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Immunohistochemical detection of the hMLH1 and hMSH2 proteins in hereditary and sporadic colon cancer tissues

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Backround: Germline mutations of hMLH1 and hMSH2 mismatch repair genes are associated with hereditary nonpolyposis colon cancer (HNPCC). Their somatic impairment can be detected in sporadic colon cancers. Tumours arisen due to inactivation of these genes are characterised by microsatellite instability (MSI) and loss of the protein product. We have studied correlation of the results of immunohistochemical detection of hMLH1 and hMSH2 protein expression with results of mutational and microsatellite instability analyses.

Material and methods: There were included: 19 colon cancer (CC), 1 stomach, 1 ovary, 1 endometrial, and 1 kidney tumour samples from hMLH1 or hMSH2 germline mutation carriers, 36 CC samples from patients without a germline mutation, and 16 CC samples from patients not subjected to mutational analysis. Immunohistochemistry was performed in paraffin-embeded tissues using mouse monoclonal antibodies (anti-hMLH1, clone G168-15; anti-hMSH2, clone G219-1129, Pharmingen, USA). MSI was analysed in tumour tissue after PCR amplification by multiplex analysis at ABI Prism 310 Genetic Analyzer. Mutational analysis was performed by sequencing of gene parts with abnormal mobility in DGGE.

Results: Loss of the appropriate protein expression was detected in all but one tumour (21/22 hMLH1, 1/1 hMSH2) from hMLH1/hMSH2 mutation carriers, all of whom exhibited a high level of MSI (MSI-H), and in 8 (5 hMLH1 and 3 hMSH2) of 11 MSI-H tumours from the other patients. None of the 41 tumours with low level MSI or stable microsatellites lossed the protein. Immunohistochemical analysis was able to detect MSI-H tumours with 100% specificity and 82% sensitivity. All tumours from hMLH1 mutation carriers expressed the hMSH2 protein; however, in 3 of them, hMSH2 expression was lower than in normal colon glands, whereas all the samples containing normal colon tissue from the other groups expressed the hMSH2 protein more intensively than normal colon glands.

Conclusion: Immunohistochemical detection of hMLH1 and hMSH2 proteins is a specific method of detection of tumours with DNA mismatch repair gene inactivation. It might be used as a screening method of HNPCC. The 4 cases with a high level of MSI and retained expression of the proteins might be explained by expression of a non-functional protein or by a defect in another DNA mismatch repair gene. The defect in the hMLH1 gene may be associated with an impaired synthesis of the hMSH2 protein in the tumour in some cases.

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Differential expression of CXC chemokine receptor 4 (CXCR4) in colorectal carcinomas

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Background: Chemokines and their receptors are known to play an important role in the process of chemotaxis. Evidence suggests that these proteins can also regulate non-chemotactic functions such as angiogenesis, apoptotic cell death and proliferation and thence, malignant tumour growth. In this study, we examined CXC chemokine receptor 4 (CXCR4) in both human colorectal carcinomas (CRC) and normal mucosa to evaluate its potential role in CRC progression.

Material and methods: Cancer tissues and normal mucosa were obtained from 56 patients with diagnosed CRC who underwent surgery at our department between 1999 and 2002. Quantitative Real-Time RT-PCR was performed to determine CXCR4 mRNA in paired tumour and normal samples using gene-specific Taqman® probes. Differences in mRNA were quantitated as relative amounts normalised for ^{2}-globin gene and expressed as a Tumor/Normal (T/N) ratio. Up or down regulation was defined as a 50% CXCR4 mRNA change in tumor tissue compared to paired normal mucosa. (T/N ratio > 1.5 for upregulation or < 0.5 for downregulation respectively). For protein expression, a peroxidase technique using monoclonal antibodies against CXCR4 was employed and the number of CXCR4-expressing tumor cells was compared with the number of CXCR4-expessing normal cells in 15 HPF in the matched section. Significant differences in mRNA and protein expression between matched pairs were tested by the Wilcoxon test, and also by a pair wise fixed randomization test. After logarithmic transformation, comparison between groups for CXCR4 expression was performed by T test or ANOVA whenever appropriate.

Results: CXCR4 mRNA expression was found to be significantly downregulated in cancer tissue as compared to the corresponding normal tissue in 37 (66.1%) of the cases. The mean T/N ratio was 0.553 (mean downregulation factor: 1.808). Protein expression correlated well with mRNA expression ($p<0.05$) and showed great heterogeneity in tumors compared to normal tissues. Significant differences were found between T/N mRNA and protein expression and T category ($p<0.01$), tumour stage ($p<0.01$) and tumour differentiation ($p<0.01$) with a CXCR4 expression trend to have a higher level of downregulation in advanced wall invasion, advanced stage and poorly differentiated tumours.

Conclusions: Our results suggest that CXCR4 downregulation may be a key factor for tumour development, growth and progression in colorectal carcinomas.

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Sentinel node technique for colorectal carcinoma: colon vs rectum

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Background: Recently, we started using the sentinel node (SN) technique for all patients operated for colorectal carcinoma in our clinic. Since there are major anatomical differences between the mesocolon and mesorectum, we evaluated the SN technique for colon and rectal carcinoma respectively.

Patients and methods: Since May 2002, all patients operated for colorectal carcinoma, in an elective setting and with the intention to cure, were included. We used the so-called ex-vivo' technique. After standard resection of the colon or rectum, 1 2 cc Patent Blue V was injected in the peritumoural, submucosal layer. Within few minutes lymphatics stained blue and the first 1 3 blue lymph nodes were identified as SN. In colon specimens these SN were excised and separately sent to the pathologist. In rectum specimens these SN were only marked by a suture in the mesorectum. This to keep the circumferential mesorectum intact for adequate pathological examination. All lymph nodes (non-SNs and SNs) were examined in a single HE-stained section. If the SN was negative for metastasis, two additional sections were immunostained with keratine CK 7/8, to reveal micrometastasis.

Results: 25 patients with colon carcinoma underwent the procedure. 3 patients were excluded because of peroperatively extensive lymphatic metastasis. In all 22 remaining patients, the SN was identified (identification rate 100%). In 7 cases the SN contained metastasis (7/22 = 33%), including 2 micrometastasis. This suggests an upstaging of 12% (2/17). In one patient the SN was false negative (accuracy 95% (21/22), sensitivity 88% (7/8)).

14 patients with rectal carcinoma underwent the procedure. In 11 patients, the SN was identified (identification rate 79% (11/14)). In 1 case the SN contained metastasis, and in 4 cases the SN was false negative (lymph node metastasis 36% (5/14), accuracy 80% (7/11), sensitivity 20% (1/5)). In 2 false negative cases there was a large lymph node containing metastasis, suggesting a bypass of the lymphatic drainage to second echelon nodes. The other 2 false negative cases had a minor tumour-burden adjacent to the primary tumour, these small tumour deposits count as lymph node metastasis.

Discussion: Extensive lymph node metastasis is harder to recognise peroperatively in a rectum specimen compared to a colon specimen. As well, the SN procedure for rectum carcinoma may be negatively affected by the preoperatively given radiotherapy, which possibly obliterates the lymphatic vessels. Furthermore, lymphatic drainage from the rectum may go via collateral pathways to the parailiacal and paracaval lymph nodes.

Conclusions: 1) The SN technique for colon carcinoma is feasible with an identification rate of 100% and an accuracy of 95%. 2) This results in an upstaging of 12%. 3) The SN technique for rectum carcinoma is in this setting not yet reliable.