Experimental Pathology/Biomarkers

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TGF-β1 but not VEGF expression is regulated at the protein level in endometrial cancer

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Vascular endothelial growth factor (VEGF) is the major stimulus for endothelial cell proliferation in endometrial carcinomas and is, therefore, associated with high angiogenesis. The role of transforming growth factor-β1 (TGF-β1) in angiogenesis and cancer development is complex, and involves aspects of tumor suppression at the initial steps of oncogenesis as well as tumor promotion as tumors evolve. In the present study we evaluated the mRNA expression pattern of VEGF and TGF-β1 by Real-Time PCR in tissue samples of 20 patients with endometrial cancer, 4 patients with complex atypical endometrial hyperplasia (AEH) and adjacent normal tissues of all patients. Western blot analysis was performed to evaluate VEGF and TGF-β1 protein levels. Transcript levels of VEGF were found to be significantly elevated in 25% of AEH cases and in 35% of endometrial cancer cases. VEGF mRNA underexpression was observed in 25% and 5% of AEH and cancer cases respectively. VEGF protein levels correlated with mRNA levels in most cases. TGF-β1 transcript levels did not differ between pathological and adjacent normal tissues, except for one case of AEH (25%) and one case of endometrial cancer (5%) where downregulation was observed. Interestingly, TGF-β1 protein levels were substantially lower in tumor samples compared to controls in 50% of AEH and 43% of cancer cases respectively. TGF-β1 protein expression was not detected in 50% of AEH and 29% of cancer tissues while it was detectable in the adjacent normal tissues. 21% of endometrial cancer tissue-pairs were found not to express TGF-β1 protein at all. Overexpression of TGF-β1 protein in the malignant compared to the adjacent normal tissue was observed in one case of advanced endometrial cancer, supporting the hypothesis of TGF-β1's tumorigenic role as tumors evolve. Post-transcriptional mechanisms seem to control TGF-β1 expression in endometrial cancer according to our findings.

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Evaluation of VEGF and TGFB1 mRNA expression profiles as markers of malignant transformation in cytological cervical specimens

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Tumor angiogenesis has been described in almost all human cancer types comprising malignancies of the female genital tract. Based on previous findings (Soufla G, et al., Cancer letters 221: 105-11, 2005) indicating the correlation between VEGF, TGFB1 and TGFBR1 mRNA expression levels in cervical tissues with the malignant transformation of the uterine cervix,we investigated whether similar expression profiles of the above genes could also be detected in cytological cervical specimens obtained during a PAP test examination. Furthermore, we examined whether the altered mRNA patterns of the above angiogenic markers could reflect an early prognostic significance for the clinical progression of the disease. Transcript levels were assessed by real-time PCR analysis in 20 cytological cervical samples with cervical intraepithelial neoplasia (CIN) and cervical cancer, compared to that of normal cervical specimens (n=20)and correlated with the clinical stage of the disease. Our findings were in accordance with the findings obtained by using cervical tissues. A highly significant increase of VEGF mRNA expression was found upon cervical neoplastic transformation (P=0.008). Low-grade squamous intraepithelial lesions as well as high-grade lesions and CA samples exhibited higher VEGF mRNA levels compared to normal specimen group (P=0.038, 0.036, 0.03, respectively), however no significant increase in mRNA expression was observed with increased severity of the lesion. In contrast, TGFB1 transcript levels were found significantly elevated only in CIN specimen group compared to the normal (P=0.008), whereas the CA samples didn't reveal considerable differences in terms of TGFB1 expression compared to normal and CIN groups. This lack of association could be attributed to the overexpression of Ying Yang 1, a negative regulator of TGFB1 transcription during tumor progression; however the significance of this inhibition should further be investigated. Summarizing, disruption of expression patterns of the factors included in the study, in the CIN and CA specimen groups compared to controls, suggests a transcriptional dysregulation during cervical cancer development, which can easily and untimely be detected in cytological cervical material obtained during a routine PAP test examination. However, additional studies are needed to elucidate the potential use of mRNA expression profiles of the above angiogenetic factors as progression indicators in cervical carcinogenesis.

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Angiotensin converting enzyme inhibitors decrease the incidence of pancreatic cancer: a study of half a million US veterans

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AIM: To investigate the effect of Angiotensin Converting Enzyme (ACE) Inhibitors use in reducing the incidence of pancreatic cancer in the US veteran population. BACKGROUND: ACE Inhibitors are commonly used antihypertensive and nephroprotective agents. Vascular Endothelial Growth Factor (VEGF) is believed to play a major role in angiogenesis in human tumors. Blocking the VEGF inhibits angiogenesis and suppresses tumor growth. ACE inhibitors cause suppression of VEGF in experimental models, leading to their anticancer effect. ACE Inhibitors have been noted to suppress tumor growth by inhibiting tumor angiogenesis in several animal and experimental models. METHOD: US Veterans Health Administration

(VHA) is organized into 21 administrative regions called Veterans Integrated Service Networks (VISN). VISN 16 provides health care treatment to >1.4 million veterans in an eight state region in south central United States. The network, an integrated health care system, includes ten medical centers, 33 community-based outpatient clinics, seven nursing homes, and two domiciliary. The data was queried from Oct 1998 to June 2004, using a retrospective case control design. Statistical analysis was performed using SAS software version 9.0 (Chicago, IL). Multiple logistic regression analysis was used with calculation of odds ratios and 95% confidence intervals. The data was adjusted for age, race, gender, BMI, smoking, alcohol use, diabetes and statin use. Patients were placed in the ACE inhibitor user group if they were using ACE inhibitors prior to the diagnosis of pancreatic cancer. RESULTS: A total of 483,733 patients were included in the analysis. 185,852 (38.43%) of those were using ACE inhibitors. Pancreatic cancer (ICD-9 code 157) was seen in 475 (0.1%). ACE inhibitor users were 48% less likely to develop pancreatic cancer (Odds ratio 0.484; 95% CI 0.386-0.607, $p < 0.01$). The dose, duration and type of ACE inhibitor used were not factored into the analysis. The protective effect of ACE inhibitors was independent of statin use.

Conclusion: ACE inhibitors are associated with a 48% reduced incidence of pancreatic cancer. The limitations of our data are the Veteran population, the database and the fact that this is a case control study.

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Apoptosis induction by sulforaphane is a consequence of G2/M cell cycle arrest in cultured 40-16 human colon carcinoma cells

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Sulforaphane (SFN) [CH3S(O)(CH2)4-N=C=S)] is a naturally occurring cancer chemopreventive isothiocyanate found as its glucosinolate precursor in cruciferous vegetables like broccoli. Apart from its ability to modulate carcinogen metabolism SFN also acts by antiproliferative and apoptosis-inducing activities. As an example, treatment of the human colon carcinoma cell line 40-16 with SFN at a 15 μM concentration led to the cleavage of PARP [poly (ADP-ribose) polymerase] as a marker of apoptosis induction, mediated by the activation of caspase-3, -7, -8 and -9, detected by Western Blotting experiments. To analyze whether cytotoxic activity was accompanied by inhibitory effects on cell cycle progression, unsynchronized SFN-treated 40-16 cells were stained with propidium iodide and analyzed by flow cytometry. Treatment with 15 μM SFN for 12 h induced a marked G2/M phase arrest, which persisted until 24 h. After 48 h incubation, a sub-G1 peak was detected, indicating apoptosis induction. To further characterize time-dependent cell cycle changes by SFN we performed kinetic experiments. After a pre-incubation phase of 24 h, cells were treated with 15 μM SFN for 3, 6, 12 and 24 h, respectively, and allowed to recover with SFN-free medium for up to 12, 24 and 48 h. Treatment for 3 and 6 h resulted in a transient G2/M arrest, which was detectable after 12 h. During prolonged incubation under SFN-free conditions, the cell cycle arrest was reversible and cells recovered from SFN treatment. In contrast, incubation with SFN for 12 h led to an irreversible G2/M phase arrest after 24 h and apoptosis induction after 48 h. In order to investigate whether activation of the caspase cascade is essential for the induction of apoptosis by SFN in 40-16 cells, we treated the cells simultaneously with 15 μM SFN and the pan caspase inhibitor z-VAD-fmk. Importantly, SFN-mediated apoptosis induction, detected by PARP cleavage and the occurrence of a sub-G1 peak in flow cytometry after 48 h, was completely inhibited by co-treatment with z-VAD-fmk. Instead, a marked G2/M arrest could be observed. Based on these data we conclude that the primary antiproliferative mechanism of SFN is a cell cycle arrest in G2/M, which is reversible after short term incubation for 3 to 6 h. After longer incubation times, the cell cycle arrest becomes irreversible and is followed by caspase-dependent apoptosis induction.

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The effect of the dyes used in sentinel lymph node biopsy localization on immunocytochemical determination of hormonal receptors in MCF-7 breast cancer cell line

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Introduction: Sentinel Lymph Node Biopsy (SLNB) is the procedure of choice for staging the axilla in breast cancer patients who are clinically and radiologically node negative. Node localization is achieved with the use of injected dye, radioactive tracer or both. The biological effect of using these materials has not been fully investigated.

Aims: To determine the effect of the added dyes (Patent Blue V(PBV), Methylene Blue (MB) and Indigocarmine (IDC)) on the immunocytochemical expression (ICC) of estrogen receptor (ERα) and progesterone receptor (PR) in monolayer cultures of breast cancer cells.

Methods: MCF-7 cells (a malignant breast cell line which is known to be positive for both ERα and PR) were cultured and treated with the above dyes. The dyes were 2.5% PBV, 1% MB and 0.4% IDC and were diluted further using RPMI-1640 media with 5% Fetal Calf serum in three dilutions, 1 in 10, 1 in 100 and 1 in 1000. The cells were treated with the dyes for 4hours and 24hours. Formalin fixed paraffin embedded blocks were prepared and ICC for both ERα and PR performed. The slides were scored blindly using the Alred quick score by two breast histopathologists.

Results: The results of the scoring showed decrease of both ERα and PR scores with higher concentration of MB dye (1 in 10 dilution of the 1%MB preparation) particularly if exposed for 24hrs. PBV and IDC did not show a significant reduction.

Conclusion: The addition of MB can interfere in vitro with the results of ER and PR receptor assessment by ICC. If the effect occurs in vivo it could lead to inappropriate antihormonal treatment ch