second in Europe and the number of new cases is rapidly growing. Due to systemic character of the disease, local recurrence or generalization are diagnosed in about 70% of patients. The individual sensitivity to anticancer drugs plays a key role in cancer therapy outcome. A large part of resistance of tumours to chemotherapy is caused by ATP-binding cassette (ABC) transporters, the ATP-dependent drug efflux pumps. The main aim of this study was to investigate expression levels of all, so far identified, human ABC transporter genes in tissue specimen from colorectal cancer patients and to follow their role in chemotherapy outcome.
Materials and Methods: Expression profile of 49 ABC transporter genes was evaluated in 19 pairs of tumour and distant unaffected mucosa tissues from patients undergoing predominantly the FOLFOX (based on 5-fluorouracil and oxaliplatin) palliative chemotherapy treatment. The analysis was performed by real-time PCR with TaqMan Gene Expression Assays. Stability of 24 reference genes was assessed and four reference genes were then used for normalization. Results were evaluated by REST2009 and SPSS programs.
Results: Significant differences in expression profiles of the examined ABC transporter genes between tumour and non-tumour tissues and between patients with remission vs. progression were observed. Significant upregulation of *ABCA12*, *ABCA13*, *ABCC1* and *ABCE1* gene expression in tumours vs. non-tumours suggested their possible role in outcome of the chemotherapy. More than 40% of ABC transporter genes were downregulated in tumours.
Conclusion: Our study suggests that ABC transporters may play an important role in outcome of colorectal cancer chemotherapy. Candidate genes will be further followed by a larger and more comprehensive study.
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6022 POSTER

Plasma Levels of Heparanase as Marker of Tumour Aggressiveness and Stage of Disease in Patients With Colorectal Cancer

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Background: Heparanase enzyme upregulation was documented in large number of tumours, including colorectal cancer. The aim of the study was to evaluate plasma heparanase level in colorectal cancer patients, as a screening tool for diagnostic and disease monitoring purposes and to examine the correlation between plasma heparanase levels and clinical and pathological parameters, such as tumour burden and response to antineoplastic treatments, in patients with colorectal cancer.
Materials and Methods: Plasma heparanase was evaluated in 92 colorectal cancer patients, that were treated and followed-up in the Department of Oncology, Rambam Health Care Campus, Haifa, Israel. The patients were divided into 3 groups, according to their tumour burden. The 1st group was comprised of 47 patients with recurrent or metastatic disease. In this group of patients blood samples were collected at the start of the treatment and at restaging procedure. The 2nd group included 27 patients without evidence of disease up to 6 months after surgery. The 3rd group included 18 patients without evidence of disease at least for two years after surgery. Plasma heparanase levels were measured by enzyme linked immunosorbent assay. Tumour heparanase expression was evaluated by immunohistochemistry in 37 patients.
Results: The median and the mean serum heparanase concentrations in the first sample of the entire population of patients were 0 pg/ml and 179.6±595.3 pg/ml, respectively. In the 1st, 2nd and 3d group of patients the mean plasma heparanase levels were 221.9±703.8 pg/ml (n-47), 28.3±102.6 pg/ml (n-27), and 295.8±696.4 pg/ml (n-18), respectively. There was a trend for higher mean serum heparanase levels among the patients with active disease (1st group) in comparison with the patients without evidence of disease (2nd + 3d group), 221.9+703.8 pg/ml and 135.3+459.5 pg/ml, respectively, ($p = 0.1$). In univariate analysis, smoking history ($p = 0.004$), lymph node sampling ($p = 0.02$), and oxaliplatin-based chemotherapy ($p = 0.007$) were independent predictors of plasma heparanase levels. A trend for higher serum heparanase concentration among the patients with metastatic disease ($p = 0.2$), and high grade tumours ($p = 0.3$) was observed, also the trend for lower plasma heparanase concentration in oligometastatic disease ($p = 0.08$) was seen. Moreover, the non-significant correlation between response to oncological treatment and plasma heparanase alterations was observed ($p = 0.18$). No correlation was observed between tumour heparanase expression and serum heparanase concentration.
Conclusions: The positive, but non-significant correlation between plasma heparanase level and tumour aggressiveness and response to oncological treatment in patients with colorectal cancer was observed. Smoking history, lymph node sampling, and oxaliplatin-based chemotherapy were independent predictors of plasma heparanase level. Larger study is required in order to validate plasma heparanase as a marker of colorectal cancer aggressiveness.

6023 POSTER

Immunohistochemical Expression of CD133 is Associated With Tumour Regression Grade After CRT in Colorectal Cancer

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Background: The Cancer stem cell (CSC) model suggests that CSCs are involved in tumorigenesis, metastasis, resistance to treatment and poor prognosis. CD133 has been identified as a putative CSC marker in various cancers including colorectal cancer. We investigated the relationship between CD133 expression and the clinicopathological features as well as the survival of patients with colorectal cancer and those with rectal cancer after preoperative chemoradiotherapy (CRT).
Material and Methods: The expression of CD133 was immunohistochemically evaluated on surgical specimens of 225 patients with colorectal cancer who underwent curative resection as well as 78 patients with rectal cancer who received preoperative CRT followed by curative resection. The latter patients received 50.4 Gy irradiation with oral administration of the prodrug of 5-FU and leucovorin during the entire course of radiotherapy. Expression of CD133 was defined as positive when CD133 staining was found in more than 5% of the entire of the tumour. The correlation between the CD133 expression and the clinicopathological features, tumour recurrence as well as the overall survival was analyzed.
Result: Among the 225 colorectal cancer patients, 93(41.3%) were positive for CD133 expression. However, CD133 was positive in 47 (60.3%) of 78 cases receiving CRT, which was significantly higher than non-CRT specimens ($p = 0.05$). Positive expression of CD133 significantly correlated with the histological tumour regression grade ($p < 0.01$). By multivariate analysis, CD133 expression remained as the most important factor associated with the tumour regression grade ($p < 0.01$) in cases with CRT. However, CD133 expression was not significantly associated with either the recurrence-free or the overall survival in both groups.
Conclusions: CD133 expression may be one of the key factors associated with resistance to chemoradiotherapy in colorectal cancer.

6024 POSTER

E2F2 Transcription Factor as a Possible Genomic Marker in Colon Cancer Initiation/Progression: Impact of Its Altered Expression on a Human Colon Cancer Cell Line

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Background: In order to identify molecular markers pronostic of initiation and/or progression of human colon cancer (CC), a genome-wide analysis was perfomed and highlighted a micro-deletion at the 1p36.11−12 region in 23% (n = 115) and 47% (n = 59) of adenomas and carcinomas, respectively. Within the micro-deleted region, a potential target gene, E2F2, is described as either oncogenic or tumour suppressor, depending on the tissue or cell type. E2F2 deletion incidence depends on tumour stages (60% in early stages whereas only 34% in metastatic stages of distal CC) and further clinical analysis showed that patients with deleted E2F2 had a lower rate of recurrence and a better overall survival. Also, RT-QPCR evidenced that E2F2 transcript expression level decreases in human CC. Thus, the aim of this study was to specify the functions of E2F2 in CC, and the impact of the E2F2 deletion in human CC process.
Material and Methods: E2F2 transcript expression was down-regulated by transitory transfection with siRNA in the human epithelial CC cell line Caco-2/TC7. Consequences were evaluated at the morphological level by immunocytochemistry for proteins involved in the cell architecture and in cell-cell and cell-matrix junctions, and at the expression level by RT-QPCR and Western Blot analyses. Functional analyses were assessed for the migratory potential with the wound healing assay, for proliferation with the MTS assay, and for adhesion on substrates such as laminin, collagen I and fibronectin.
Results: E2F2 down-regulation reduced proliferation and induced severe morphological modifications, associated with relocalization of structural members of adherens junctions (beta-catenin, APC), tight junctions (Claudin-1, ZO-1) and cytoskeleton (F-Actin, Cytokeratin-19). The integrins alpha5, alphaV, alpha2 and beta-1, were downregulated and the adhesion properties on laminin-111, but not on collagen I or fibronectin were lost. More interestingly, inhibition of E2F2 expression leads to a decrease of