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

the migration potential. Additionnaly, the paxillin, a central protein of focal adhesion contact points, is highly downregulated when E2F2 is inhibited.
Conclusions: This study showed that inhibition of E2F2 gene expression leads to morphological rearrangements and the proliferation and migration potentials are reduced. These effects could result from a reduced expression of integrins and paxillin which are structural compounds of focal adhesion contributing to cell adhesion and motility. These results will be comforted in *in vivo* xenograft experiments to ascertain the good prognostic value of E2F2 deletion and strengthen the hypothesis that E2F2 expression deregulation could play a key role in human colon tumour initiation/progression but not dissemination.

6025 POSTER

Association Between ESR1 and ESR2 Polymorphisms and Risk of Colorectal Cancer in Chinese Han Population

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Background: Epidemiologic and biologic evidence suggests that estrogen may play an important role in pathological progress of colorectal cancer (CRC) and lung cancer. As the action of estrogens is regulated by the estrogen receptor (ESR), the objective of this study is to investigate whether variants *ESR1* and *ESR2* genes confer genetic risk to CRC and lung cancer, and different genetic effect between males and females in the Chinese Han population.
Material and Methods: Two SNPs rs2234693, rs9340799 in *ESR1* and two SNPs rs1256049, rs4986938 in *ESR2* were genotyped. For CRC, two independent studies including 331 cases and 378 cases with a shared common controls with 747 subjects were enrolled. For the lung cancer, 609 patients and 700 controls were selected for analysis.
Results: The minor allele T of *ESR2* rs1256049 was associated with increased CRC risk (adjusted $P = 0.025$, $OR = 1.21$). More specifically, when cases were divided into two groups by gender, variation in the *ESR2* rs4986938 was associated with an increased risk of CRC in men (adjusted $P = 0.005$, $OR = 1.57$), but it did not contribute to the disease susceptibility in women. Lacking of association was observed between lung cancer and *ESRs*.
Conclusions: This study shows that *ESR2* rs4986938 polymorphisms may be linked with increased CRC susceptibility and furthermore, this association is gender specific. This study also indicates that *ESR2* rs1256049 confers a significant risk of CRC. Our findings further suggest a possible role of *ESR2* variants on CRC.

6026 POSTER

MDGA1 Expression and Promoter Methylation Analysis in Colorectal Cancer

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Background: Human *MDGA*1 gene encodes a Glycosylphosphatidylinositol (GPI) anchored protein containing a MAM domain (Meprin, A5 protein, receptor-protein tyrosine phosphatase m). This gene was isolated in our laboratory and genomic organization as well as gene expression patterns in normal human tissues and tumours has been reported. MDGA1 protein is a 955 aminoacids glycoprotein (37 kDa) attached to the cell membrane by a GPI anchor and localized in lipid rafts. We have also reported that MDGA1 expression increases cell motility and cell-cell adhesion and reduces adhesion to extracellular matrix proteins in MDCK cells.
In the present study we have analysed *MDGA*1 expression level and promoter methylation status of the gene in colorectal cancer. Patients and methods: Forty-three primary colorectal tumours were obtained from patients who underwent surgery at San Carlos Hospital in Madrid (Spain). As control samples, a pool of eight-ten normal tissues from colon was used. *MDGA*1 expression was analysed in all these samples by real time quantitative PCR using the TaqMan® gene expression system. For *MDGA*1 methylation analysis genomic DNA was treated with sodium bisulfite by using BisulFlash® DNA modification kit. The methylation status of *MDGA*1 was then determined by Methylation-Specific Polymerase chain reaction (MSP). Results: Our results shown a significant down regulation of *MDGA*1 gene expression, as compared to normal tissues, in 25 of the 43 colorectal tumours analysed (58%). We next analyzed the methylation status of *MDGA*1 promoter in tumour tissues to establish a potential relationship with gene expression.
Conclusion: Expression of *MDGA*1 is downregulated in human colorectal tumours. To our knowledge, no study of *MDGA*1 promoter methylation and gene expression has been reported so far.

6027 POSTER

RON is Associated With Colorectal Cancer Progression via the Inhibition of Apoptosis and Cell Cycle Arrest Through the Modulation of Akt, MAPK and β-catenin Pathways

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Background and Aims: Recepteur d'Origine nantais (RON) is associated with the induction of oncogenic properties including malignant transformation, migration and proliferation. Moreover, overexpression of RON has been observed in various human epithelial cancers. The aims of current study were to evaluate whether RON affects tumour cell behaviors and oncogenic signaling pathways in human colorectal cancers, and to examine the relationship of its expression with various clinicopathological parameters and patient survival.
Methods: To study the biological role of RON on tumour cell behavior and oncogenic signaling pathways in human colorectal cancer, we used small interfering RNAs (siRNA) to knockdown endogenous RON gene expression in human colorectal cancer cell lines, SW480 and DLD1. To study the role of RON in human colorectal cancer progression, we have used an immunohistochemical technique to localize RON protein in paraffin-embedded tissue blocks obtained from 161 colorectal cancer patients.
Results: Knockdown of RON by siRNA diminished invasion of human colorectal cancer cells. The proportion of apoptotic cells induced by transfection of RON siRNA was greater than that induced by transfection of the scramble siRNA. Knockdown of RON resulted in an arrest in the G0/G1 phase of the cell cycle. Knockdown of RON activated cleaved caspase-3, cleaved PARP and down-regulated the expression of survivin and XIAP leading to induction of apoptosis. Knockdown of RON decreased Akt and MAPK signaling proteins. Knockdown of RON blocks β-catenin activation and down-regulated c-myc and cyclin D1 gene expression. RON expression was significantly associated with lymphovascular invasion, lymph node, distant metastasis, tumour stage and poor survival.
Conclusions: These results indicate that RON is associated with human colorectal cancer progression via the inhibition of cell cycle arrest and apoptosis through the modulation of Akt, MAPK and β-catenin signaling pathways.

6028 POSTER

Mechanical Activation of Myc and Twist Oncogenes in Mouse Colon Pre-Tumoral Tissues

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Our understanding of multi-cellular tissue morphogenesis and homeostasis is being challenged by increasing evidence demonstrating the involvement of a mechano-sensitive interplay between shape-related strains and state of expression of the genome in tissues. Even though such mechanical cues have been demonstrated to be critically involved in key steps of early embryonic development *in vivo*, as well as during organogenesis, the associated primary mechano-transduction sensors and the underlying molecular mechanisms remain unknown. We show here first experimental evidence of a role of the multi-cellular tissue pressure, potentially associated to external pressure (associated to intestinal transit) or to internal pressure (associated to tumour growth), in the expression of tumour progression genes, with direct mechanical manipulation and perturbation of the tissue mimicking environmental pressure. Genetically predisposed pre-tumoral APC1638N+/− mice colon explants (Adenomatous Polyposis Coli protein, mice carrying one mutant allele APC1638N) were subjected to a mechanical deformation in a tissue compression device (1.2 mm depth for control and 0.3 mm depth for compressed). This mechanical deformation causes the Src-family kinase dependent phosphorylation of the site Y654 of interaction of the beta-catenin with E-cadherins, leading to the release of a pool of beta-catenin into the cytoplasm, which is not fully degraded due to the defect of APC expression in the APC+/− colon tissues. We observe also the nuclear translocation of beta-catenin, with activation of Twist and c-Myc target oncogenes expression. Finally, we