

Results: Among 162 colon cancer subjects, K-Ras 12 codon mutation was detected in 55 cases (34.0%) and K-Ras 13 codon mutation was detected in 16 cases (9.9%). BRAF mutation was detected in 26 cases (16.0%). It was observed that K-Ras 12 or 13 mutations was not associated with the age, gender, TNM stage, the lymph node metastasis, differentiation. In the Kaplan-Meier survival analysis, K-Ras mutation was not significantly associated with 5-year survival rate ($p = 0.73$, $p = 0.52$). In cases expressing BRAF mutation, it was shown to be not associated with the age, gender, TNM stage, lymph node metastasis, differentiation. Nevertheless, in the Kaplan-Meier survival analysis, 5-year survival rate of cases with BRAF mutation was significantly decreased ($p = 0.02$).

Conclusion: Taken together, it appears that K-Ras mutation is not associated with various clinicopathological factors of colorectal cancer patients, and it does not correlate to survival rate. Nonetheless, it was confirmed that survival rate was reduced in colorectal cancer patients expressing BRAF mutation, and it is considered that BRAF mutation could be a prognostic factor in colorectal cancer patients.

6139

POSTER

Incidence of VEGFR-2, PDGFR α and PDGFR β Mutations in Colorectal Cancer and Potential Value as Prognostic Markers

P. Estevez Garcia¹, A. Castaño², A.C. Martín³, F. Lopez-Rios⁴, J. Iglesias⁵, I. Lopez-Calderero¹, S. Molina-Pinelo¹, M.D. Pastor¹, L. Paz-Ares¹, R. Garcia-Carbonero¹. ¹Hospital Virgen del Rocío/Instituto de Biomedicina de Sevilla (IBIS), Medical Oncology, Sevilla, Spain; ²Hospital de Fuenlabrada, Pathology Department, Madrid, Spain; ³Bionostra, Aplicaciones Biotecnológicas S.L.U., Madrid, Spain; ⁴Centro Integral Oncológico Clara Campal, Pathology Department, Madrid, Spain; ⁵Bionostra, Aplicaciones Biotecnológicas S.L.U., Madrid, Spain

Background: Angiogenesis plays an essential role in tumour growth and metastasis, being a major target in cancer research. Vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR) families are largely involved in this process, but incidence of mutations in these receptors and their potential value as prognostic or predictive markers in colorectal cancer (CRC) has not been fully assessed.

Methods: VEGFR2, PDGFR α and PDGFR β mutations were evaluated by sequencing their tyrosine kinase domains in 8 CRC cell lines (T84, LOVO, LS174T, HT 29, LS180, SW48, COLO205) and in 92 samples of patients with colorectal adenocarcinoma. Potential correlations with clinicopathological features and survival of these patients were analyzed.

Results: Three genetic variations were identified in PDGFR α and one in PDGFR β , all of them corresponding to single nucleotide polymorphisms (SNP 11A, 12A, 16A and 19B), whereas no VEGFR-2 mutations were detected. SNP 11A and 16A were present in 100% of cell lines and tumour samples in homozygosity. SNP 12A was detected in 2 colorectal cell lines (LS174T, LS180) and SNP19B in 4 (LS174T, LS180, SW48, COLO205). Patient characteristics were representative of a standard CRC population: median age 68 years, 63% males, 75% colon and 25% rectal cancer, and typical stage distribution (I:9%,II:24%,III:26%,IV:40%). SNP 19B was found in 45 patients (49%) and SNP 12A in 13 (14%). Five-year overall survival was significantly higher for patients with PDGFR-B19 wild type (WT) tumours than for those harbouring SNP B19 (54% vs 22%; $p = 0.047$), but no survival difference was observed according to PDGFR-A12 status. Multivariate analysis revealed PDGFR SNP B19 ($p = 0.037$), age ($p = 0.002$), TNM stage ($p < 0.001$) and CEA ($p = 0.012$) as independent prognostic factors for decreased survival.

Conclusions: PDGFR SNP B19 is associated with poorer survival and could be a promising new prognostic marker in colorectal cancer patients. Further studies to validate our data are warranted.

6140

POSTER

Cetuximab-mediated Immune-enhancing Effects in Vitro and in Metastatic Colorectal Cancer (mCRC) Patients

P. Corrales¹, C. Botta¹, M.G. Cusi², E. Bestoso¹, S. Apollinari¹, M. Caraglia³, M.T. Del Vecchio⁴, A. Abbruzzese³, A. Aquino⁵, P. Tagliaferri⁶. ¹S. Maria alle Scotte" Siena University Hospital, Department of Oncology, Siena, Italy; ²S. Maria alle Scotte" Siena University Hospital, Department of Molecular Biology Microbiology Section, Siena, Italy; ³II University of Naples, Department of Biochemistry and Biophysics, Naples, Italy; ⁴S. Maria alle Scotte" University Hospital of Siena, Section of Pathological Anatomy Department of Human Pathology and Oncology, Siena, Italy; ⁵"Tor Vergata" University, Department of Neurosciences Pharmacology Section, Rome, Italy; ⁶Magna Graecia University and Tommaso Campanella Cancer Center Catanzaro, Medical Oncology, Catanzaro, Italy

Introduction: Cetuximab is a chimeric human-murine monoclonal IgG₁ to the Epidermal-growth-factor-Receptor, approved for colorectal cancer

treatment with chemotherapy. Its human constant fragment (Fc), throughout receptor binding, trigger additional immune-mediated effects, which may offer significant contribute to the final therapeutic effect. We investigated cetuximab ability *in vitro* to promote colon cancer cell phagocytosis and antigen-cross-priming by dendritic cells (DCs) to tumour-specific cytotoxic-T-cell (CTL) precursors. We also carried-out an immunological study in 26 mCRC patients enrolled in an on-going phase 2 trial, receiving an experimental biochemotherapy (GILFICet) regimen combining gemcitabine, irinotecan, fluoruracil, levofolinate, cetuximab, and metronomic sc. aldesleukine.

Material and Methods: Transmission-electron-microscopy (TEM) and Flow cytometry were used to evaluate the susceptibility of multiple colon cancer cell lines to DC-mediated phagocytosis. Human DCs loaded with cetuximab-coated colon cancer cells, exposed or not to a combination of anti-cancer drugs, were used to *in vitro* sensitize human PBMCs from normal donors and cancer patients collected at baseline and after 3 GILFICet courses. T-cell cultures were characterized for immune-phenotype and tumour-antigen specific CTL activity by Flow cytometry, LDH release and IFN γ -ELISPOT.

Results: ILF (irinotecan+ folinate+5-fluorouracil) and GILF (gemcitabine+ILF) poly-chemotherapies confirmed their ability to induce antigen remodelling and danger signals in colon cancer cells. After exposure to poly-chemotherapy and cetuximab these cells became highly susceptible to Fc-receptor-mediated phagocytosis/trogocytosis by human DCs, promoted their activation, and increased their ability to elicit a highly efficient CTL response on human PBMCs *in vitro*. Our study on the PBMCs of colon cancer patients enrolled in the GILFICet trial, revealed a significant treatment-related increase in naïve and central-memory-T-cells, activated-CTLs, NK/NKTS, mature-activated DCs and IFN γ -releasing cells. Substantial differences were observed in T-cell lines generated from patients' PBMCs taken before and after biochemotherapy. In the latter group there was in fact, a remarkable increase in proliferating CD8⁺Ki67⁺CTLs and tumour-antigen specific CTLs' precursors.

Discussion: These results suggest that cetuximab may exert immune-enhancing effects with potential antitumour activity.

6141

POSTER

Frequent Molecular Alterations in Brain Metastases From Colorectal Tumours

M. Denis¹, D. Loussouarn², S. Theoleyre¹, D. Vansteene³, J. Bennouna³. ¹CHU Nantes- Hotel Dieu, Laboratoire de Biochimie, Nantes, France; ²CHU Nantes- Hopital Laennec, Anatomie Cytologie Pathologiques, Nantes, France; ³Institut de Cancérologie de l'Ouest, Oncologie Médicale, Saint Herblain, France

Background: Brain metastases associated with colorectal cancer are relatively rare, but a frequent cause of death. The aim of our study was to analyze brain metastases from colorectal tumour, and identify cancer characteristics which were associated with their development.

Material and Methods: We performed a retrospective study of 19 patients (11 males and 8 females, 50-88 years, mean 67 years) who underwent brain surgery. DNA was extracted from FFPE sections following macrodissection using an automated iPrep system (Invitrogen). RNA was extracted from serial sections using the RNeasy FFPE kit (Qiagen), and cDNA was synthesized using the Quantitect reverse transcription kit (Qiagen). KRAS and BRAF mutations were tested using allele-specific PCR. PTEN expression was determined by immunohistochemistry. Amphiregulin expression was measured by real-time PCR.

Results: KRAS mutations were found in 13 (68.4%) brain metastases: c.35G >T, c.35G >A, c.35G >C and c.38G >A mutations were found in 5, 3, 3 and 2 samples, respectively. One tumour (5.3%, KRAS wt) presented the BRAF p.V600E mutation. PTEN was detected by immunohistochemistry in 12 tumours (63.2%), but 7 cases (36.8%) were negative for PTEN expression. Of these 7 tumours, 5 presented a KRAS mutation, and 1 was BRAF V600E. Amphiregulin expression was found to be very low in 10 tumours, intermediate in 6 samples, and high in the remaining 3 tumours. Two of these 3 tumours presented a mutated KRAS and the third one was PTEN negative. KRAS, BRAF and PTEN status were fully concordant between primary cancer and brain metastasis from the same individual. However, we did not find any correlation in the expression level of amphiregulin between the primary tumour and the metastatic site.

Conclusions: KRAS mutation prevalence was high in this series of patients presenting with cerebral metastases. Loss of PTEN was strongly associated with KRAS/BRAF alterations. Altogether, the overwhelming majority of the tumours tested (16/19, 84.2%) presented at least one molecular alteration. Further characterization of these tumours might yield better insight into their development, and potentially the treatment of these patients with poor prognosis.

Acknowledgements: samples were kindly provided by Drs S. Martin, M.F. Heymann, G. Aillet, N. Rioux-Leclercq, C. Magois, and J.-J. Auger.