Data were compared with 21 pts with mCRC who have received CT alone as first-line treatment (M/F: 16/5, median age: 56 yrs) and with 40 healthy subjects (M/F: 20/20, median age: 40 yrs). The immunological profile of our pts was evaluated by flow cytometric analysis of different PB lymphocyte and DC subsets.
Results: With respect to normal donors, a significant decrease of absolute lymphocyte number, CD4 T lymphocytes, CD19 and CD20 B-lymphocytes, NK cells and DCs was evidenced in mCRC pts treated with CT alone. Bevacizumab addition to CT didn't affect the B lymphocyte and the NK compartments. With respect to mCRC pts treated with CT alone, a statistically significant increase of CD4 T lymphocytes was observed ($p < 0.003$). At the same time, Bevacizumab administration was associated with a significant increase of absolute DC number and of their cellular subset ($p < 0.001$), with a decrease of DC humoral subset ($p < 0.002$).
Conclusions: First-line Bevacizumab-based therapy in mCRC pts seems to improve the in vivo T-cell mediated response, because of the increase of DC cellular subset. As evidenced in murine models, the VEGF blockade could have a synergistic effect in cancer immunotherapy programs for mCRC pts.

3035 POSTER

Levels of survivin splice variants correlate to degree of differentiation in colon cancer

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Background: Survivin is a member of the inhibitors of apoptosis family and plays an important role in cell division and proliferation by inhibition of apoptosis. There are five splice variants of survivin (survivin, survivin-2B, survivin-3B, survivin-ΔEx3 and survivin-2α) which appear to have different functions and take part in the regulation of the action of survivin. The aim of this study is to assess the role of these five splice variants in colon cancer.
Materials and Methods: Matched neoplastic and normal colonic tissue was obtained from 20 consecutive patients who underwent surgery for therapeutic reasons at the University Hospital of Patras. Total RNA was isolated and quantified using Ribogreen (Molecular Probes) before being reverse transcribed with Stratascript (Stratagene). Expression levels of the 5 splice variants were assessed with variant-specific primers and Taqman probes by quantitative PCR.
Results: Survivin-2α and survivin-2B, the two isoforms with pro-apoptotic function, were the isoforms most frequently expressed in normal tissue (100 and 90%, respectively). Survivin and survivin-3B were expressed in 80% whereas survivin-ΔEx3 in 60% of the normal colonic tissue. In neoplastic tissue, survivin, survivin-2B, survivin-3B and survivin-2α were expressed in all samples, whereas survivin-ΔEx3 was expressed in 95% of the samples. The levels of expression of all splice variants were significantly higher in neoplastic than in normal tissue ($p < 0.001$). Additionally, they were higher in stage B than in C although the difference was statistically significant only for surivivin-2α ($p = 0.019$). Moreover, all 5 survivin isoforms were expressed in higher levels in well compared to moderately differentiated tumors ($p < 0.05$). Furthermore, the ratios of the expression of the different isoforms were assessed. Survivin-3B/survivin-2α was higher in normal tissue than in neoplastic ($p < 0.05$). None of the ratios changed with the level of differentiation or the stage of the disease.
Conclusion: All 5 isoforms are expressed in both normal and neoplastic tissue although with different frequencies. Anti-apoptotic survivin-ΔEx3 expression exhibited the highest difference suggesting a role in colon cancer. All 5 isoforms are expressed at higher levels in neoplastic than in normal tissue. Furthermore, the higher levels of expression in well-differentiated tumors may suggest a role for survivin isoforms in the initial stages of colon carcinogenesis.

3036 POSTER

Tipification of genes related to oxaliplatin (OXA) sensitivity in a panel of 14 human colorectal cancer (CRC) cell lines by using microarray technology and real time quantitative PCR (QRTPCR)

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Background: Platinum drugs resistance is a complex process based in the alteration of genes that belong to several pathways related to drug metabolism. To clarify the multifactoriality of these mechanisms, we analyzed gene expression profile in fourteen CCR cell lines with different sensitivity to oxaliplatin. The aim of this work was to compare gene expression profile between high IC50 (IC50 > 1 mM) and low IC50 (IC50 < 1mM) cells and to validate the results by QRTPCR to determine genes that could play a role as a marker in oxaliplatin sensitivity.
Methods: Gene expression profile was analyzed through microarray technology (Human 19K oligo; labeled with Genisphere; data analysis by Genesis 1.5.0). We calculated logratio (OXA 24h treated cells vs untreated cells) for each cell line and analyze changes in gene expression comparing high (LOW OXA sensitivity) versus low IC50 (HIGH OXA sensitivity) groups. To study selected genes we used QRTPCR (Taqman®), considering as a positive validation, those genes that showed significant differences at expression level between high and low OXA sensitivity groups (ANOVA $p < 0.05$)
Results: By microarray analysis we obtained 51 candidate genes. Twelve of these genes were selected and 2 of them were positively validated after QRTPCR analysis: DUSP11 and VPS33A were upregulated in LOW OXA sensitivity group).
Conclusions: In our model, 2 genes showed expression changes between two OXA sensitivity groups. DUSP11 is a member of the dual specificity protein phosphatase subfamily that have been related to regulation of MAPK. VPS33A (vacuolar protein sorting 33A) is involved in vesicle-mediated protein transport. According to these results, both genes must be validated as a potential OXA sensitivity markers in CRC patients.

3037 POSTER

Two pathways of carcinogenesis in patients with colorectal cancer less than 45 years old

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Background: Colorectal cancer (CRC) arising from a complex series of molecular changes that involve at least in two different pathways. These include microsatellite instability (MSI) pathway and chromosomal instability (CIN) pathway. The aim of this study was the determination of predominant pathway involved in carcinogenesis of patients with CRC less than 45 years old with and without family history (FH) of CRC.
Method: In our study surgical pathology specimens of 108 patients with CRC less than 45 years old were immunostained for DNA mismatch repair proteins (MMRP) including hMLH1, hMSH2, hMSH6 and PMS2. Beta-catenin and P53 were also examined for CIN pathway.
Results: Totally 108 patients with median age of 40(20 to 45) were evaluated. Fifty seven patients were male and 51 were female. The site of tumor in 84 patients was colon and in 14 were rectum. Among 96 patients with known family history, 33(34.4%) had positive FH. The overall rate of abnormal immunostaining were MLH1 8.3%, MSH2 18.5%, MSH6 8.3%, PMS2 11.1%, P53 74.1% and beta catenin 35.2%. Meanwhile abnormal staining for hMSH2 and hMSH6 were significantly more seen in patients with positive family history ($p = 0.008$ and $p = 0.032$ respectively). Patients with positive FH for CRC had significantly more abnormal MMRP (54.5% vs. 20.6%, $p = 0.001$) and less positive p53 (54.5%vs. 81%, $p = 0.006$) than patients with negative FH. Patients with early T, N stage tumor had at least one more abnormal MMRP than advance T, N stage ($P = 0.050$ for T and $P = 0.030$ for N stage). Among different factors abnormal hMSH2 had significant association with lower cancer related death ($P = 0.060$). Patients with rectal cancer had more abnormal MMRP than patients with colon cancer but not significantly (35.7% vs. 29.8%, $p = 0.655$) and positive p53 staining for rectal and colon cancer were 71.4% and 72.6% respectively. Both in colon and rectal cancer patients with negative family history had more prevalent positive p53 (80.4% vs. 56.7%, $p = 0.022$ for colon and 81.8% vs. 33.3%, $p = 0.099$ for rectal cancer).
Conclusion: Our study indicate that even in CRC less than 45 years old, the main pathway for carcinogenesis in patients with negative family history is CIN, but in positive family history MSI is as effective as CIN.However main pathway in both colon and rectal cancer is CIN.