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Investigation of genes that are related to colorectal cancer and liver metastasis

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The initial observation of this study was based on cDNA microarray analysis of CC531 colon cancer cells, which had metastasized to rat liver following injection into the mesenteric vein of syngeneic rats and then had been re-isolated at different time points. From the 2919 genes, which showed deregulated expression in response to metastasis formation, some candidates from promising gene families were chosen for further analysis using different *in vitro* models and human CRC samples. These gene families included the claudins, insulin like-growth factor binding proteins, the matricellular proteins as well as the metalloproteinases and the homeobox genes.

For claudins, it was shown that the initial phase of rat CRC cells homing into the liver involves a transient down-regulation of at least *Cldn1*, *Cldn3*, *Cldn4*, *Cldn9* and *Cldn12*. The transcription repressor *Snail*, which regulates most claudins, was concomitantly up-regulated during the early stages of metastasis before returning to normal expression levels. It was also shown that hepatocytes and Kupffer cells can be excluded as the main cause of the initial claudins down-regulation during early metastasis. By preventing the cell-cell contacts and the adherent-state of the cells, physical forces negatively influenced the expression of *Cldn4* and *Cldn12*, but not of *Cldn1*, *Cldn3* or *Cldn9*. Further, siRNA silencing of *Cldn1* and *Cldn4* increased migration and reduced colony formation, with these phenotypes being consistent with metastatic homing. These model results were paralleled in human CRC tumor samples, which showed increased *Cldn1* and *Cldn4* expression in UICC stages l-lll, and significantly reduced expression in stage IV and in liver metastasis. Additionally, the IHC analysis revealed that the expression of CLDN3 was high in all 32 CRC specimens, while the expression of CLDN1 and CLDN4 was high in 94% and 87.5% of all tumor specimens, respectively. Nevertheless, liver metastases showed lower expression of CLDN1, CLDN4 and CLDN3 than the corresponding primary carcinomas. Therefore, it is suggested that primary CRC tumors have an initial growth advantage from increased claudin expression, whereas metastasizing cells require a transient reduction in claudin expression to be liberated from the primary tumor and then to initiate metastatic growth in the liver.

With regard to insulin-like growth factor binding proteins, it was shown that IGFBP3 and IGFBP7 are multi-functional genes, deregulation of which is intimately related to the metastatic behavior of CRC tumor cells. In CRC patients, a significant correlation between the expression levels of these genes was noticed, but no relation to overall survival was registered. Further, the patients' age was inversely related to the expression of IGFBP3. Silencing of IGFBP3 and/or IGFBP7 in human SW480, Caco2 and rat CC531 colorectal cancer cell lines reduced proliferation, colony formation, and for IGFBP3, also migration. These results indicate that IGFBP3 and 7 cannot be simply assigned to the group of tumor suppressors, but have additional properties, which become evident only in the context of cancer progression and metastasis formation.

It was also shown, that *Sparc* and *Hevin* were ≥2-fold up-regulated during early metastasis (3^{rd} and 6^{th} day of metastasis formation) and the expression of these genes showed the same pattern of regulation, with a more pronounced increase in *Sparc* than in *Hevin* expression. The co-culture of CC531 cells with hepatocytes induced expression of *Hevin* and *Sparc*. Hevin expression was also induced in CC531 cells in response to siRNA silencing of *Sparc*.

Moreover, siRNA-mediated knockdown of *Sparc* resulted in significantly decreased proliferation, migration and (large) colony formation of CC531$^{si.Sparc}$ cells. In CRC patients, *SPARC* mRNA was mainly up-regulated, whereas *Hevin* was down-regulated. Despite this, statistical analysis showed a moderate correlation between the expression levels of both genes. In addition, silencing of *Hoxa2* in CC531 cells caused induction of *Sparc* expression in tumor cells, suggesting *Hoxa2* as a transcriptional repressor for *Sparc*. *Hoxa2* knockdown caused significantly decreased proliferation, migration and (large) colony formation of CC531$^{si.Hoxa2}$ cells. Interestingly, siRNA silencing of *Opn* caused up-regulated expression of *Hoxa2* mRNA in CC531$^{si.Opn}$ cells, suggesting further interaction of *Hoxa2* with *Opn*, which in turn could explain the correlation between *Sparc* and *Opn*. In addition, *Opn* expression was found to be up-regulated after the 6th day of metastasis formation in rat liver and its suddenly increased expression *in vitro* thereafter was related to the confluence-dependent-regulation of *Opn* found in this study. The metalloproteinase *Mmp12* had a similar expression profile as *Opn* during metastasis formation and then *in vitro*. Also, siRNA silencing of *Opn* in CC531 cells caused up-regulation of *Mmp12*, suggesting that OPN may bind to MMP12 in a way similar to its known binding to MMP3. In summary, these results show that the model detected temporal changes in gene expression, which are related to metastasis formation of CRC cells in the liver. The therapeutic importance of the gene families identified in this study will be assessed in future experiments.