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

doi:10.1136/gut.2011.239301.238

J Giannios* Translational Cancer Medicine, Erasini Oncology Hospital, Athens, Greece

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 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.