## 6. SUMMARY

Over 90% of pancreatic carcinomas exhibit functional inactivation of the cell cycle inhibitor $p16^{INK4a}$ (p16) as well as constitutive activation of Kirsten-Ras (K-Ras). Our previous studies showed a restitution of sensitivity to anoikis by reexpression of p16 in human pancreatic cancer cells so that the basic molecular mechanisms of this possibly therapeutic relevant reversion were to be elucidated in the present thesis work. Exemplary in the human pancreatic carcinoma cell line Capan-1 an essential function of oncogenic K-Ras for anoikis resistance and a participation of Caspase-8 in p16 mediated anoikis was given evidence.

Capan-1 pancreatic carcinoma cells mainly express the K-Ras isoform to which all detected Ras activity could be attributed. Cultivating cells in suspension led to a marked increase in K-Ras activity. Interestingly, Capan-1 clones with stable reexpression of p16 exhibited not only a complete loss of K-Ras activity under adhesive as well as suspension conditions, but also a pronounced decrease in the cellular K-Ras content as compared with control cells. Reduction of Ras expression in Capan-1 cells with K-rasV12 antisense oligonucleotides led to an increased rate of anoikis, whilst vice-versa restitution of oncogenic K-ras in Capan-1/p16 cells highly reduced their anoikis fraction. Besides, the ability of Capan-1 cells to form colonies in soft agar, reduced by p16 reexpression, was restored by stable expression of oncogenic K-ras. The Capan-1/p16 acquired loss of tumorigenicity in nude mice, however, remained in spite of K-Ras substitution. In place of the transcriptional level, p16 mediated regulation of K-Ras expression was disclosed in lowered protein stability of the Ras isoform, probably by direct association with p16 and consequently accelerated degradation. The regulation of K-Ras by p16 in a second pancreatic carcinoma cell line and a colon carcinoma cell line points to an underlying general principle.

These observations document a functional relevant regulation of the oncoprotein K-Ras by the tumorsuppressor p16 for the first time and characterize the inhibition of K-Ras as an essential event in the context of p16 mediated anoikis. This new, functional interaction of the most prominent genetic alterations in human pancreatic carcinoma probably determines the typical aggressive progress of the disease by the central tumorbiological phenomenon of anoikis resistance.