γ-H2AX as a Predictive Biomarker of Individual Radiosensitivity

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A small percentage of cancer radiotherapy (RT) patients develop abnormally severe side effects to RT, and this small subgroup dictates the RT dose schedules that we use in routine clinical practice. An effective predictive assay that identified such individuals prior to commencing RT would enable a personalized treatment approach. While the genetic basis of radiosensitivity (RS) is complex and not completely understood, an empirical functional assay of RS offers the best option, and the γ-H2AX response to ionising radiation provides the basis for such an assay. The γ-H2AX assay enables screening for functional integrity of DNA double strand break (DSB) repair, and a broad range of genetic and epigenetic variations affecting DSB repair can be detected. The aim of this project is to develop a predictive test that can be used in routine clinical practice to assess the individual RS of cancer patients scheduled for RT.

We have established a database of RS patients comprising individuals who developed abnormally severe late radiation toxicity following RT. For each RS patient, we have identified a corresponding control patient matched for approximate age, gender, tumor type, radiation dose, and time period of RT. In this study, we apply the γ-H2AX assay as the rapid "readout" of DNA damage, to primary peripheral blood lymphocytes and eyebrow hair follicles, by following the γ-H2AX response to *ex-vivo* irradiation. We have shown that the kinetics of γ-H2AX foci and co-localization of these foci with other repair proteins, provides a functional assay to measure individual DNA DSB repair capacity. In the current retrospective validation phase of the project, assay results from RS patients are compared with those from matched control patients. The second stage of the project will be a prospective evaluation of RT patients prior to commencing treatment, and comparison of assay results with subsequent clinical observations.

The results obtained for 16 RS and 12 control patients investigated to date are best described in the context of the four main features of the γ-H2AX response to radiation: the peak response (to various radiation doses), repair rate, fraction of unrepaired component, and co-localization efficiency of γ-H2AX and 53BP1 foci following *ex vivo* exposure of lymphocytes and hair follicles to ionizing radiation. In all of the 16 RS patients, defects were observed for all, or most, of these criteria when compared to control patients.

The rapid read-out of the γ-H2AX assay enables the investigation of DNA repair capacity in *ex vivo* irradiated fresh tissue samples and provides the basis for a reliable biomarker to predict individual patient RS.