Conclusion Male babies and twin pregnancies increased risk AFLP. A high index of suspicion is needed in patients with abdominal pain, malaise and high ALT in the last trimester, the Swansea score should be applied to aid diagnosis, higher Swansea scores indicate greater risk. Recent years have shown improved maternal and foetal survival[2] but correct identification and early obstetric intervention prevents complications such as liver failure.

Competing interests None declared.

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PTU-042 DNA-PK OR ATM INHIBITION INHIBITS NON-HOMOLOGOUS END JOINING AND ENHANCES CHEMO- AND RADIO SENSITIVITY IN HEPATOCELLULAR CANCER CELL LINES

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L Cornell,* J Munck, N Curtin, H Reeves. *Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, UK*

Introduction Hepatocellular carcinoma (HCC) is chemotherapy resistance possibly due to dysregulation of DNA damage signalling and repair. DNA double-strand breaks (DSBs) are the most cytotoxic lesions induced by ionising radiation (IR) and anticancer drugs such as topoisomerase II poisons (eg, doxorubicin). DSBs are repaired by non-homologous end joining (NHEJ), initiated by DNA-dependent protein kinase (DNA-PK), and homologous recombination (HR), reportedly initiated by *Ataxia telangiectasia mutated* (ATM). DNA-PK is up-regulated in HCC (GEO profiles), possibly contributing to anticancer therapy resistance. To assess DNA-PK and ATM as therapeutic targets for chemo- and radio-sensitisation in HCC we determined the effect of their inhibition in HCC cell lines.

Methods DNA-PK and ATM protein levels and activation by IR (Western blot), DSB levels (γ-H2AX foci), HR (RAD51 foci), cell growth (DAPI fluorescence) and cytotoxicity (colony formation) following exposure to IR or doxorubicin was determined in a panel of 6 hepatoma cell lines (HepG2, Hep3B, Huh7, SNU-182, SNU475 and PLC/PRF/5). Studies were performed in the presence and absence of the DNA-PK inhibitor NU7441, and the ATM inhibitor KU55933.

Results DNA-PK protein concentration and activity were high in all HCC cell lines. In contrast, ATM expression varied, and was lowest in Hep3B cells. Cell-specific sensitivities to IR and doxorubicin correlated with ATM expression (highest in HepG2 and lowest in Hep3B). NU7441 sensitised all cells to doxorubicin (average PF_{50} 4.3±3.0) and IR (average PF_{50} 3.9±1.1), significantly increasing growth inhibition and reducing survival (4.8 to 3.3-fold; colony forming assays). KU55933 significantly potentiated cytotoxicity in HepG2 cells (eightfold) but had little effect on cytotoxicity in Hep3B cells. Following exposure to IR, both NU7441 and KU55933 delayed DSB repair (~50% clearance γH2AX foci at 4 h vs only 10%–15% in presence NU7441). NU7441 also enhanced HR (threefold increase in RAD51 foci), while KU55933 had little effect.

Conclusion DNA-PK levels were high in all HCC cells and its inhibition with NU7441 was associated with significant chemo- and radio-sensitisation. Potentiation by ATM inhibition varied in the cell lines, reflecting the level of ATM expression. Both inhibitors substantially impaired the rapid phase of DNA repair commonly attributed to NHEJ. Notably, KU55933 had little effect on HR, suggesting that ATM is not central to this repair pathway. We propose that these inhibitors will increase the effectiveness of lower safer doses of cytotoxic therapies, amplifying tumour toxicity, and that DNA-PK and ATM levels in tumour and non-tumour liver will predict those patient likely to benefit.

Competing interests None declared.

PTU-043 DEVELOPMENT OF A LIVER-SPECIFIC, TUMOUR-SELECTIVE RECOMBINANT ADENO-ASSOCIATED VIRUS VECTOR TO TARGET HEPATOCELLULAR CARCINOMA

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[1]M Della Peruta,* [2]C Binny, [2]D Nathwani, [2]J McIntosh, [2]C Rosales, [3]A M Davidoff, [1]R Williams, [1]S Chokshi, [2]A C Nathwani. *[1]The Institute of Hepatology, Foundation for Liver Research, London, UK; [2]Department of Haematology, UCL Cancer Institute, London, UK; [3]Department of Surgery, St. Jude Children's Research Hospital, Memphis, Tennessee, USA*

Introduction Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and is currently one of the most difficult to treat. Surgical resection and liver transplantation are considered curative therapies but they are feasible for only a small number of patients. Other therapeutic options, such as radiofrequency ablation and arterial chemoembolization, are effective only in small tumours. Recombinant adeno-associated virus (rAAV) vectors are ideally suited for gene transfer-based therapeutic approaches for HCC because of their safety profile and remarkable tropism for the liver, with >90% of the vector particles being detectable in hepatocytes following a single systemic administration in non-human primates.

Methods Sequence complementarity to *miR-122a* (122aT) was cloned downstream of luciferase under the control of a new liver specific promoter/enhancer element, HLP, and flanked by the AAV-inverted-terminal-repeats (ITRs). AAV8 capsid-pseudotyped AAV8-HLP-LUC±122aT vector stocks were prepared by 293T-based three-plasmid transient transfection, and used for: in vitro transduction of HCC cell lines HUH-7 (miR-122a-positive) and SNU-387 (miR-122a-negative); in vivo tail vein injection of tumour-bearing SCID and B6 mice.

Results A single tail-vein injection of rAAV8 in tumour-bearing mice resulted in selective transduction of the liver as well as of HCC, but not neuroblastoma xenografts. We cloned the 122aT into our expression cassette to improve the transgene expression for HCC, taking advantage of the differential expression of miR-122a (abundant in normal liver but down regulated in most HCC). Normalised transgene expression with AAV8-HLP-LUC-122aT was at least 26.5-fold higher in SNU-387 when compared to HUH-7 cell lines. Tail-vein injection of rAAV8-HLP-LUC resulted in high transgene expression in liver, but mice transduced with rAAV8-HLP-Luc-122aT had little or no luciferase expression despite the proviral DNA presence. Subcutaneous HCC xenografts in mice showed a strong luciferase signal following tail-vein injection of both of these vectors.

Conclusion Inclusion of 122aT in the expression cassette allows regulation of rAAV mediated transgene expression in HCC cell lines and normal liver, and reduces expression in miR-122a positive cells (eg, normal hepatocytes). This novel vector, therefore, has the potential to deliver therapeutic transgenes to HCC in preference to normal hepatocytes in the liver, thus limiting toxicity.

Competing interests None declared.