Poster Presentations (Sun, 25 Sep, 09:30−12:00)
Gynaecological Cancer

8008 POSTER

The Immunomodulatory Role of Endogenous Glucocorticoids in Ovarian Cancer

A.A. Seida[1], S.H. Sebastian Häusler[1], C.H. Claudia Heidbrink[1], J.W. Jörg Wischhusen[1]. [1]*Universitäts-Frauenklinik Würzburg, Department of Obstetrics and Gynecology Junior Research Group Tumour Progression and Immune Escape, Wuerzburg, Germany*

Background: Tumour-infiltrating myeloid-derived suppressor cells (MDSC) or tumour-associated macrophages (TAM) which are abundant in ovarian cancer show a high expression of the enzyme 11Beta-Hydroxysteroid dehydrogenase I (11b-HSD1). This enzyme is essential for the conversion of biologically inactive cortisone into active cortisol which has been detected in ascitic fluid and tumour exudates from ovarian cancer patients. Considering that cortisol has strong suppressive effects on all kinds of immune cells, we hypothesize that the activation of endogenous glucocorticoids by MDSC or TAM may contribute to the immune escape of ovarian cancer.
Material and Methods: Using immunohistochemistry, real-time PCR, luminescent immunoassays (LIA), Immunofluorescent double staining and adoptive transfer of glucocorticoid receptor knock out immune cells into immune deficient mouse model for ovrain cancer.
Results: We found that 11b-HSD1 enzyme is highly expressed in human and murine ovarian cancer tissue. Luminescent immunoassays (LIA) showed elevated cortisol levels in serum, ascites and tissue exudates from ovarian cancer patients as compared to healthy controls. Immunofluorescent double staining revealed a co-localization of 11bHSD1 with CD14, CD68, and CD85, but not with EpCAM. Expression of 11b-HSD1 can thus be attributed to tumour associated macrophages (TAM) or myeloid derived suppressor cells (MDSC). To test our hypothesis about activation of endogenous glucocorticoids by immune cells may contribute to the immune escape of ovarian cancer is now being tested in PTENloxP/loxP; loxP-Stop-loxP-krasG12D mice which spontaneously develop ovarian cancer after intra-bursal injection of adenoviral Cre recombinase. The ongoing experiments involve adoptive transfer of glucocorticoid receptor knock out immune cells as well as pharmacological inhibition of 11b-HSD1 which shall be combined with various immune stimuli. In a first functional in vivo assay, the adoptive transfer of glucocorticoid receptor-deficient T cells led to increased immune cell infiltration of the tumour tissue − which did not translate into prolonged survival. Instead, infiltrating T cells assumed mostly a Foxp3+ (regulatory) phenotype and survival was even shortened.
Conclusion: We thus propose that endogenous glucocorticoids exert immunomodulatory functions in ovarian cancer. Their putative role in tumour immune escape, however, needs to be assessed in context of further tolerogenic mechanisms that may be simultaneously present.

8009 POSTER

The Determination of the Gene Amplification for Human Telomerase (hTERC) in Cervical Intraepithelial Neoplasia and Cervical Carcinoma in the Czech Republic

L. Moukova[1], P. Kuglik[2], V. Vranova[2], I. Slamova[2], M. Kissova[2]. [1]*Masaryk Memorial Cancer Institute, Department of Gynaecological Oncology, Brno, Czech Republic;* [2]*Masaryk University Faculty of Sciences Institute of Experimental Biology, Laboratory of Molecular, Brno, Czech Republic*

Background and Aims: The incidence of carcinoma of the uterine cervix is 20/100 000 women in the Czech Republic. It is known that carcinoma are characterized by series of cytogenetic abnormalities. Gain of hTERC (3q26) was found in cervical carcinoma and cervical intraepithelial neoplasia. The amplification can be a predictive factor of malignant transformation and progression from precancers to cervical carcinoma.
Methods: From 2007 we studied copy number changes of the chromosomal region of hTERC gene by interphase fluorescent in situ hybridization (FISH) in cytological smears. From 2009 we have been using a new DNA probe for simultaneous identification of HPV infection and examination of copy number changes of hTERC.
Results: 35 women are currently in our file with carcinoma of the uterine cervix, 4 women with cervical carcinoma in situ, 21 women with cervical intraepithelial neoplasia (11 − CIN III, 7 − CIN II, 3 − CIN I). Gain of hTERC gene was found in 53% patients. Patients with lymph nodal metastases had proven the amplification in 75%. Positive amplification was detected only in cervical intraepithelial neoplasia CIN III. New DNA probe for simultaneous identification of HPV was used for more accurate detection.
Conclusions: The results of genetic analysis could select patients with high risk of progression from precarcinoma to carcinoma of the uterine cervix. Patients with gain of hTERC can have more aggresive therapy in the future.
The pilot project is rising by support of Experimental intention MZ0MOU2008 and IGA research grant project NT 11089−4/2010.

8010 POSTER

Visual Inspection of Cervix With Acetic Acid, a Low Cost Tool

M. Jain[1]. [1]*Disha Fertility and Surgical Center, Obst. and Gynaecology, Indore, India*

Organized cervical cytology screening programs are not feasible in many developing countries like us India where cervical carcinoma is most common cancer in women. Evaluation of visual inspection of the cervix with acetic acid (VIA) for screening cervical intraepithelial neoplasia is a low cost method. We compared visual inspection of the cervix after application of 3−4% acetic acid with cytology as methods for the detection of cervical carcinoma and its precursors.
Three hundred ten women were screened using pap smear, visual inspection with acetic acid (VIA) and colposcopy in a one year duration. In positive case with any of screening methods underwent large loop excision of suspected zone. Sensitivity and specificity of different screening methods was analyzed.
The sensitivity of VIA (92%) was much higher than that of pap smear (68%), and a higher colpo scopy (99%). The specificity of VIA (45%) was lower than pap smear (96%) and colposcopy (99%).
Results indicate that VIA and cytology had similar performance in detecting moderate dysplasia or more severe lesions in this study. VIA merits further evaluation as a primary screening test in low-resource settings with added advantage of ease to use and it immediate result. Main limitation of VIA is a high rate of false positive result.

8011 POSTER

Self-production of CCL5 in Ovarian Cancer Stem Cells Contributes to Its Invasion and Migration Through NF-κB-mediated MMP-9 Upregulation

B. Zhu[1], H.X. Long[1], R.K. Xie[1], T. Xiang[1], S. Lin[1]. [1]*Xinqiao Hospital Third Military Medical Univisity, Institute of Cancer, Chongqing, China*

Background: The concept of cancer stem cells (CSCs) propose that solely CSCs are capable of generating tumour metastases. However, how CSCs maintain the ability of invasion and migration, the most important properties of metastatic cells, still remains to be determined. The present study is aimed to elucidate the underlying mechanisms of metastasis for CSCs.
Material and Methods: Ovarian cancer stem cells were obtained from A2780 cell line by serum-free culture selection and from primary tumour tissues of ovarian cancer by CD133-labeled maganetic activated cell sorting. Cancer stem cells were identified by stem cell surface marker (CD133), stem cell molecular marker (OCT-4, Nanog, etc), self-renewal, tumorigenesis in NOD/SCID mice. The difference of chemokines and receptors expression between CSCs (A2780-derived CD133-positive cells) and non-CSCs (A2780-derived CD133-negative cells) were determined by human inflammation-related factots RT^2 Profiler™ PCR Array, and then verified by real-time PCR, ELISA and flow cytometric analysis in primary ovarian cancer stem cells. Transwell experiments were used to determine the ability of invasion and migration of CSCs before and after blocking at different concentration of anti-CCL5, CCR1, CCR3, CCR5 antibody and NF-κB inhibitor. MMP-9 expression in CSCs were tested with the use of real-time PCR before and after inhibition of NF-κB.
Results: We found that human ovarian cancer cell line and cancer tissue contains cancer stem cells denied by CD133 expression that displayed stem cell properties in vitro and highly tumorigenic in vivo. The PCR-array data demonstrated that the expression of chemokines ligands (CCL2, CCL5, CLL27, CXCL16, etc.) and chemokine receptors (CCR1, CCR3, CCR5, CXCR4, CCR7, etc) are much stronger compared to non-CSCs. These upregultaed genes were successfully verified by real-time PCR, ELISA for chemokine ligands, flow cytometric analysis for chemokine receptors in primary ovarian cancer stem cells from five patients. Especially, the results of CCL5 and it's receptors (CCR1, CCR3 and CCR5) expression were consistent among five patients. The invasion and migration of CSCs could be blocked by anti-CCL5, CCR1 or CCR3, but not CCR5 blocking antibody, and indicated a dose-denpendent manner. Interestingly, we found the down-regulation of MMP-9 and NF-κB activity decrease in CSCs when blocking CCL5, CCR1 or CCR3, which is consistent to their effect on invasion and migration. Furthermore, NF-κB inhibitor not only significantly decreased the migration and invasion of CSCs, but also down-regulated MMP-9 expression in CSCs.
Conclusions: Our results suggest that the binding of self-production of CCL5 with CCR1 and CCR3 on ovarian cancer stem cells leading to NF-κB activation and MMP-9 upregulation is the main mechanism of metastatic property of ovarian cancer stem cells.