cyclin-dept kinase 4 and 6 preventing their interaction with cyclin D. PCR analysis has showed downregulation of cyclin D1 and K-Ras. Subsequent treatment with docetaxel has exerted a synergistic antimitotic effect exhibited by BrdU and Ki-67. Flow-cytometry has reported diploid DNA. A biochemical assay showed activation of caspase-3/CPP32 pathway which led to electron cytological signs of D2 apoptotic stage forming apoptotic bodies which were phagocytosed by adjacent tumour cells leading to a bystander killing effect.

Conclusion Concluding, by restoring wild-type p16 protein in chemoresistant adenosquamous carcinoma cells, we have achieved to block cyclin D1 which led to inactivation of K-Ras allowing induction of apoptosis after the antimitotic action of docetaxel.

Competing interests None.

Keywords adenoviral transfection, p16cDNA, pancreatic adenosquamous Ca, K-Ras, hypermethylation.

PTU-110 CHEMOGENE TREATMENT CONSISTING OF RECOMBINANT ADENOVIRAL TRANSFECTION OF P16CDNA (SVN-22/3), AND DOCETAXEL ERADICATES CHEMORESISTANT ANEUPLOID PANCREATIC ADENOSQUAMOUS CA CHARACTERISED BY OVEREXPRESSION OF K-RAS AND HYPERMETHYLATION OF CPG ISLANDS OF P16

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Introduction Adenosquamous Ca is an aggressive and highly metastatic variant of adenocarcinoma with both glandular and squamous differentiation. Usually, it occurs in chemoradiated patients.

Methods Tumour cells were obtained from a resected pancreatic adenosquamous Ca which already had metastasised to regional lymph nodes. Methylation-specific PCR (MSP) detected methylated DNA template of p16. The methylated CpG islands in a promoter of p16 inhibited transcription by preventing RNA polymerase and the RNA transcription machinery from producing messenger RNA leading to gene inactivation. SSCP analysis has detected mutated K-Ras. We constructed an adenovirus p16 expression vector and we inserted p16cDNA into a cassette cosmid containing an adenovirus type 5 genome. Subsequently, we produced a recombinant adenovirus termed as SVN-22/3 by cotransfection of expression cosmid and adenovirus DNA-terminal protein complex into cells by calcium phosphate precipitation.

Results After 1 h treatment with SVN-22/3, pancreatic Ca cells expressed high levels of p16 gene mRNA according to NB hybridisation analysis. The adenoviral mediated gene transfer of wt p16-INK4A formed a heterodimer with