

EP300—a miRNA-regulated metastasis suppressor gene in ductal adenocarcinomas of the pancreas

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Genetic and epigenetic alterations during development of pancreatic ductal adenocarcinomas (PDACs) are well known. This study investigates genetic and epigenetic data together with tumor biology to find specific alterations responsible for metastasis formation. Using 16 human PDAC cell lines in a murine orthotopic PDAC model, local infiltration and metastatic spread were assessed by standardized dissemination scores. The cell lines were further classified into 3 hierarchical groups according to their metastatic potential. Their mRNA and microRNA (miRNA) expression was profiled *via* mRNA-microarray as well as Taqman Low Density Array, and validated by single quantitative RT-PCR and Western blotting. In the highly metastatic group, a significant induction of EP300 targeting miRNAs miR-194 (fold change: 26.88), miR-200b (fold change: 61.65), miR-200c (fold change: 19.44) and miR-429 (fold change: 21.67) ($p < 0.05$) was detected. Corresponding to this, decreased expression of EP300 mRNA ($p < 0.0001$) and protein ($p < 0.05$) were detected in the highly metastatic PDAC cell lines with liver metastases compared to the nonmetastatic or marginally metastatic cell lines, while no correlation with local tumor growth was found. In conclusion, epigenetic alterations with upregulated EP300 targeting miRNAs miR-194, miR-200b, miR-200c and miR-429 are related to reduced EP300 mRNA and protein in PDAC. These results demonstrate that miRNAs might be able to modulate the expression of metastasis-specific suppressor genes and metastatic behavior in PDAC, suggesting diagnostic and therapeutic opportunities for EP300 and its targeting miRNAs in PDAC.

Pancreatic ductal adenocarcinoma (PDAC) is among the most malignant tumors with a highly unfavorable prognosis. The 5-year survival rate of all patients is below 5%, and the median survival time after diagnosis is ~6 months. Even af-

ter operation with curative intention, the 5-year survival rate in specialized centers is below 15%, increasing to only 25% when adjuvant chemotherapy is applied.^{1,2} The cancer's aggressive nature, the lack of methods for early detection and the limited response to available treatments contribute to its high mortality rate. Pancreatic cancer is characterized by modifications in gene expression due to mutations, deletions and amplifications of genes critical for tumor development and progression. Although these alterations are purely genetic, epigenetic mechanisms such as shifts in DNA methylation patterns can also contribute to the induction and maintenance of pancreatic cancer.^{3,4} Epigenetic mechanisms are modifiers of gene expression that are heritable but potentially reversible and do not involve changes in the DNA sequence. At the post-transcriptional level, epigenetic regulation can take place *via* microRNAs (miRNAs). miRNAs are small noncoding RNAs that are cleaved from 70- to 100-nucleotide hairpin pre-miRNA precursors in the cytoplasm by RNaseIII Dicer into their mature form of 18–23 nucleotides.⁵ Single-stranded miRNAs bind messenger RNAs (mRNA) of potentially hundreds of genes at the 3'un-translated region with perfect or near-perfect complementarity, resulting in degradation or inhibition of the respective target mRNA. Recently, miRNAs have gained attention as another family of molecules involved in cancer development.^{6,7} Upon binding to their target mRNAs, miRNAs initiate post-transcriptional

Key words: pancreatic cancer, metastasis, metastasis suppressor genes, epigenetics, microRNA

Abbreviations: ANOVA: analysis of variance; CDKN2a: cyclin-dependent kinase 2a; EP300: E1A binding protein p300; miRNA: micro ribonucleic acid; miRISC: miRNA-associated, multiprotein RNA-induced silencing complex; PDAC: pancreatic ductal adenocarcinoma; qRT-PCR: quantitative reverse transcriptase polymerase chain reaction; RNAi: RNA-mediated interference; SERPIND1: serpin peptidase inhibitor, clade D, member 1; TLDA: Taqman low density array

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