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A novel nuclear phenotype-based high-throughput screen to identify essential genes involved in proliferation and migration of human aortic smooth muscle cells

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Proliferation, migration and apoptosis of vascular smooth muscle cells (VSMCs) have a vital role in the pathophysiology of vascular diseases. Few therapies have been developed which target these cells effectively. We therefore performed a siRNA screen on human aortic smooth muscle cells to identify genes essential for growth and migration of these cells. 290 siRNAs significantly influenced cell proliferation. After pathway analysis, 38 candidate genes were selected for further study. In a secondary screen 23 genes were confirmed as relevant. These genes affected mostly proliferation. 13 genes induced apoptosis in the synthetic phenotype of HaoSMCs. Furthermore, 11 genes were found to induce aberrant nuclear phenotypes and which therefore were involved in cell mitosis. 4 genes were identified which affected migration in HaoSMCs. In the bioinformatics network analysis, 11 genes showed an indirect or direct interaction. Our screen illustrates the applicability of an optimized high-throughput screen approach to identify genes which are implicated in the regulation of cell biological processes of live non-transformed cells such as HaoSMCs. These genes are by themselves potential drug candidates or may lead to the development of lead compounds for the development of novel medical approaches.

In part two of this thesis we investigated whether single nucleotide polymorphisms (SNPs) of EGF 61*A/G, TGF-β1-509*T/C and TNF-α -308*A/G are associated with the survival rate after pancreatic cancer surgery and/or with the frequency of postoperative complications such as leakage of the pancreatic anastomosis, leakage of the biliodigestive anastomosis and wound infections. The EGF 61*A/G, TGF-β1-509*T/C and TNF-α -308*A/G genotypes were analyzed in patients who underwent pylorus preserving pancreaticoduonectomy for pancreatic carcinoma. SNPs were determined by means of PCR-RFLP. A significantly lower median survival time was found in EGF 61*A/A homozygote patients as compared to the A/G heterozygote group. There was also a significantly lower median survival time in the TNF-α -308* A/A homozygote group as compared to the A/G and GG group. Survival time in patients with the TGF-β1-509*T/C polymorphism was unchanged. There was a significantly lower median survival time in the TNF-α -308* A/A homozygote group as compared to the A/G and GG groups in a Cox hazard proportional model. Patients with the TGF-β T allele had a higher frequency of leakage of the pancreatic anastomosis. Patients with a postoperative leakage of the biliodigestive anastomosis showed frequently the TGF-β TC allele and not the TGF-β CC allele. The frequency of the TGF-β TT+TC genotype was significantly higher in patients with a postoperative leakage of the biliodigestive anastomosis as compared to TGF-β CC genotypes. In a Cox hazard proportional model only wound infection had a significant influence on the long term survival time for pancreatic cancer patients and was associated with an adverse prognosis. This study may help in future to identify high risk patients.