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**Background:** Pdcd4 (programmed cell death protein4) is a potential tumor suppressor, expression of which is downregulated in various human tumor types. Additionally, Pdcd4 is able to inhibit the neoplastic transformation in the JB6 mouse model. Pdcd4 has been shown to inhibit translation of diverse regulatory factors important for neoplastic transformation. Moreover, it was shown that AKT phosphorylates Pdcd4, causing nuclear translocation and inactivation of the latter. This suggests that Pdcd4 activity is dependent on its cellular localisation. Up to now, Pdcd4 protein expression and cellular localisation has not been analysed in a large series of patients with colorectal cancer (CRC).
**Methods:** We investigated the expression pattern and localisation of Pdcd4 tumor suppressor protein in resected tumor and corresponding normal tissue in a series of 41 CRC patients (32 R0-resected) who did not receive neoadjuvant treatment, by Western blotting (WB) and immunhistochemistry (IHC). A separate semiquantitative score for ICH staining of the cytoplasm and nuclei was established. Preliminary analysis of Pdcd4 expression and localisation was correlated with patient's clinical tumor stage (UICC) and with recurrence-free survival.
**Results:** In WB high overall Pdcd4 amounts were detected in normal tissue in comparison to the tumor samples where the signal was significantly decreased ($p = 0.025$, Wilcoxon). IHC analysis revealed strong nuclear presence of Pdcd4 in the apical cryptal epithelium of normal tissue, as opposed to the complete loss of nuclear expression in tumor tissue ($p = 0.001$, Wilcoxon). In normal tissue, loss of Pdcd4 nuclear expression/increase of cytoplasmic Pdcd4-staining was significantly associated with advanced UICC stages ($p = 0.027$, $\chi^2$). Preliminary Kaplan–Meier-analysis (median recurrence-free survival time: 38 months, range: 1–74 months) showed a trend for loss of Pdcd4 expression in the nuclei of the normal tissue to be associated with poor recurrence-free survival ($p = 0.09$, Breslow log rank).
**Conclusion:** This is the first clinical study that demonstrates a potential relevance of Pdcd4 expression and localisation in resected colorectal tumors and corresponding normal tissue, for tumor diagnosis and progression. Further analysis of colorectal adenomas will be performed to study the role of Pdcd4 localisation as a potential clinical marker for carcinogenesis in CRC.

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## P6. PROTEINASES ACTIVATED RECEPTORS: EXPRESSION AND QUANTIFICATION IN COLON AND PROSTATE HUMAN CANCER

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**Background:** Proteinases activated receptors (PARs) are important members of the G protein-coupled receptors family. The proteolytic cleavage of their extracellular domain by serine proteases such as trypsin or thrombin, generates an autoactivating tethered-ligand. Thus, the corresponding activated G proteins trigger a cascade of downstream events leading to diverse cellular outcomes such as calcium signalling, cell adhesion, cell migration and mitogenesis. The role of proteases in promoting invasion of cancer cells and tumorigenesis have prompted us to study the expression level and localisation of the four known members of the PARs family and explore their role in cell proliferation.
**Methods:** First, by using quantitative RT-PCR, we have quantified: (i) the expression of each PAR in colon and prostate cancer cell lines, (ii) the PARs expression in 40 patients samples (colon or prostate normal tissue and cancer). Second, we have studied PARs tissue localisation in colon and prostate, by using immunohistochemistry staining on TMA samples. Finally, we investigated the role of the four members of PAR family (PAR1-4) in cell proliferation by stimulating cell lines in different conditions (various thrombin concentrations).
**Results:** Our results show that the expression profile of the PARs is different for each PAR and depend mostly of the cell lines characterisation (origin and stage). However, we demonstrate a constant overexpression of two PARs in every tested cell line, that was also found in tumor samples.

Immunohistochemichal analysis shows PAR1 endothelial expression which was stronger in malignant tissues (prostate and colon samples), and weaker (prostate) or absent (colon) in normal tissues.
**Conclusion:** These results confirm the importance of PARs in cancer, whose expression pattern is likely to influence cancer cell behaviour in tumors.

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## P7. COMPLETE COMPILATION OF MATRIX METALLO-PROTEINASE EXPRESSION IN HUMAN MALIGNANT GLIOMAS

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**Background:** Glioblastomas are the most common malignant brain tumors in adults characterized by very aggressive local growth and invasiveness. Tumor invasion into surrounding brain tissue is facilitated by increased expression and activity of matrix metalloproteinases (MMPs), which may be marker for tumor aggressiveness. However, for several of the 23 human MMPs there are no or only very limited literature data available concerning expression by glioblastomas. Therefore, we screened an extensive panel of 15 low-grade astrocytomas and 15 glioblastomas in order to fill the gaps in our knowledge about MMP expression by these tumors.
**Methods:** Expression of MMPs was analysed by semiquantitative RT-PCR and immunostaining. Total RNA was used as template for RT-PCR. Immunostaining was performed on cryosections.