Could Antisense Oligonucleotides Targeted to K-*ras* Inhibit the Tumor Growth, Invasiveness and Expression of MMP-2 and MMP-9 *in vitro* and *in vivo* in hamster experimental pancreatic câncer model?

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BACKGROUND: Matrix metalloproteinases (MMP), especially MMP-2 and MMP-9, are thought to play major roles in pancreatic cancer growth and metastasis. Ras activates a multitude of downstream activities with roles in cellular processing, including invasion and metastasis. Therefore, antisense oligonucleotides (ASO) targeting this K-*ras* gene may be a therapeutic approach. Hamster pancreas is similar to human genetically, anatomically, and immunologically. K-*ras* point mutation is found on codon 12 in hamster pancreatic cancer cell line, as in humans. Orthotopic implanted pancreatic cancer in hamsters could mimic the features of the disease with hepatic metastases rate of 60% in our model.

HYPOTHESIS: Our aim was to clarify the effectiveness of this gene therapy in hamster experimental cancer model.

METHODS:HaP-T1,a cell line derived from BHP-induced pancreatic cancer was used.Transfection with antisense oligonucleotides (ASO) were performed. MTT and MTT agarose assays were done. Chemoinvasion assay was performed. MMP-2 and MMP-9 production by the cell lines was determined by gelatin zymography. For *in vivo* experiments, subcutaneously resected tumors were implanted orthotopically in Syrian golden hamsters.They were divided in 3 groups:1.Positive control (PC),2.Sense treated hamsters (STH),and 3.Antisense treated hamsters (ATH).Oligonucleotides were administered for 2 weeks.Follow up was done. Five animals of each group were sacrificed at Days 10,17,24,31,and 38,to study the local response and metastatic sites. Five animals of each group were left to study the survival time.Specimens were studied histopathologically.Orthotopic pancreatic tumor MMP production was measured by gelatin zymography.

RESULTS: ASO inhibited the tumoral growth and invasiveness.They downregulated active forms of MMP-2 and MMP-9 in a dose dependent manner *in vitro.*Positive controls,STH,and ATH survived in average 72.7,74.3,and 82.7 days, respectively. Spontaneous lymph node metastases were found from 31 days in ATH group, while PC and STH groups showed metastases and direct invasion to adjacent organs from 17 days.After death, metastatic sites were similar in 3 groups.ASO downregulated the activation of MMP-9, more than MMP-2 *in vivo.*

CONCLUSIONS: These experiments suggest that ASO targeted K-*ras* point mutation may be a good choice in the management of pancreatic cancer because of the suppression of tumor growth and invasiveness *in vitro* and *in vivo.* ASO also downregulated the activation of MMP-9 and MMP-2 *in vitro* and *in vivo.*