissues, cancers and ovarian cancer cell lines, as would be expected for genes with essential cell functions, such as ribosomal RNA biosynthesis, suppression of apoptosis and ribosome assembly.

These observations indicate that the activation of retrogenes, normally expressed only during spermatogenesis, maybe a general phenomenon in ovarian cancer. Given their highly restricted tissue specific expression pattern, these retrogenes are of great interest as they may provide diagnostic markers, tissue-specific targets for therapy and offer insight into the nature of cells from which ovarian cancers originate.

Gene expression profiling of ovarian tumors obtained prior to and after chemotherapy: identification of putative markers implicated in mechanisms of chemoresistance

Sylvain L'Esperance,[1] Ion Popa,[1] Magdalena Bachvarova,[2] Bernard Tetu,[1] Dimcho Bachvarov[1,3]

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[1] CHUQ, Centre de recherche de l'Hôtel-Dieu de Québec
[2] Centre de recherche de l'Hôtel-Dieu de Québec (CRHDQ) and Université Laval (Québec, Canada)
[3] Dept. of Medicine, Laval University

Introduction: Chemotherapy resistance, both intrinsic and acquired, represents a very serious problem in the treatment of ovarian carcinomas, as the underlying mechanisms and pathways leading to this resistance seem complex and presently not well understood. We tried to identify genes implicated in the mechanisms of ovarian cancer chemoresistance by comparing gene expression profiles in tumor samples obtained prior to, and after chemotherapy from six ovarian cancer patients. The Agilent Human 1A Oligo Microarray (v2) containing 20,173 genes was used for gene expression studies. The differential gene expression of some putative chemoresistance markers was further validated by semi-quantitative RT-PCR. **Results:** A list of 244 genes was identified to be differently expressed (=1,5 fold) following chemotherapy treatment in all 6 patients studied. Upregulated genes upon chemotherapy are mostly implicated in cellular growth regulation, cell communication and cell metabolism (including metallothionein 2A, ATP6AP1, MUC1, peroxyredoxin 2A and COX7b), while genes that were downregulated following treatment are predominantly involved in regulation of gene expression and apoptosis (including fibromodulin, tumor rejecting 1, BAG-1 and paranoplasic antigen 1). Some of these genes (MUC1, metallothioneins 2A, COX7b, fibromodulin) have been previously associated with mechanisms of chemoresistance. Additionally, the individual gene expression profiles (prior to and after chemotherapy) of the 6 patients studied allowed their separation in two distinct groups (each including 3 patients). This grouping was further confirmed by a cluster analysis, as some genes associated with tumor suppression (i.e. PKC binding protein, THY-1, OVCA), some tumor antigens and DNA repair genes were downregulated in group 1 while upregulated in group 2. Analysis of the clinical charts of the 6 patients showed some differences in certain clinical parameters (p53 status, chemotherapy treatment) that could potentially explain this grouping. **Conclusions:** Gene expression profiles of ovarian tumors obtained prior to- and after chemotherapy from 6 patients has led to the identification of putative markers involved in the process of chemoresistance. To our knowledge, this is the first gene expression comparison in ovarian tumor samples taken prior to, and after chemotherapy from the same patient. Several potential chemoresistance markers (metallothionein 2A, MUC1, COX7b, fibromodulin) were identified that have already been associated with mechanisms of chemoresistance and their differential expression was confirmed by semi-quantitative RT-PCR. These markers will need further functional validation in model systems. An understanding of the molecular mechanisms of drug resistance could contribute to the development of novel treatment strategies designed to prevent its emergence.

Expression Analysis in Serous Epithelial Ovarian Cancer Associated with Intrinsic Resistance to Chemotherapy

Chung-Hae Lee,[1] Mona Prasad,[1] Jan Hauspy,[1] Marcus Bernardini,[1] Allan Covens,[2] Barry Rosen,[1] Joan Murphy,[1] Pat Shaw,[1] Heather Begley,[1] Pascale Macgregor,[1] Jeremy Squire[1]

[1] University Health Network
[2] Sunnybrook Health Science Centre

Introduction: Ovarian cancer is the second most frequently diagnosed gynecological malignancy, and causes more deaths than any other cancer of the reproductive system. The lack of reliable methods of early detection and the absence of specific symptoms result in late stage diagnosis in 75% of patients. Although initial response rates to conventional chemotherapy among advanced stage patients are high, resistance to chemotherapy remains the primary factor accounting for the low 5-year survival in this patient population. The current project utilized expression microarray data analysis to improve our knowledge of sporadic Serous Epithelial Ovarian Cancer (SEOC), and to ultimately identify a signature of differentially expressed genes that are predictive of treatment failure and poor prognosis. **Methods:** Snap frozen tissue samples from 22 sporadic SEOC naïve to chemotherapy that had at least 75% tumor content were selected from the Toronto Ovarian Tissue Bank and Database. Based on their CA 125 levels before, during, and after treatment, ten SEOC samples were selected from the 22 patients who exemplified extreme cases of differential response to standard carboplatin/taxol therapy. Total RNA extracted from each tumor was processed for microarray hybridization using cDNA spotted arrays. Agglomerative hierarchical cluster analysis based on Pearson correlation coefficient, Significance Analysis of Microarrays (SAM), and Prediction Analysis for Microarrays (PAM) were performed on LOWESS normalized data. **Results:** Unsupervised analysis of the 22 samples displayed a group of sensitive and resistant samples segregating into opposite nodes but the two main clusters also contained samples of the opposite class. Unsupervised analysis of the ten extreme responders showed clear separation of the two groups. Supervised analysis with the ten samples identified a group of genes whose differential expression was significantly associated with drug resistance. Within this group five genes (GAPD, HMGB2, HSC70, GRP58 and HMGB1) were previously shown to form a nuclear complex associated with resistance to DNA-conformation altering chemotherapeutic drugs in in vitro systems. Real time RT-PCR confirmed lower levels of the five gene transcripts in the resistant compared to the sensitive group. **Conclusions:** Five genes (GAPD, HMGB2, HSC70, GRP58 and HMGB1) identified with supervised analysis and data mining may represent a novel class of genes associated with in vivo drug response in ovarian cancer patients. We are currently validating the expression patterns of the nuclear complex as well as further exploring its role in drug resistance in SEOC.

CA125 influences the sensibility of NIH:OVCAR-3 ovarian cancer cells to genotoxic anticancer drugs

Marianne Boivin,[1] Denis Lane,[1] Julie Beaudin,[1] Claudine Rancourt[1]

[1] Université de Sherbrooke

Introduction: Cancer antigen CA125 is overexpressed in more than 80% of epithelial ovarian cancers and its function is still unknown. Data from our laboratory suggest that CA125 is involved in carcinogenesis and in the metastatic process. Based on an analogy with MUC1, which confer resistance to apoptosis induced by genotoxic agents, we hypothesized that CA125 can modulate ovarian cancer cell sensitivity to anticancer agents. **Methods:** The objective of this study was to determine the influence of CA125 in ovarian cancer cells sensitivity to cytotoxic agents. The methodology used consists of NIH:OVCAR-3 derived stable cell lines expressing an anti-CA125 single chain antibody (scFv) that is targeted and retained to the endoplasmic reticulum (ER). Sequestration of CA125 within the ER mimics a CA125 knockdown by preventing its proper cell surface localization. **Results:** Cell sensitivity to anticancer drugs was tested on CA125 positive cells (parental cell line OVCAR-3 and scFv control) in comparison to CA125 knockdown cells (1:9#9 and 1:9#7 clones) by XTT assays. Such drugs included: DNA damaging agents (cisplatin, doxorubicin, cyclophosphamide), topoisomerase II inhibitor (etoposide) and microtubule dynamic inhibitors (taxol, vinorelbine). CA125 knockdown cells (1:9#9 and 1:9#7) were 3 to 5 times more sensitive to cisplatin, cyclophosphamide, doxorubicin and etoposide when compared to controls. No differential effect was observed with taxol and vinorelbine. Western-blot analyses of endogenous levels of proteins involved in the apoptotic mitochondrial pathway (Bcl-2, Bcl-XL, Bax, Xiap, procaspase-3, procaspase-9) showed no significant difference. There was an increase in the cleavage of caspase-3 and caspase-9 in CA125 knockdown cells when exposed to cisplatin, confirming that cells died by apoptosis. **Conclusions:** These results indicate that CA125 regulates genotoxic drug sensitivity: CA125 knockdown cells are more affected by drugs affecting DNA (covalent binding or inhibition of topoisomérase II) than control cells. CA125 seems to have no role in cell sensitivity to microtubule inhibitor drugs. The enhanced sensitivity to genotoxic drugs could be caused by events upstream caspase-9 activation that could be differentially regulated in CA125 knockdown cells. Our data suggest that CA125 negatively regulates DNA damage induced apoptosis, and led us to propose that CA125 tumor status could influence chimiotherapy response of ovarian cancer patients.

CA125 tumor antigen modulates TRAIL-induced apoptosis in NIH:OVCAR-3

Martine Migneault,[1] Denis Lane,[1] Roxanne Grondin,[1] Claudine Rancourt,[1] Alain Piché[1]

[1] Université de Sherbrooke

Introduction: TNF-Related Apoptosis Inducing Ligand (TRAIL) and Fas Ligand (FasL) are a cytotoxic cytokines from the Tumor Necrosis Factor (TNF) family. Recently, the use of a soluble form of TRAIL or of agonist antibodies against TRAIL receptors has been proposed as a potential new therapeutic agent for treatment of various types of cancer, including epithelial ovarian cancer. We demonstrated that many human ovarian cancer cell lines and primary cultures are partly or completely resistant to TRAIL-induced apoptosis. 80% of epithelial ovarian cancers overexpress the MUC16 mucin, better known as CA125. We therefore hypothesized that the resistance or sensitivity of ovarian cancer cells to TRAIL and FasL could be linked to the expression and functions of CA125. **Methods:** We developed an inhibitor of CA125 which consists of an anti-CA125 single chain antibody (ScFV) that is targeted to and retained within the endoplasmic reticulum (ER). Stable clones expressing CA125 inhibitor (CA125 knockdown clones) and controls were derived from NIH:OVCAR-3 human ovarian cancer cells which express high levels of CA125. Sequestration of CA125 within the ER mimics a CA125 knockdown by preventing its proper cell surface localization. **Results:** The goal of this study was to determine whether CA125 influence the in vitro sensitivity of NIH:OVCAR-3 cells to apoptosis induced by cytotoxic cytokines. CA125 knockdown clones were compared to controls for their sensitivity to apoptosis induced by TRAIL, FasL and TNF-alpha using XTT based cytotoxicity assays. Results demonstrated that CA125 knockdown clones are more sensitive to FasL and TRAIL-induced apoptosis than the control cells. No effect was obtained with TNF-alpha as the parental NIH:OVCAR-3 cells did not respond to TNF-alpha. Western blot analyses showed that in CA125 knockdown cells, TRAIL induced more procaspase-8, procaspase-3 and PARP cleavages suggesting an increase in caspase-8 and caspase-3 activity. An augmented DEVDase activity supported the increased caspase-3 activity. Apoptosis was confirmed by flow cytometry analysis of PI-stained cells. **Conclusions:** Taken together our results demonstrate that, in NIH:OVCAR-3 cells, CA125 knockdown increases sensitivity to apoptosis induced by TRAIL and FasL. We therefore suggest that the expression and functions of CA125 can be linked to the resistance or sensitivity of ovarian cancer cells to these cytotoxic cytokines.

c-erbB-2, p53, and nm23 Proteins as Prognostic Factors in Patients with Epithelial Ovarian Carcinoma

Stanko Jukic,[1] Snjezana Tomic,[2] Jadranka Ilic Forko,[3] Damir Babic,[3] Drazen Jukic[4]

[1] Institute of Pathology
[2] Dept of Pathology, Split University Hospital Center and School of Medicine Split
[3] Dept of Gynecological and Prenatal Pathology, Zagreb University Hospital Center and School of Medicine Zagreb
[4] UPMC, Shadyside Hospital, Pittsburgh, USA

Introduction: To demonstrate immunohistochemical expression of p53, c-erbB-2, and nm23 proteins in ovarian cancer and to establish their correlation with such predictive factors as clinical stage, grade, and vascular invasion. The effect of protein overexpression on patients' overall survival was also assessed. **Methods:** We performed immunohistochemical analysis of formalin-fixed, paraffin-embedded specimens from 80 ovarian carcinomas, using the anti-nm23, p53, and c-erbB-2 monoclonal antibodies. Immunohistochemical results were scored semiquantitatively. All patients were staged according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO) staging system (I-IV). Carcinomas were graded as low-or high-grade, according to the modified grading system recommended by Shimatzu and Silverberg. For univariate analysis, survival time was analyzed by Kaplan-Meier method, and the log-rank test was used to assess the differences between the groups. For multivariate analysis, Cox proportional hazard regression model was used to examine several parameters simultaneously. **Results:** Univariate analysis showed that advanced clinical stage ($p<0.001$); positive staining for nm23 ($p<0.001$), p53 ($p=0.021$), and c-erbB-2 ($p=0.003$) protein; high histological grade ($p<0.001$); and vascular invasion ($p=0.006$) were associated with shorter overall survival. Multivariate analysis revealed only clinical stage as an inependent prognostic parameter ($p=0.014$). Multivariate analysis for early-stage disease showed that only the presence of vascular invasion was significantly associated with shorter survival ($p=0.008$), whereas none of the parameters analyzed for the advanced-stage disease showed independent predictive value for prognosis. **Conclusion:** The overexpression of p53, nm23, and c-erbB-2 proteins was associated with other parameters characteristic of aggressive tumors, such as advanced clinical stage, high grade, and/or presence of vascular invasion. However, this overexpression had no independent prognostic value either for overall survival or survival corrected by clinical stages.

Gene expression profiling predicts therapeutic response to chemotherapy treatment in patients with serous papillary adenocarcinoma of the ovary

Dimcho Bachvarov,[1,2] Sylvain L'Esperance,[2] Ion Popa,[2] Magdalena Bachvarova,[3] Bernard Tetu[2]

[1] Dept. of Medicine, Laval University
[2] CHUQ, CR-HDQ
[3] Centre de Recherche de l'Hôtel-Dieu de Québec (CRHDQ) & Université Laval (Québec, Canada)

Introduction: Ovarian carcinoma is a leading cause of gynecologic cancer death in women. Early-stage ovarian cancer tends to be asymptomatic and most ovarian cancers are diagnosed at advanced stages. Despite treatment, a large number of women with ovarian cancer eventually relapse and die of the disease. Drug resistance is a major cause of treatment failure, resulting in death for more than 90% of patients with metastatic disease. We hypothesized that differences in gene expression before treatment could distinguish serous adenocarcinoma patients with short versus long time to recurrence after chemotherapy. Serous adenocarcinomas comprise the majority (approximately 80%) of all epithelial ovarian cancers. Moreover, we wanted to use a more homogeneous set of tumor samples for our gene expression analyses, since it was previously shown that different histological subtypes of ovarian cancer display significant differences in chemoresistance and molecular markers **Results:** We performed gene expression profiles of 34 primary surgically resected tumors from women with advanced stage, high grade serous papillary adenocarcinoma of the ovary using the Agilent Human 1A Oligo Microarray (v2) containing 20,173 genes. Supervised learning algorithms were applied in an effort to develop a binary classifier that could discriminate women at risk for early (=20 months) versus late (>20 months) relapse after initial chemotherapy. A 42-gene predictive model was developed using a duplicate set of training samples (n = 26) and subsequently tested using an independent set of test samples (n = 8). The 42-gene set cross-validated the training set with 100% accuracy. Hierarchical clustering of the 42 genes correlated with chemotherapy response for all tumor samples included in the training set. This model correctly predicted the outcome of all 6 test samples (100% accuracy). The differential expression of some functionally-related to disease progression and chemoresistance genes was validated by semi-quantitative RT-PCR and immunohistochemistry, using tissue arrays. **Conclusion:** Predictive markers for early recurrence can be identified for chemotherapy in primary ovarian serous adenocarcinoma. The proposed 42-gene model needs to be validated independently with additional primary tumor samples obtained from ovarian serous adenocarcinoma patients as well as from patients with other types of epithelial ovarian cancer to more precisely determine its specificity as well as its more general application in ovarian cancer treatment. If future studies validate this model, stratification based on pretreatment gene expression would be feasible in patients with ovarian cancer, thereby allowing women who are destined for early relapse to receive alternative primary treatment strategies.

Chk2 Activation by Thr-68 Phosphorylation Is Regulated by p53 in Response to Cisplatin Treatment in Human Ovarian Cancer Cells

Jing Jie Yu,[1] Xiaobing Liang,[2] Heimo Riedel,[3] Eddie Reed[2]

[1] West Virginia University
[2] WVU Mary Babb Randolph Cancer Center
[3] WVU Biochemistry & Mol Pharma

Introduction: The protein kinase Chk2 and tumor suppressor p53 are central mediators of the DNA damage response to cellular stress and cytotoxic reagents. A signaling pathway represented by a linear sequence of ATM/ATR – Chk2 – p53 had been proposed earlier to activate various targets. However, this linear model has been called into question recently by various reports from different groups. These studies indicate that Chk2 is not required for a p53 response in human cancer cells; that wild-type p53 suppresses mRNA and protein levels of Chk2 in human osteosarcoma Saos2 (p53 null) cells; and that p53 mutations increase Chk2 expression in human gastric carcinoma. **Methods:** Human ovarian cancer A2780 and SKOV-3 cell lines were used in the current investigation. Some cells were treated with cisplatin for 1 hr at IC50 doses (3 micromolar for A2780 and 75 micromolar for SKOV-3) alone; others were transfected with siRNA against p53; or transient-transfected with plasmid pC53-SN3 or pCMV-Neo-Bam for 24 hours, prior to cisplatin treatment. Treated and untreated (control) cells were subjected to western blot analysis with antibodies of anti-Chk2 phosphothreonine-68, anti-Chk2, anti-p-p53 phosphoserine-15, anti-p-p53 phosphoserine-20, anti-p53, anti-p21 and anti-beta-actin. **Results:** We observed that cisplatin activates p53 at Ser-15 and Chk2 at Thr-68 by phosphorylation, and that the levels of phosphorylated p53, of phosphorylated Chk2, and of total p53 protein were all increased after cisplatin treatment in a time-dependent manner. The observed cisplatin-induced increase in p53 phosphorylation precedes (by about 12 hours) the observed increase in Chk2 phosphorylation. Increased expression of p53 in human ovarian cancer A2780 cells by cDNA transfection at least doubled the amount of observed Chk2 phosphorylation 48 hours after cisplatin treatment, whereas treatment with p53-specific siRNA reduced Chk2 phosphorylation. **Conclusions:** Our data suggest that Chk2 phosphorylation at Thr-68 is regulated by p53 in the DNA damage response pathway to the chemotherapeutic reagent cisplatin in human ovarian cancer cells.

4D-CT Respiratory-Gated Whole Abdominal Intensity-Modulated Radiotherapy (WAIMRT) for Ovarian Cancer: A Feasibility Study

Adam Garsa,[1] Dwight Heron,[1] Sushil Beriwal,[1] Regiane Andrade,[2] Hayeon Kim,[1] Edward Brandner,[1] Hank Chen,[1] Kristina Gerszten[1]

[1] University of Pittsburgh
[2] Radiation Oncology Department, University of Pittsburgh

Introduction: Whole-abdominal radiotherapy has a controversial role in the management of ovarian cancer. Tolerance of the abdominal organs and bone marrow limits its routine use. This study assesses the feasibility and implementation of respiratory-gated WAIMRT. **Methods:** A GE Light speed CT-scanner and Varian RPM Respiratory Gating System were used. Scan data were acquired over an entire respiratory cycle. The 4D data set was binned and sorted into 6-8 phases to reduce organ motion to less than 5 mm. The planning target volume (PTV1) includes the entire peritoneal cavity plus a 1 cm margin, and a pelvic field boost was created (PTV2). Both kidneys were excluded from the target volume, although their peritoneal surfaces (~1-2 mm) were partially included in the PTV1. Optimization was designed to spare liver, bone marrow, and kidneys. Three patients were treated utilizing this clinical pathway. The dose prescribed to PTV1 was 30 Gy followed by 14.4 Gy to PTV2. **Results:** Dosimetric results are as follows: The mean dose for PTV1 receiving 100% of the prescribed dose was 90%. The mean PTV1 receiving 110% of the prescribed dose was 48% and mean PTV1 receiving 120% was 4.7%. Mean V30 for the liver was 54%. For kidneys mean V20 was 19% and for bone marrow, mean V20 was 74%. Acute toxicities were anemia (grade 1: 1/3, grade 2: 1/3), leukopenia (grade 1: 1/3, grade 3: 2/3) and thrombocytopenia (grade 1: 1/3, grade 3: 2/3), one patient required a treatment break due to hematologic toxicity, though. Two patients experienced mild diarrhea and nausea, which were successfully treated with medication. One patient could not complete whole-abdomen field after 19.5 Gy because of persistent nausea. **Conclusions:** 4D WAIMRT reduced treatment volume significantly, by reducing the margin for diaphragmatic excursion. This technique demonstrated excellent coverage to the target with reduced dose to the abdominal organs and bone marrow. WAIMRT is a novel and feasible technique for ovarian cancer treatment. Further studies are needed to determine the efficacy and overall toxicity of this method.

Pathways to development of serous epithelial ovarian cancer: combining genetics and epidemiology

Melissa Merritt,[1] Glen Boyle,[1] Tanya Newton,[1] Penny Webb,[1] Adele Green,[1] Peter Parsons[1]

[1] Queensland Institute of Medical Research

Introduction: Ovarian cancer is a major cause of morbidity and mortality among women. For the most common subtype of epithelial ovarian cancer (serous), there are still basic questions about the development of this disease. It has been suggested that benign and low malignant potential (LMP) tumours may be precursors to low grade malignant tumours. However, most serous malignant tumours are high grade, late stage, and have no apparent precursor lesion. **Methods:** To further our understanding of the development of serous ovarian cancer, we applied cDNA microarrays to four normal ovaries and 43 serous ovarian tumours representing a range of tumour types (benign to malignant). The possibility also exists that aberrant gene expression in ovarian cancer tissue may be related to exposure to risk factors for ovarian cancer. Using cDNA microarrays, we investigated whether a patient's exposure to risk factors for ovarian cancer influenced the gene expression patterns in 34 LMP and malignant tumours. **Results:** Significant differences in gene expression were identified between normal/benign and LMP/malignant tumours. When exposure to risk factors was considered, significant differences in gene expression were identified in LMP/malignant tumours of patients grouped by their body mass index, use of the oral contraceptive pill or hormone replacement therapy and smoking status. In contrast, no differences in gene expression were detected when age, menopausal status, or history of tubal ligation/ hysterectomy were investigated. The most promising genes were selected from these analyses for confirmation using other laboratory techniques (e.g. real-time PCR and immunohistochemistry). Once confirmed, selected genes will be evaluated in a larger set of tissue blocks to determine their importance on a larger scale. **Conclusions:** This research has brought together genetic and epidemiological data to learn more about the causal molecular pathway leading to the development of serous ovarian cancer. Key findings of this research will be the identification of novel genes involved in cancer of the ovary. With a better understanding of the causal molecular pathways that lead to serous ovarian cancer, we will contribute to an improvement in diagnostic and preventative strategies, thereby leading to a decrease in ovarian cancer incidence.

Molecular mechanisms of the antiproliferative action of Nutlin in tumor cells

Geneviève Synnott,[1] Magdalena Bachvarova,[2] Lyubomir Vassilev,[3] Dimcho Bachvarov[4]

[1] Centre de Recherche de l'Hôtel-Dieu de Québec (CRHDQ)
[2] Centre de Recherche de l'Hôtel-Dieu de Québec (CRHDQ) & Université Laval (Québec, Canada)
[3] Roche Research Center (Nutley, NJ)
[4] Dept. Medicine, Laval University & CHUQ-CR-HDQ

Introduction: MDM2 protein binds the tumor suppressor protein p53 with high affinity and modulates negatively its activity and its stability. A newly discovered imidazoline analog Nutlin can block the p53-binding pocket in the MDM2 protein and thus can potentiate the p53 pathway in cancer cells, leading to cell cycle arrest and apoptosis. We are studying the therapeutic potentials of Nutlin in cancer treatment and the molecular mechanisms of its antiproliferative action. **Methods:** MTT assays and FACS analysis have permitted to verify the Nutlin effect on cell cycle arrest and apoptosis in HCT-116 cell lines (colon cancer) and in different ovarian cancer cell lines (A2780, OVCAR-3, OV-4, OV866, TOV21, TOV112, TOV155, TOV1592, UCI-100). The level of protein expression (p53, p21 and MDM2) has been measured by Western Blot. Gene expression analyses were performed using the Agilent human 1A (v2) oligonucleotide microarray (containing 20,173 genes) in order to better elucidate the molecular mechanisms antiproliferative and cytotoxic action of Nutlin. **Results:** Initial in vitro analysis using MTT assays confirmed the potent cytotoxicity effect of the Nutlin in different cancer primary cell lines, including ovarian cancer primary cultures, carrying a wild-type p53 gene. Western analysis showed increase in p53, p21 and MDM2 protein levels in these cell lines upon Nutlin administration. Different genes involved in cell cycle control and cellular proliferation were down-regulated following Nutlin treatment, while a number of genes from the p53 pro-apoptotic pathways were up-regulated. No effects were observed in cell lines containing mutated p53 gene. Nutlin displayed an intermediate inhibitor effect in the HCT-116 p21-mutant cell line, indicating that p21 is not the only molecule implicated in the antiproliferative action of p53. Twenty-four hours following Nutlin treatment, most cancer cell lines displayed G1+G2 cell cycle arrest, including different clones of the HCT – 116 cell line; however one HCT-116 clone repeatedly displayed G1 cell cycle arrest. Gene expression comparison between G1+G2 growth-arrested HCT-116 cells and G1growth-arrested HCT-116 cells has indicated the possible implication of several cell cycle regulator genes, including cyclin G2 and the aryl hydrocarbon receptor. **Conclusion:** Our results confirm the strong therapeutic potential of Nutlin for tumors (including ovarian tumors) that have retained wild-type p53.

New ligands for targeting the high affinity folate receptors

Archana Nigam,[1] Patricia Griffin,[1] Michael Zhuravel,[2] Jacqueline Jones-Laughner,[3] Lori Kubasiak,[2] Julie DeLoia,[3] Donald Tomalia,[2] Erik C. Wiener[1]

[1] Hillman Cancer Center, University of Pittsburgh Cancer Institute
[2] Dendritic Nanotechnologies
[3] Magee-Women's Research Institute

Introduction: A variety of human tumors of epithelial origin express a high affinity for Folic acid, composed of a pterin ring, para-aminobenzoic acid, and glutamic acid due to the expression of the high affinity folate-receptor (hFR) that binds folate with a dissociation constant of about 10-9. Several cancer diagnostic and therapeutic agents have been targeted to the hFR by attaching these agents to folate molecules. Attachment of these molecules must occur at the active gamma-carboxylate of the glutamate moiety in order to achieve similar affinities for the hFR. Once bound to the hFR, these agents enter the cells via receptor-mediated endocytosis. We developed a new strategy to target the hFR based on the hypothesis that any alpha-amino acid linked to the 4-aminobenzoic acid and pterin ring such that the alpha-acid remains free will target the hFR. We coupled cysteine to cystamine core polyamidoamine (PAMAM) dendrimers followed by pteroyl-azide. The pteroyl-cysteine conjugate specifically binds to hFR-positive cells; this binding is significantly higher in cells expressing higher levels of the FOL1-R m-RNA; binding increased with upregulation of the hFR, is inhibited by free folic acid. **Methods:** The precursor pteroyl-azide was synthesized by cleaving off the glutamate moiety from folic acid with trifluoroacetic anhydride followed by treatment with hydrazine, trifluoroacetic acid with KSCN, and tert-butylnitrous acid and was characterized by nuclear magnetic resonance and mass spectroscopies. The pteroyl-azide was then reacted with a polyamidoamine point dendron with either cysteine or cystamine linked to the attachment point via a disulfide bridge and 8 surface fluoresceines. The pteroyl-cysteine/cystamine conjugates were characterized by IR-spectroscopy. The folate-receptor positive cell lines, KB and C2Bbe1 were grown in a continuous culture with folate free media. The cells were incubated with a 40nM fluorescent-labeled pteroyl-cysteine conjugate dendrimer. For the competitive-inhibition studies, the cells were incubated in an excess molar concentration of free folic acid. **Results:** Coupling of pteroyl-azide with cysteine/cystamine dendrimers was confirmed by IR-spectroscopy. The flow-cytometry results showed that pteroyl-cysteine conjugate binds with the cells expressing hFR. The competitive-inhibition studies also revealed that free folate inhibits the binding of the pteroyl-cysteine conjugates. The kinetic studies show that the cells accumulating pteroyl-cysteine conjugate in a receptor-mediated manner. The fluorescence microscopy studies confirmed the specific binding of pteroyl-cysteine to the cells expressing hFR. **Conclusion:** Pteroyl-azide was coupled with cysteine/cystamine dendrimers to give pteroyl-cysteine/cystamine conjugates. Only the pteroyl-cysteine conjugate binds to cells expressing hFR. Our data support our hypotheses that any alpha-aminoacid conjugated to pteroyl-azide will target the hFR.

BORIS, a novel cancer-testis antigen is a potential target for immunotherapy in epithelial ovarian cancer

Jeannine Villella,[1] Feng Qian,[1] Deborah Driscoll,[1] Jonathan Skipper,[2] Andrew Simpson, [2] Lloyd Old,[2] Shashikant Lele,[1] Kunle Odunsi[1]

[1] Roswell Park Cancer Institute
[2] Ludwig Institute for Cancer Research

Objective: Cancer-testis antigens (CTA), are selectively expressed in various types of human neoplasms but not in normal tissues other than testis. This characteristic feature of CTA makes them promising targets for cancer-specific immunotherapy. Brother of Regulator Imprinted Sites (BORIS), a novel CTA located on chromosome 20q13.2, is involved in epigenetic re-programming. The aims of this study were to determine the frequency of aberrant expression of BORIS in epithelial ovarian cancer (EOC) and examine its correlation with clinical outcome. **Methods:** One step RT-PCR was performed with RNA from various normal and 94 EOC tumor tissues obtained from 1995 to 2002. A 270bp BORIS PCR product was amplified using specific sense 5'-CAGGCCCTACAAGTGTAACGACTGCAA and antisense 5'-GCATTCGTAAGGCTTCTCACCTGAGTG primers. Glyceraldehyde-3-phosphodehydrogenase (GAPDH) specific PCR amplification was used as control The Chi square test was used to analyze the distribution of BORIS expression and clinical outcome. Survival distributions were calculated by the Kaplan-Meier method and statistical significance was determined with the log-rank test. **Results:** Aberrant expression of BORIS was present in (63/ 94) 67% of EOC primary tumors, and undetectable in other normal tissues including brain, heart, kidney, liver, lung, skeletal muscle, spleen, uterus. The normal ovarian surface epithelial cell lines, IOSE and HOSE were negative for BORIS while 3/4 (75%) ovarian cancer cell lines (OVCAR3, OVCA432, SKOV3) were positive. The median follow up of the patient population was 25 months (range 0.5-119). Tumor expression of BORIS did not correlate with stage, grade or histology. The median survival for BORIS positive patients was 49 months (CI:35,64) compared with 40 months (CI:26,54) for BORIS negative patients (not significant). **Conclusion:** BORIS is a novel CTA with high frequency expression in EOC. Although the expression of this antigen does not appear to influence survival, the tissue restricted expression pattern, role in epigenetic re-programming, and potential immunogenicity makes BORIS an attractive target for antigen-specific immunotherapy in EOC. The characterization of additional CTA with a frequent expression in EOC is warranted to allow for the development of polyvalent vaccines.

Increased IGF1 signaling contributes to paclitaxol resistance in ovarian cancer cells

Ting Chen,[1] Christopher Taylor[2]

[1] Georgetown University, Dept. of CellBio
[2] Georgetown University

Introduction: A common complication of many cancers in general and ovarian cancer in particular is the development of drug resistance. We have developed both mouse and human paclitaxel resistant cell lines (ID8-TaxR cells and CaOV3-TaxR cells) by continual exposure to increasing sub-lethal concentrations of paclitaxel. Src inhibition effectively re-sensitizes the paclitaxel resistant cells. This effect appears to be MDR1 independent, although MDR1 is over-expressed in ID8-TaxR cells and CaOV3-TaxR cells relative to parent paclitaxel sensitive cells. The goal of present study is to identify signals involved with Src re-sensitization of drug-resistant ovarian cancer cells. **Methods:** Cell lysates extracted from ID8 cells, ID8-TaxR cells and PP2 pre-treated ID8 TaxR cells were subjected to gene microarray analysis. Reverse transcription PCR and immunoblot analysis were performed to confirm gene microarray results. Cytotoxicity of different reagents was determined 48 hours later by direct cell counts. Signal transduction pathway involved was determined by luciferase assay, immunoblot analysis and co-immunoprecipitation. **Results:** IGF1 gene level and mRNA level significantly increase in ID8-TaxR cells compared to ID8 cells, while Src inhibition decreases this signal in ID8-TaxR cells. Contrarily, IGFBP4 gene level and mRNA level decrease in ID8-TaxR cells compared to ID8 cells. Stat1 gene level and protein level dramatically increase in ID8-TaxR cells and CaOV3-TaxR cells relative to parent sensitive cells, respectively. IGF1 promotes cell survival under paclitaxel treatment, while IGFBP4 suppresses the survival of ID8-TaxR cells treated with paclitaxel. IGFBP4 and paclitaxel combinational treatment causes significant caspase 3 processing in ID8-TaxR cells, while IGF1 decreases paclitaxel-induced caspase 3 processing in ID8 cells. Interestingly, caspase 3 processing is also increased in dominant negative Stat1 transfected ID8-TaxR cells under paclitaxel treatment compared to EGFP transfected ID8-TaxR cells with same treatment. Luciferase assay shows that IGF1 can up-regulate Stat1 activity, while addition of PP2 returns Stat1 activity to basal level. Moreover, either IGFBP4 or PP2 alone can decrease Stat1 basal activity. There is direct protein-protein interaction between Src and Stat1, and this interaction is decreased by Src inhibition. **Conclusions:** IGF1/Stat1 signal promotes drug resistance in ovarian cancer cells. Src inhibition probably can decrease Stat1 activity via down-regulating IGF1 level. But it is also possible that Src can directly regulate Stat1 activity. Re-sensitization of ovarian cancer cells by Src tyrosine kinase inhibition provides an effective strategy for the treatment of disseminated and recurrent drug resistant ovarian cancer.

The immune response of an ovarian carcinoma anti-idiotype minibody *in vitro* and *in vivo*

Zhu Honglan,[1] Li Yi,[2] Cui Heng,[2] Chang Xiaohong[2]

[1] Peking University People's Hospital
[2] Gynecologic Oncology Center, People's Hospital, Peking University

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Introduction: An anti-idiotypic 6B11 minibody with optimal antigenicity mimicking ovarian cancer antigen was investigated for the pre-clinical research in vitro and in vivo. **Methods:** Using gene engineering technique, prokaryotic expression vector was constructed by genetic fusion of 6B11scFv to human IgG1 hinge and CH3 region, and was named 6B11 minibody, which can mimic human ovarian cancer associated antigen OC166-9. Six groups (n=6) BALB/c mice were immunized with 150ug/100ug/50ug of 6B11 minibody or mouse IgG, human IgG and PBS every two weeks for three times. Indirect ELISA and inhibition ELISA tests were used to analyze the characterization of anti-anti-idiotypic antibody (Ab3). Boost after Ab3 declined and the mice were sacrificed after Ab3 elevated again. The spleen cells were used as effect cells and SKOV3 cells (human ovarian cancer cell line which is OC166-9 positive) as target ones, ADCC and CDC were measured using 51Cr-release assay. Twenty human-PBL-SCID mice bearing ip SKOV3.ip1 cells (subline of SKOV3 which leads to ascites) were divided into two groups (10 per group), 10 mice immunized repeatedly with 6B11 minibody. The latent period of ascites growth and the mean survival time were observed, respectively. CD4+ and CD8+ T cells from the spleen of immunized mice were assayed by flow cytometry. **Results:** In BALB/c mice, the Ab3 level gradually elevated and peaked 1 week after the second booster and maintained at high level for about 6 weeks then declined dramatically at 7 weeks after the second booster. Ab3 level was highest in 100ug group, but no significant difference. In the human-PBL-SCID mice model, the ratio of CD4+/CD8+ was highest at the 13th day after last vaccination. The latent period of ascites growth was (37.7±5.5) days in control group, and (48.6±14.3) days in minibody treated group (P=0.04); whereas the mean survival time was (42.5±1.8) days in the control group and (59.4±16.8) days in the minibody group (P=0.011), respectively. **Conclusion:** Antigenicity of 6B11 minibody was increased without adjuvants and partial humanization was realized. Minibody can induce humoral and cellular anti-idiotypic immune responses against ovarian carcinoma in vitro and in vivo. The ascites formation was delayed or prevented and the survival was prolonged in minibody treated group. We suggested that 6B11 minibody may be used as tumor vaccine to ovarian carcinoma in the future clinical trails.

Chemotherapy-induced serine protease HtrA1 associates with microtubules and induces cell death by targeted-disruption of microtubules

Jeremy Chien,[1] Aletti Giovanni,[1] Ravi Shridhar,[2] Robert Crowl,[3] Keith Bible,[1] Lynn Hartmann,[1] Scott Kaufmann,[1] Viji Shridhar[1]

[1] Mayo College of Medicine
[2] Wayne State University
[3] Novartis Pharmaceuticals

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Introduction: Despite optimal surgery and systemic chemotherapy, usually with a taxane and a platinum agent, the vast majority of patients with disseminated (stage III or greater) ovarian cancer die of their disease due to re-emergence of drug resistant disease. This observation highlights the need for improved understanding of drug resistance in ovarian cancer. In this report we have investigated a novel mechanism by which the serine protease HtrA1 participates in cisplatin- and paclitaxel-induced cytotoxicity. **Methods:** We investigated HtrA1 association with tubulin by immunohistochemistry, co-sedimentation and immunoprecipitation analyses. The ability of HtrA1 specifically to induce apoptosis in response to cisplatin and taxol treatment was determined with siRNA mediated downregulation, colony formation and apoptosis assays. Finally, using Tissue Microarray (TMA) containing 60 primary ovarian tumors, we determined whether expression of HtrA1 correlated with chemosensitivity. **Results:** We showed that HtrA1 associates with microtubules. Furthermore, it co-fractionates and co-immunoprecipitates with microtubules. We have shown that HtrA1 is upregulated and activated by drug treatment and participates in chemotherapy-induced cytotoxicity by targeting the proteolytic degradation of tubulins during cell death. This upregulation results in limited auto-proteolysis and activation of HtrA1. Active HtrA1 associates with microtubules (MTs), targets tubulins for degradation, and contributes to chemotherapy-induced cell death. Accordingly, down-regulation of HtrA1 attenuates cisplatin and paclitaxel cytotoxicity while forced expression of HtrA1 enhances cytotoxicity. Finally, patients with tumors expressing high levels of HtrA1 show 90% response rate (27/30), whereas those with weak/moderate levels of HtrA1 expression show 63% response rate (19/30) ($p < 0.015$, Pearson's Chi-square test). **Conclusion:** We have identified tubulin as a substrate of HtrA1 and uncovered a novel mechanism by which this serine protease mediates programmed cell death. These findings uncover a novel mechanism by which HtrA1 mediates paclitaxel- and cisplatin-induced cytotoxicity. More importantly our analysis of HtrA1 expression on a TMA indicates that high levels of HtrA1 expression significantly correlate with better therapeutic response to chemotherapy. These results suggest that loss of HtrA1 in ovarian cancer may have prognostic significance in the effectiveness of chemotherapy.

Antiproliferative Effects of Phenethyl Isothiocyanate Against Ovarian Cancer Cells via Inhibition of Epidermal Growth Factor Receptor

Sivakumar Loganathan,[1] Ruifen Zhang,[1] Ian Humphreys,[2] Sanjay K. Srivastava[1]

[1] University of Pittsburgh Cancer Institute
[2] University of Pittsburgh

Ovarian cancer is one of the leading causes of gynecologic cancer-related deaths among women of all ages in the United States. The cause of ovarian cancer is not clear and is often detected at an advanced stage. The overall prognosis of ovarian cancer is very poor despite significant advances in surgical and therapeutic management. Genetic alterations including overexpression of oncogenic epidermal growth factor receptor (EGFR) (~60% of ovarian tumors) may play a crucial role in the proliferation and cell survival of these cancer cells. Therefore, EGFR family of receptor tyrosine kinases (EGFR or ErbB-1 and ErbB-2) represents useful targets for novel anticancer therapeutics. The contribution of diet and nutrition status to cancer risk and conversely to the prevention and treatment of cancer has been a major focus of research as well as public health policy. Several recent epidemiological studies have clearly suggested that higher consumption of cruciferous vegetables reduces the risk of ovarian cancer. Our present studies demonstrate that phenethyl isothiocyanate (PEITC), a constituent of cruciferous vegetables such as broccoli, significantly inhibits the proliferation of NIH-OVCAR human ovarian cancer cells in culture with an IC50 of about 20 μM (concentration that may be achieved clinically through dietary intake of cruciferous vegetables) and induces apoptosis in these cells. Exposure of NIH-OVCAR cells to PEITC for 24h resulted in significant inhibition of the protein expression of EGFR, ErbB-2, as well as the phosphorylation of Akt at serine 473, in a dose-dependent manner. In addition, PEITC treatment also causes down-regulation of the anti-apoptotic protein Bcl-2. Furthermore, apoptosis induced by PEITC in these cells was associated with significant cleavage of caspase-3 and PARP. Taken together, our results demonstrate for the first time that PEITC exerts antiproliferative activity against human ovarian cancer cells by targeting the EGFR/ErbB-2/AKT pathway. Based on our findings, it is logical to speculate that PEITC will find its place as novel dietary agent for the prevention and/or treatment of ovarian cancer.

Patient as Researcher: A Unique Perspective on Quality of Life After a Diagnosis of Ovarian Cancer

Cyndee DePastino,[1] Heidi Donovan[2]

[1] National Ovarian Cancer Coalition Pittsburgh Division
[2] University of Pittsburgh School of Nursing

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Introduction: There are multiple studies evaluating the physical,functional, emotional, and social consequences of a diagnosis of ovarian cancer. This study presents a unique perspective, however, in that it was conducted as an undergraduate thesis project while the researcher was undergoing chemotherapy for a new diagnosis of Stage IIIC ovarian cancer. The purpose of this report is to present the quantitative findings of the study integrated with the qualitative experience of conducting research while undergoing the same experience as study participants. **Methods:** In order to evaluate the quality of life of women with ovarian cancer, 36 women were recruited from an out-patient gynecologic oncology clinic and an ovarian cancer support group. Women completed the Functional Assessment of Cancer Therapy – Ovarian (FACT-O), a valid, reliable, multi-dimensional assessment of Health-Related Quality of Life. **Results:** Quantitative findings were similar to those found in the literature. As a group, participants were maintaining a surprisingly high quality of life after a diagnosis of ovarian cancer. However, there was a subset of women who scored below the midpoint on the FACT-O subscale scores indicating marked impairments in quality of life. These prevalence rates ranged from 8% of women for social well-being to 19% for emotional well-being. The principal investigator will reflect on her dual roles as researcher and cancer patient to discuss how quantitative assessments of quality of life can and cannot capture the essence of quality of life after a diagnosis of ovarian cancer. **Conclusions:** This integrative report from the perspective of patient as researcher can inform others conducting quality of life research in ovarian cancer.

Evolution of a Statewide Ovarian Cancer Public Policy Agenda

Karen Greendale,[1] Elizabeth Berberian,[2] Carol Leslie Brown,[3] Maureen Killackey,[4] David Hoffman[2]

[1] Bureau of Chronic Disease Services, NYS Department of Health
[2] New York State Department of Health
[3] Memorial Sloan-Kettering Cancer Center
[4] Mary Imogene Bassett Hospital

Introduction: Ovarian cancer (OC) is a significant health problem in New York State (NYS) with more than 1600 women diagnosed and nearly 1000 deaths annually. In 1996, NYS Public Health Law was amended to create the NYS Ovarian Cancer Program, the first in the nation, along with an Advisory Council to provide expert guidance. The program's mission has evolved from its original focus on public and provider education to include a wide array of activities with significant public policy implications. **Methods:** NYSDOH has utilized a multifaceted approach to raise the level of awareness about OC around NYS. The state has mobilized a diverse group of survivors, advocates, clinicians and researchers to inform its response to OC as a public health issue. The program has generated an active dialogue via an electronic list-serve and annual meetings of key stakeholders. Strategies have included involvement of the Lieutenant Governor in program events; annual designation of Ovarian Cancer Month by Governor Pataki; creation of NYS OC Quilts to personalize the disease's impact; funding of community-based educational programs; inclusion of a letter from the Commissioner of Health in a mailing to providers across NYS; collaboration with the statewide cancer screening program in efforts to reach underserved women; and inclusion of OC in the strategic NYS Comprehensive Cancer Control Plan. With improved survival in patients surgically managed and treated by gynecologic oncologists well documented, the program is now collaborating with the SUNY Albany Center for Health Workforce Studies to study distribution of these sub-specialists and access to their services across the state. **Results:** The program's 14 pilot projects have demonstrated the success of a community-based approach, serving large numbers of rural, urban, minority and underserved women. The program has been able to facilitate collaboration among a diverse group of stakeholders with a common goal. **Conclusions:** This multi-faceted program has generated national interest and can serve as a model for other states considering public health approaches to OC. A small program with limited funds can expand its reach by working with other stakeholders. While a state can serve as a convener, program partners can advocate in ways that state agencies cannot. Survivorship issues can be addressed in conjunction with more traditional public health messages focusing on prevention of OC.