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EGFR downstream signaling pathway in primary colorectal tumours and related metastatic sites: optimizing EGFR targeted treatment options

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Although multiple therapeutic options targeting the EGFR-driven molecular pathway have been recently proposed for the treatment of advanced colorectal tumours, the biologic mechanisms underlying the activity of these drugs "in vivo" are still to be fully investigated. In fact, most of the scientific data available so far were unable to correctly identify molecular markers predicting response (or resistance) to EGFR-targeted agents.
After our previous findings of a lack of EGFR status correlation, we analysed the expression of phosphorylated Akt and MAPK in primary tumours and corresponding metastatic sites with the aim to better define the EGFR-related molecular profile of colorectal cancer, in order to serve as a tool for treatment selection Ninety-nine cases (paired primary tumours and metastases with an already determined EGFR status) were available for our study, to date we completed Akt and MAPK analysis in 52 primary tumours and 55 paired metastases. In primary tumours immunohystochemically determined phosforilated Akt and MAPK were positive in 33 (63%) and 32 (62%) cases respectively, whereas Akt and MAPK were positive in 32 (58%) and 36 (65%) metastatic sites respectively. Interestingly EGFR negative primary tumours (27 cases, 52%) expressed Akt and MAPK in 16 (59%) and 17 (63%) cases. Accordingly with these findings also in EGFR negative metastases Akt was expressed in 19 (59%) and MAPK was expressed in 24 (75%) metastatic samples. Akt expression in primary colorectal tumours changed from positive to negative in 11 (21%) paired metastases and from negative to positive in 6 (11%) related metastatic sites. MAPK status in primary tumours changed from positive to negative in 8 (15%) paired metastases and from negative to positive in 11 (21%) related metastatic sites. Taken together our findings suggest that akt and MAPK status in primary tumours does not correlate with Akt and MAPK status in corresponding metastases. Moreover EGFR downstream signaling pathway can be over-activated even in the absence of EGFR expression in a considerable proportion of patients, thus making the use of anti-EGFR treatment with monoclonal antibodies at least theoretically inappropriate in these tumours. On the contrary, in these cases the use of a treatment strategy including small tyrosine kinase inhibitors that can interfere with the downstream pathway of the EGFR, seem more appealing. Complete analysis of all 99 cases will be presented at the meeting.

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Analysis of the SDF-1/CXCR4 chemokine-chemokine receptor axis and downstream effector pathways in human colorectal carcinomas

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Introduction: We have previously reported (ECCO12) that the CXC chemokine receptor 4 (CXCR4) is mostly downregulated in colorectal carcinomas (CRC) and that this is correlated with tumor progression. In order to further elucidate the factors involved in this phenomenon, we aimed to: a) evaluate the status of the ligand (SDF-1); b) dissect receptor-ligand downstream signalling pathways and c) evaluate the SDF-1/CXCR4 relationship with standard clinicopathologic variables including patients' survival.
Methods: CXCR4 and SDF-1 mRNA and protein expression was examined by real time RT-PCR, immunohistochemistry and western blot analysis in 134 tissue samples including 56 perfectly matched tumour and normal tissues, normal and metastatic lymph nodes (n = 16) and normal and metastatic liver tissues (n = 6). Protein expression was also analysed in 60 independent samples (tissue microarray) including primary and metastatic tumours and in 30 different human CRC cell lines. Gene expression differences between up, down and unregulated CXCR4 groups were evaluated by a low density oligonucleotide microarray approach using unsupervised clustering analysis and relevance gene networks. Analysis of the phosphorylation status of the MAPK family members ERK 1/2, JNK and P38 at the CXCR4 downstream pathway was performed by western blot.
Results: CXCR4 mRNA and protein were found to be 1.8 and 3.3 fold downregulated as compared with the corresponding normal tissue in 37 (66.1%) of the cases ($P < 0.0001$ and $P < 0.05$ respectively) with no significant changes in SDF-1 levels. Amongst the 90 genes analysed by microarrays, 20 (22%) were found to be differentially expressed between the CXCR4 up and downregulated cases. Genes involved in the MAPK pathway were the most differentially regulated, with differences ranging from 2 to 10 fold in change. These changes were confirmed at the protein level with significant low phoshorylation levels for the ERK 1/2 MAP kinase and higher phosphorylation status for the JNK and P38 MAP kinases resulting in different proliferation-apoptosis patterns. Finally, patients with tumors expressing low CXCR4 levels showed poorer survival probabilities when compared to the upregulated cases.
Conclusion: Our results show that CXCR4 is mostly downregulated in CRC without change in SDF-1 levels. The alterations in downstream signaling pathways following CXCR4 downregulation are likely to be responsible for the different tumor progression profiles observed.

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Indomethacin and Wnt signalling in HT-29 colon cancer cells

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Nonsteroidal anti-inflammatory drugs (NSAIDs) lower the incidence of and mortality from colon cancer. Although there is much evidence from epidemiological and laboratory studies that NSAIDs have antitumor activity and reduce the incidence of colon cancer, the mechanism of action remains unknown. In this paper, we present the effect of indomethacin on growth inhibition, induction of apoptosis, and alterations in the expression of several genes involved in Wnt signalling in HT-29 colon cancer cells.
We have shown that indomethacin reduces the proliferation rate of HT-29 colon cancer cells and induces apoptosis. Concentrations of indomethacin from 10^{-4} to 10^{-3} M strongly inhibited the growth of HT-29 cells. The inhibition of growth, as well as induction of apoptosis was dose and time dependent. The treatment of cells with 4×10^{-4}M indomethacin caused strong inhibition of cell growth (about 70%), enhanced expression of APC, decreased expression of beta-catenin and induced expression of E-cadherin proteins. Expression of beta-catenin was not markedly reduced instead, beta-catenin was translocated from the nucleus and cytoplasm to the plasma membrane. These results were confirmed by real-time RT-PCR analysis on mRNA level. At a concentration of 4×10^{-4} M indomethacin there was increased expression of *APC* gene (10.9-fold induction; delta delta Ct = 3.43) and *E-cadherin* gene (3.5-fold induction; delta delta Ct = 1.79).
These results suggest the antiproliferative effect of indomethacin may contribute to enhanced cell adhesion through increased expression of E-cadherin and translocation of beta-catenin from the nucleus to the cell membrane.

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Intraperitoneal application of the trifunctional antibody catumaxomab (anti-EpcAM x anti-CD3) for the treatment of peritoneal carcinomatosis due to GI cancer: Results of a phase I trial

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Background: Peritoneal carcinomatosis (PC) due to GI cancer is associated with a survival of 3–6 months. Due to the lack of effective therapies no standard treatment is currently recommended. The trifunctional antibody (trAb) catumaxomab (anti-EpCAM x anti-CD3) belongs to a new class of intact antibodies. While binding simultaneously to EpCAM+ tumor cells, T cells, and via the intact Fc region to accessory cells, it is able to induce effective tumor cell killing. In a phase I trial the intraperitoneal application of catumaxomab is being investigated in patients with PC due to GI cancer.
Patients and methods: 17 patients after diagnosis of EpCAM+ PC (7 gastric-ca, 9 colon-ca, 1 CUP) have been evaluated up to now. Treatment consisted of 4 escalating doses of catumaxomab administered intraperitoneally within 10 days. The MTD was defined for each of the 1st, 2nd, 3rd and 4th infusion according to a dynamic escalation schedule. Anti-tumor efficacy was evaluated by immunocytochemical analysis of peritoneal lavages before and after treatment. Patients were free to get any further chemo- or radiotherapy after trAb treatment and were frequently followed up. Patient enrollment into additional subgroups is ongoing. Currently 23 patients have been treated. In these patients, shorter duration of infusion with first pharmacokinetic (pk) data and further dose escalation with dexamethasone premedication is investigated.