P3. COMBINATION ANALYSIS OF ACTIVATOR PROTEIN-1 FAMILY MEMBERS, Sp1 AND AN ACTIVATOR PROTEIN-2α-RELATED FACTOR BINDING TO DIFFERENT REGIONS OF THE UROKINASE RECEPTOR (u-PAR) GENE IN RESECTED COLORECTAL CANCERS

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Introduction: Studies on the transactivation of genes via promoter elements have mostly been done on cell lines rather than resected tissues. This, however, is essential to address an in vivo or clinical relevance. We have previously shown tumor-specific binding of Sp1and an activator protein (AP)-2α related factor to promoter region -152/-135 of the metastasis-related u-PAR gene in 60% of in vivo resected cancer tissues. Cell lines have implicated an additional role, and potential synergism, of an AP-1 region (-190/-171) in u-PAR regulation. This study was done to (a) analyze AP-1 binding to this region in resected tumor and normal tissues, and define subgroups in which it is tumor-specific, and (b) to analyze transcription factor binding patterns to both promoter motifs in resected tissues, supporting synergism, and draw first prognostic conclusions.
Methods: In 103 patients with colorectal cancer, electrophoretic mobility shift assay/supershift analysis for u-PAR promoter region -190/-171 was done in tumors and normal tissues. In 71 patients, region -152/-135 was also analyzed. U-PAR protein was measured by ELISA.
Results: Tumor-specific AP-1 binding to region -190/-171 of the u-PAR promoter was found in 40% of patients. Subgroup analysis showed tumor-specific binding for c-Fos in 58%, for c-Jun in 50%, for JunD in 39%, and for Fra-1 in 4% of cases. AP-1 binding correlated significantly with u-PAR protein amounts in both normal and tumor tissues ($p < 0.001$), in contrast to a tumor-specific correlation with u-PAR of the AP-2/Sp1 region. In analyses for both promoter regions, 62% of cancers showed simultaneous binding for AP-1, AP-2, and Sp1, 11% for AP-1 and AP-2, 16% for AP-2 and Sp1, 4% for AP-2 only, 3% for AP-1 only, and 0% for Sp1 only. The binding of AP-1, AP-2, and Sp1 correlated significantly with each other ($p < 0.001$), the combination of AP-1 and AP-2 showing the highest correlation with u-PAR ($p = 0.008$). Preliminary survival analysis indicated a trend for poorer prognosis for binding of all three transcription factors.
Conclusion: This is the first study differentiating transcription factor binding to two important u-PAR promoter regions in a large series of resected tumors and normal tissues. The AP-1 site seems to be a less tumor-specific regulator than the Sp1/AP-2 motif. Nevertheless, data corroborate the hypothesis of synergism between both elements in resected tumors.

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P4. TUMOR SUPPRESSOR Pdcd4 INHIBITS INVASION AND REGULATES UROKINASE-RECEPTOR (u-PAR) GENE EXPRESSION VIA Sp-TRANSCRIPTION FACTORS

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Background: Tumor-suppressor Pdcd4 has never been investigated as to a potential role in invasion/metastasis. The urokinase receptor (u-PAR) promotes invasion and metastasis and is associated with a poor cancer-patient survival. The present study was conducted: (1) to implicate a role of Pdcd4 in invasion and u-PAR-regulation, (2) to describe first mechanisms by which this is achieved.
Methods: Diverse gastrointestinal carcinoma cell lines were screened for Pdcd4- and u-PAR expression and protein amounts by Northern-, Western blot analysis respectively RT-PCR. To determine a potential regulation of the u-PAR promoter by Pdcd4, CAT- and luciferase reporter assays using diverse u-PAR wildtype and deletions mutants were undertaken and potential *cis*-element were screened for transcription factor binding by EMSA. Invasion and intravasation influenced by Pdcd4 were tested by using different invasion assay approaches.
Results: In colon/gastric cancer cell lines, a reciprocal expression of u-PAR and Pdcd4 was observed. RKO and HCT116 colon cancer cells made to express Pdcd4 showed a reduction in u-PAR-mRNA and -protein, this being paralleled by an inhibition of invasion/intravasation. A CAT-reporter driven by the wildtype u-PAR-promoter was reduced in constitutive activity with increasing Pdcd4-expression. Deletion of a region containing a putative Sp-1 binding site at -402/-350 inhibited u-PAR-promoter regulation by Pdcd4, this being paralleled by a reduction of Sp1 binding to this region in *pdcd4*-transfected cells. *Pdcd4*-transfected cells showed an increase of Sp3 binding to u-PAR-promoter region -152/-135, and the deletion of this region reduced the ability of Pdcd4 to suppress u-PAR-promoter activity.
Conclusion: These data suggest Pdcd4 as a new negative regulator of invasion and the invasion-related gene u-PAR. Furthermore, it is the first study to implicate Pdcd4-induced gene expression via Sp3 induction and Sp1 suppression.

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P5. EVIDENCE OF Pdcd 4 AS A NOVEL MARKER FOR TUMOR DIAGNOSIS AND PROGRESSION IN RESECTED COLORECTAL CARCINOMAS

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