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

show a putative involvement of the kinase Ret (known to phosphorylate the Y654 site of beta-catenin) upstream in the pathway activated by the mechanical deformation. Treatment during compression with Sunitinib, a specific inhibitor of Ret, impairs Ret pY1062 phosphorylation, in the same way as beta-catenin nuclear translocation and Twist and c-Myc gene expression. We propose that strains associated to intestinal transit or tumour growth triggers the activation of the primary oncogene program in genetically predisposed pretumoral APC+/− mice colon tissues *ex vivo*. Nowadays our goal is to check the effect of different specific Ret kinase inhibitors on this mechano-sensitive oncogenic pathway and test its impact on tumour progression *in vivo*.

6029 POSTER

Osteopontin Enhanced Hepatic Metastasis of Colorectal Cancer Cells

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Background: Liver metastasis is a major cause of mortality from colorectal cancer (CRC). However, the underlying mechanisms are largely unknown. Osteopontin (OPN) is a secreted phosphorylated glycoprotein that is involved in tumour migration and metastasis. But the whole story of OPN relating to cancer has been far from clear to date.
Material and Methods: OPN mRNA was examined in tissues from CRC, adjacent normal mucosa and liver metastatic lesions using quantitative real-time PCR analysis. The protein expression of OPN and its receptors (integrin αv and CD44 v6) was detected with immunohistochemical (*IHC*) method. The role of OPN in liver metastasis was studied in established colon cancer Colo 205 and SW480 cells lines transfected with sense- or antisense-OPN eukaryotic expression plasmids. Florescence redistribution after photobleaching (FRAP) was used to study gap functional intercellular communication (GJIC) among OPN-transfected cells.
Results: It was found that OPN was highly expressed in metastatic hepatic lesion of CRC compared to primary CRC tissue and adjacent normal mucosa. OPN expression was also detected in normal hepatocytes surrounding CRC metastatic lesion. Two known receptors of OPN, integrin αv and CD44v6 proteins, were strongly expressed in hepatocytes of normal liver. Colon cancer cells with forced OPN expression exhibited increased heterotypic adhesion with endoepithelial cells and weakened intercellular communication.
Conclusions: OPN is playing a significant role in CRC metastasis to liver through interaction with its receptors in hepatocytes, decreased homotypic adhesion and enhanced heterotypic adhesion.

6030 POSTER

The Number of High Risk Factors is Related to Outcome in Stage II Colonic Cancer Patients

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Background: A subgroup of stage II colonic cancer patients are considered to be at high risk for recurrent/metastatic disease based on 1) tumour obstruction/perforation 2) <10 lymph nodes 3) T4 lesions and 4) lymphangio-invasion. Their prognosis is regarded as comparable to stage III colonic cancer and it is therefore strongly advised to treat them with adjuvant chemotherapy. The purpose of this study was *i)* to determine the magnitude of prognostic significance of the conventional high risk factors and *ii)* to determine whether the number of high risk factors influences outcome.
Materials and Methods. We retrospectively analyzed 212 stage II colonic cancer patients undergoing surgery between January 2002 and December 2008. No adjuvant chemotherapy was given.
Results. 154/212(73%) patients were considered to be high risk patients based on conventional high risk factors. 58 patients did not meet any high risk factor, 125 patients met 1 high risk factor and 29 patients met ⩾2 high risk factors. Median follow up was 40 months.
Multivariate analysis identified four independent risk factors for recurrent/metastatic disease: age, obstruction, perforation and lymphangio-invasion.
The three-year-DFS-rates for the low-risk group, the high-risk group with 1 high-risk-factor and the high-risk group with ⩾2 high-risk-criteria are 90.4%, 87.6% and 75.9% respectively.
Patients meeting ⩾2 conventional high risk criteria had a significantly worse three-year-disease free survival ($p < 0.002$).
Conclusions. Four independent high risk factors were identified. The number of high risk factors does influence outcome. Therefore, patients with ⩾2 high risk factors should receive adjuvant chemotherapy without any hesitation.

6031 POSTER

Combined Neoadjuvant Chemoradiotherapy With Radiosensitization Shows Good Response and Low Toxicity Rate in Locally Advanced Rectal Cancer Treatment

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Background: Resectability is a major issue for locally advanced fixed or tethered rectal cancer. The search for new ways to improve neoadjuvant treatment effect without increasing toxicity is an important research direction. The aim of this trial was to evaluate treatment response and toxicity rate after chemoradiotherapy with local hyperthermia and metronidazole as radiosensitizers.
Methods: From July 2006 to February 2011, 74 previously untreated patients were enrolled. The external dose of radiotherapy was 40 Gy given in 10 fractions 3 times per week. Oral capecitabine 650 mg/m^2 bid was given on days 1−22 and intravenous oxaliplatin 50 mg/m^2 was administered on days 3, 10, 17. Local high-frequency hyperthermia 41–45^{0}C during 60 minutes was performed on days 8, 12, 15, 17. Metronidazole 10 g/m^2 was administered per rectum on days 12 and 17. Surgery was carried out within 6−8 weeks after neoadjuvant treatment. Tumour regression was measured according to Dworak scale. Toxicity was evaluated by NCI-CTC v 3.0 criteria.
Results: Grade I-II toxic events were observed in 34 (45.9%) patients. Grade III events included diarrhea − 14.9% (n = 11), vomiting − 2.7% (n = 2) and proctitis − 2.7% (n = 2). No grade IV events were observed. Five patients (6.7%) remained inoperable. All 69 (93.3%) patients with resected tumour had R0 resection. Eight patients had grade IV regression (10.8%), 29 patients had grade III regression (39.2%).
Conclusions: Investigated treatment scheme with radiosensitization demonstrates encouraging treatment response rate, while toxicity remains comparable to standard regimens.

6032 POSTER

Colonic Tumour Localization, Clinicopathological Patterns and Incidence of Colorectal Carcinoma in Mexican Population

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Background: Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide. The aim of this study was to examine the interrelationship between the anatomical distribution of CRC by gender, age at presentation, and incidence rates for the disease in the National Cancer Institute of Mexico (INCan).
Material and Methods: A retrospective study was carried out on 403 cancer cases diagnosed in the Gastrointestinal Cancer Department of INCan, for a 6-year period (2004–2010). Data from clinical reports, computed tomography reports and surgical resection specimens were analyzed and included in a prospective database for statistical analysis. Tumours according colon-anatomy were classified as: proximal (PC), tranverse (TC), ascending (AC), descending (DC) and sigmoid colon (SC).
Results: Median age was 54 years (range 21−88 years). Distribution by gender was: 53.8% females and 46.2% males. Adenocarcinoma was the most frequent (94.9%) with moderately differentiated tumours predominantly. Twenty patients were identified as being in stage I (5%), 94 patients in stage II (23%), 132 patients in stage III (33%) 137 patients in stage IV (34%),15 patients were in recurrence (4%) and 1% (n = 5) of the patients were not classified. Colonic tumour localization was: 21.8% for PC (34%, stage I/II); 24.2% for TC (37%, stage IV), 46.6% for DC (38.2%, stage III) and 7.4% SC (53.3% stage II, III). Ninety-two (22.8%) of all cases were young patients (⩽40 years). Colonic tumour location compared with age was: young patients (⩽40 years) were mostly localized at DC (10.1%; and 31% stage IV) follow by PC (6.4%; and 46% in stage IV), and TC (4.9%). Meanwhile for patients over 40 years (77.2%), colonic tumour localizations predominantly were DC (36.4%), TC (19.1%) and AC