

Are E6 and E7 the key modulators of radiosensitivity in HPV 16 and 18 carcinoma cell lines?

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Objective: HPV-16 and 18 related malignancies lead to significant morbidity and mortality worldwide. Radiotherapy is the primary modality of treatment for these cancers, but outcomes remain suboptimal. Previous research (1) has shown that an antiviral agent, cidofovir, can abrogate E6 and E7 expression, increase p53 and pRb levels, and increase radiosensitivity in vitro and in vivo in HPV positive cells, with no effect on HPV negative cells. The aim of this study is to demonstrate if the oncoproteins E6 and E7 directly modulate radiosensitivity. **Materials and Methods** Human cervical carcinoma HPV-18 cells (HeLa) and murine lung carcinoma HPV-16 cells (TC -1) have been engineered with siRNA E6 and E7 EBV plasmid to selectively decrease oncoprotein expression, using the method developed by D Biard (2). DMEM culture with 10% foetal bovine serum, 1% Hepes Buffer and 1% penicillin and streptomycin was used, and lines were selected with Hygromycin B 125µl/ml. E6/E7 expression was monitored using PCR and Western Blot. Modulation of intrinsic radiosensitivity was assessed in vitro after exposure to 2Gy, 4Gy and 6Gy.

Results: We have confirmed successful knock down of E6 and E7 in HeLa cells, with 80% reduction in levels on PCR and an absent band on Western Blot compared to control. Initial experiments with clonogenic survival assays have shown that for E6 and E7 knocked down HeLa cells, exposure to radiation produces significantly increased radiosensitivity. The survival fraction (SF2) for the control line was normalised to 100%, and for the two E6 lines there was 60% and 11%, and for the E7 lines 38% and 41% clonal survival. Downstream effects of E6/E7 knock down are currently being assessed by measuring p53, p21, p16 and pRb levels with Western Blot. The alteration of DNA repair process and cell cycle distribution will be measured using standard techniques.

Conclusion: These early data have identified E6 and E7 as primary modulators of radiosensitivity in HPV 18 carcinoma cell lines, but further results are due. 1. Abdulkarim B, et al "Antiviral agent Cidofovir restores p53 function and enhances the radiosensitivity in HPV-associated cancers" *Oncogene* Vol 21 No 15, pp2334-2346 2. Biard DSF "Untangling the relationships between DNA repair pathways by silencing more than 20 DNA repair genes in human stable clones" *Nucleic Acids Research* 2007 Vol 35 3535-3550