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Identification of genes in ASML rat pancreatic cancer cells, as assessed by the modulations of messenger- and microRNA expression, which contribute to liver colonization

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The aim of this study was to identify pancreatic cancer genes, which contribute to liver metastasis. For mimicking liver metastasis, the rat ASML^{eGFP} pancreatic cancer cell line was chosen as model and injected into the portal vein of isogenic rats. The subsequent studies followed a twofold approach: First, ASML^{eGFP} cells were re-isolated by FACS sorting after successful implantation at early, intermediate, advanced and terminal stages of liver colonization, corresponding to 1/3, 6, 15 and 21 days of growth in the rat liver. The RNA of these cells was subjected to genome wide micro-array analysis of messenger- and microRNA species and related to the respective expression levels obtained from ASML control cells growing in vitro. Any significantly altered expression with regard to this control was considered as factor potentially contributing to metastatic liver colonization. Second, for refining these results, the mother cell line ASML was used to select nine clones by single cell deposits. All these clones were injected subcutaneously and intraportally to isogenic rats. Their growth was recorded and growing ASML^{eGFP} clones were re-isolated after 15 days of growth. These cells were also analyzed by micro-array for changes in mRNA and miRNA expressions, and the results obtained from liver and subcutaneous tissues were related to those from a clone showing no growth in rats. mRNA levels were obtained from 30508 genes by microarray. Analyses were performed by statistical means and Ingenuity pathway analysis (IPA). Clearly, there was huge amount of gene expression modulation that appeared between control cells growing in vitro as well as between the four stages of liver colonization. When interpreting these modulations, it was tried to differentiate between expression changes that occurred due to the cells' growth under in vitro or in vivo conditions or due to the tumor cells' need during the four phases of colonization. In the early stage of liver

colonization, ASML cells significantly increased the expression of genes belonging to the categories of proliferation, migration, movement, chemotaxis, accumulation of macrophages and other white blood cells, production of reactive oxy-gen species, and stimulated proliferation of fibroblasts inside the infected liver, but genes belonging to the categories homing and angiogenesis were still inactivated. In the intermediate and advanced stages, most processes were decreased, except the processes which contribute to neurodegeneration. It appears as if the tumor cells would rest before a final rise in expression. In the final stage, all processes showed increased expression, including homing, proliferation, migration, vasculogenesis, and angiogenesis.

A most noticeable modulation in gene expression was found in extracellular matrix genes, such as collagen and laminin genes, chemokine-, metalloproteinase-, and A Disintegrin & Metalloproteinase Domain genes, as well as transforming growth factor- and apolipoprotein genes. Some genes were highly expressed in early and late stages of liver colonization, which indicates that these extracellular matrix genes can be used as prognostic marker. Among the investigated 1776 miRNAs, there was also a large modulation of expression. Noticeably, there were significant depletions in the levels of miRNA- 29a, miRNA -1, miRNA-330, let-7c-1, miRNA-21, and miRNA-339-5p. Concomitantly, there were significantly increased levels of miRNA-199a-3p and miRNA-335. In view of this huge number of modified genes, the refinement experiments with ASML clones were performed. Remarkably, from 9 clones selected from the ASML mother cell line, there were only 2 clones (clones 6 and 15), which grew aggressively, i.e. colonized the rat liver completely within 15 days; three clones (clones 7, 12, and 14) grew moderately, i.e. the liver was colonized by less than 30%, and the remaining clones (clones 8, 9, 11, and 13) didn’t grow either subcutaneously or in the liver. Interestingly, there was a strong similarity between the genes, which were modulated more than 20-fold in clones 12 (less aggressive) and 15 (highly aggressive). Derived from this, it is hypothesized that the about 300 genes, which are altered in expression in a significantly parallel way, are critical for the establishing of growth in rat liver.

Among these about 300 genes, there were most notably genes from families, which are responsible for hemostasis, complement activity, and metabolism. It is reasonable to assume that their gene products contribute to create a masked environment for tumor cells for their improved survival and growth. It is anticipated that the identification of

mRNAs and miRNAs, which are modulated as established in this work, will be helpful in selecting targets for diagnostic and therapeutic procedures.