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MECHANISMS AND PREDICTION OF TUMOR RESPONSE TO RADIATION

We are pursuing two research lines, one on fundamental aspects of the radiation response and the other aiming more directly at clinical translation. The first involves how the cell handles DNA base damage and single strand breaks, an aspect of radiation damage that has indicated some novel targets for radiotherapy for cancer. The second focuses on prediction of outcome of radiotherapy, particularly using genome wide screening methods, which has also indicated some interesting potential targets.

Mechanisms and modulation of radiosensitivity

Possible involvement of polbDN in DSB induction or repair

We have shown that a polbeta dominant negative (polbDN) radiosensitized mammalian cells, of potential clinical relevance, since a significant proportion of human cancers have polbeta mutations resembling this polbDN. More residual double strand breaks (DSB) after irradiation were found in polbDN-expressing cells than in vector controls, and we therefore tested polbDN's involvement in DSB induction and repair. Studies with a DNA-PK inhibitor indicated no involvement of non-homologous endjoining (NHEJ) in radiosensitization by the polbDN, supporting previous data on DNA repair mutant cell lines. Premature chromosome condensation (PCC) assays indicated secondary double strand break formation after the initial immediate radiation-induced breaks, which was independent of DNA-PK activity. Interestingly, these extra breaks were not accompanied by γ H2AX foci. Irradiation induced more chromosome and chromatid type aberrations in polbDN-expressing cells than in wildtype cells, but an increase in only chromatid type aberrations was seen after H_2O_2 , also an oxidative damage inducer. This indicates a role for clustered damage, unique to ionizing radiation, in polbDN-induced radiosensitization.

We further showed a replication dependence of polbDN radiosensitization, since sensitization was less in confluent than in log phase cells, and could be modeled assuming only S/G₂ cells were sensitized. This is in contrast to polb-deficient (knockout) cells, which were more radiosensitive only under confluent conditions or in G₁ log-phase cells. This is presumed to be partly due to the extra inhibition of long patch repair by the polbDN, and the role and availability of the various backup pathways.

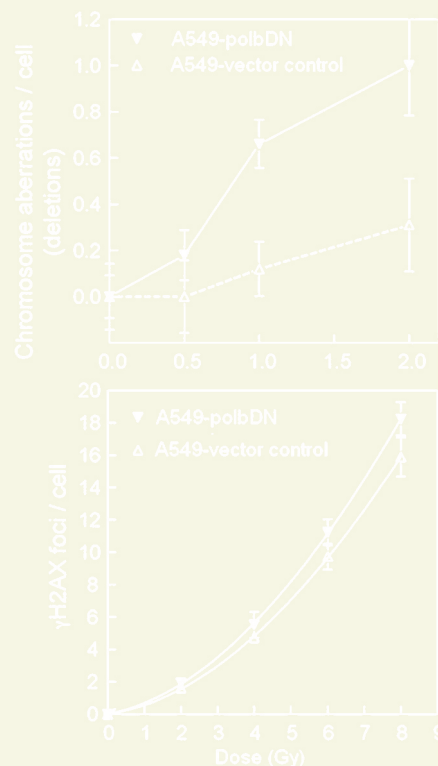


Figure 1: Expressions of a DNA polymerase beta dominant negative (polbDN), known to inhibit base excision repair, causes increased chromosome aberrations after irradiation (upper panel), indicating formation of additional double strand breaks. This is probably the cause of the observed radiosensitization. These extra double strand breaks do not appear to signal the formation of γ H2AX foci (lower panel).

Testing the hypothesis that BER-deficient cells are sensitive to HR inhibition

Synthetic lethality has been shown when both homologous recombination (HR) and base excision repair (BER) are inhibited, arising from BRCA mutations and PARP inhibition. We hypothesized that this synthetic lethality would also arise if cells with mutations in the BER pathway, frequent in human tumors, are treated with HR inhibitors. We are currently testing this hypothesis. 17AAG has been shown to reduce BRCA2 and RAD51, thus affecting HR. Indeed, polDN-expressing cells (BER deficient) were radiosensitized more by 17AAG than wildtype cells. Experiments with XRCC1-knockout and polb-knockout cells (BER deficient) are underway, including treatment with caffeine and other putative HR-associated inhibitors.

To look for novel HR or related inhibitors, we have set up a drug screening assay using the in-house robotics facility, with the goal of finding drugs which radiosensitize BER deficient more than proficient cells. This would provide tumor specific radiosensitization for those tumors exhibiting BER defects. For this, human tumor cells with and without expression of the polbDN were plated and irradiated in 384-well plates. To assess post-irradiation growth, a automated microscopy based cell count assay was found to be far more sensitive than the CellTiter blue viability assay. In order to determine survival accurately after irradiation, assay times longer than 6 days were necessary. We have begun to screen chemical libraries using this assay.

Prediction of outcome We want to understand and predict causes of failure after radiotherapy, allowing selection of patients for alternative more effective therapies. Last year we measured expression profiles of advanced (T3-4) head and neck cancers (4 sites) prior to treatment, to search for genes which predict outcome in patients treated with concurrent radiotherapy and cisplatin (collaboration with departments of Head and Neck Surgery, Radiotherapy and Pathology). We found that a previously published signature, reported to distinguish head and neck cancer patients at high risk of either local or distant recurrence (Chung C et al, Cancer Res 2006), proved to be a highly significant predictor of loco-regional recurrence after combined radio-chemotherapy in our patient series. We have now assessed the performance of this signature in a multivariable analysis with other clinical factors on 75 tumors where we also measured tumor volume at the time of treatment. The Chung signature proved to be independent of tumor site, volume and T-stage, with the signature and site being the most significant, yielding hazard ratios of around 4. This represents an independent validation of a signature, which appears to be independent of known clinical factors affecting outcome, further indicating its potential clinical usefulness. We have also completed a study on a series of larynx tumors from patients treated with radiotherapy alone and with less advanced tumors, representing a more homogenous patient and treatment group. Each patient with a local recurrence was matched for subsite and T-stage with one or two cured patients. This matched series comprised 19 recurrences and 30 cures, all with T1 or T2 tumors, in which pretreatment biopsies were taken and expression profiles measured (Illumina platform). In an hypothesis-driven approach, we tested the predictive potential of signatures known or suspected to affect response to radiotherapy. The putative stem cell marker CD44 was the most significant predictor of outcome (local control), with an acute hypoxia signature showing a strong trend. CD44 also emerged as the most significant gene in a data-driven approach searching for any set of genes distinguishing cures from recurrences. Signatures for intrinsic radiosensitivity and proliferation were not significant. We are now comparing these mRNA expression data with CD44 positivity assessed by immunohistochemistry. Further, we are measuring expression profiles on 30 more advanced (T3) larynx tumors and some oropharynx tumors, both treated by radiotherapy alone.

In parallel, in a search for an intrinsic radiosensitivity predictor, we measured expression profiles on a series of 33 head and neck carcinoma cell lines with a range of radiosensitivities both before and up to 6h after 4Gy in vitro (collaboration with R Grenman, Turku, Finland). No genes which changed expression in response to irradiation were found which correlated with radiosensitivity. In contrast, a set of 288 genes, which did not change after irradiation (remained high or low), were found which showed a significant correlation with intrinsic radiosensitivity. This represents the largest such dataset on site-specific cell lines. We are further investigating and validating the genes in this set.

Publications

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