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MECHANISMS AND MODULATION OF RADIOSENSITIVITY

Fanconi Anemia (FANC) mutations in sporadic head and neck cancer Patients with defects in the FANC pathway have an increased incidence of head and neck cancer (HNSCC) indicating an involvement of the Fanconi DNA repair pathway in the development of sporadic HNSCC. This repair pathway is essential to cell survival upon exposure to crosslinking agents such as mitomycin C (MMC) and cisplatin. Since combinations of cisplatin with radiation are the standard therapy option for patients with advanced HNSCC, this might have implications for the clinic. A panel of 30 primary HNSCC tumour cell lines derived from patients was therefore studied as basis for the evaluation of the functional integrity of the FANC pathway in sporadic HNSCC. These tumour cell lines were tested for MMC-induced G₂ cell cycle blocks, as seen in FANC pathway defective cells. Approximately 20% of the cell lines showed blocks similar to confirmed FANC A or G deficient human fibroblasts. We further analysed survival after MMC in these cells and found a considerable proportion of the cell lines showed a sensitive phenotype, comparable to the FANC-deficient controls. G₂ block data correlated well with the survival data. The cell lines are being further characterized by mutation and expression analyses in order to identify the FANC defects, which will be confirmed by further functional assays. In parallel, we are carrying out high throughput DNA sequencing on a cohort of HNSCC tumours for aberrations and mutations in FANC genes and several other DNA repair genes. For this purpose a capture array has been designed that enriches for over 500 selected genes involved in DNA repair and shown to play a role in HNSCC. First tests show good enrichment and specificity, also indicating high coverage during sequencing, thereby optimizing output and sensitivity.

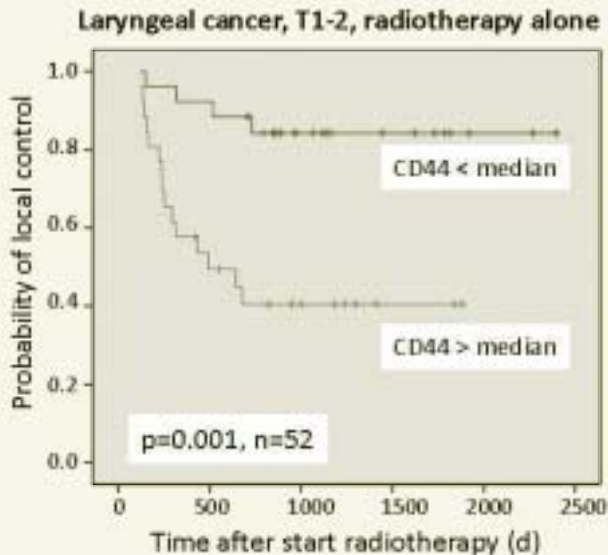


Figure 1: CD44 mRNA expression is predictive for local control after radiotherapy for early stage larynx cancer.

Prediction of outcome In a recent study of early stage larynx tumors from patients treated with radiotherapy alone, we found that expression of the putative stem cell marker CD44 was the most significant predictor of local control. This was validated in a separate series of larynx tumors in which CD44 staining intensity and frequency on a tissue microarray made from pretreatment biopsies correlated with outcome after radiotherapy. We have therefore pursued the relationship of CD44 expression with factors known to influence radiotherapeutic response. We found no relationship of CD44 expression with intrinsic radiosensitivity in a series of 9 larynx cancer cell lines. Further, we knocked down CD44 expression in two squamous cell lines using an inducible shRNA vector and again found no effect on radiosensitivity. In a study to test for a possible relationship with hypoxia, we found no relationship between CD44 expression and either CA9 or HIF-1 alpha expression

by immunohistochemistry on the larynx tissue microarray. Further, in a series of patients given the hypoxia marker drug pimonidazole, no relationship was found between pimonidazole staining and CD44 staining. Finally, a significant correlation was found between CD44 expression and plating efficiency in the 9 larynx cell lines.

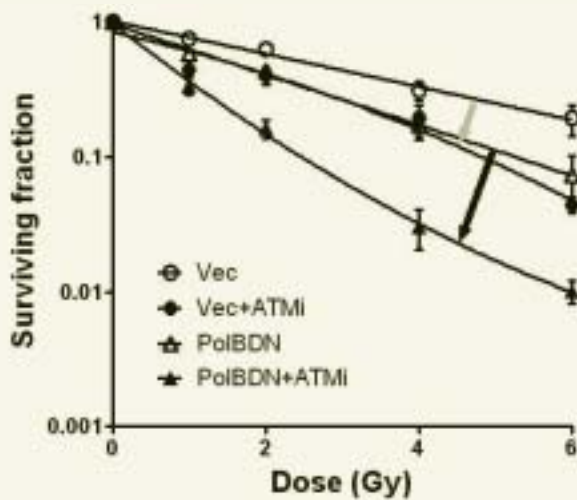


Figure 2: Example of the concept of tumor targeted radiosensitization: tumor cells deficient in base excision repair (PolBDN) show greater radiosensitization (black arrow) by an ATM inhibitor (ATMi) than repair proficient cells (Vec: grey arrow).

These data together support the idea that stem cell content is the dominant factor determining outcome of early laryngeal cancer treated with radiotherapy. In an expression profiling study on 33 head and neck carcinoma cell lines, we found a “classifier” of 288 genes showing a significant correlation with intrinsic radiosensitivity. Of interest was the presence of epithelial-mesenchymal transition (EMT) genes in this gene set. To study the role of EMT genes more directly, we expressed a mutant HIF1 α containing a deletion in the oxygen dependent degradation domain (HIF1 α -deltaODD). This forced EMT and these cells were found to be more radioresistant. In a second cell line pair, EMT forced by SNAIL expression was also accompanied by increased radioresistance. The relationship of radioresistance with EMT has to our knowledge not been previously reported.

Publications

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