COMMENTARY

Radiation and the genome: from risks to opportunities for therapeutic exploitation

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ABSTRACT. On 1 December 2009, the Radiation and Cancer Biology Committee of the British Institute of Radiology (BIR) held a one-day conference on the theme of radiation and the genome. Talks covered genomic instability (its importance for radiation-induced carcinogenesis and potential for exploitation in the development of novel chemoradiotherapy combinations) and the prospects of exploiting knowledge of the genome to understand how individual genetic variation can impact on a patient's likelihood of developing toxicity following radiotherapy. The meeting also provided an overview of stem cell biology and its relevance for radiotherapy in terms of both tumour (somatic) and normal tissue (germline) sensitivity to radiation. Moreover, the possibility of manipulating stem cells to reduce radiation-induced normal tissue damage was considered.

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Constant challenges to the mammalian genome can result in genomic instability. Exposure to reactive oxygen species, environmental genotoxic agents and radiation causes DNA damage. Failure to repair this damage, which is enhanced by defects in DNA metabolism and error-prone DNA repair, makes the genome unstable and prone to a wide variety of chromosome aberrations.

Genomic instability is a key process in carcinogenesis. Professor Eric Wright (University of Dundee, UK) gave an overview of radiation-induced genomic instability. An emphasis was placed on the non-targeted effects of radiation, that is, radiation-induced effects in cells that are descendants of irradiated cells or in cells neighbouring irradiated cells (radiation-induced bystander effects). These radiation-induced effects are genotype dependent and have properties associated with inflammatory processes. The effects correlate with macrophage stimulation in mice and the activation of a cascade of pro-inflammatory response genes, such as those encoding tumour necrosis factor and nitric oxide synthase. Stimulated macrophages appear to contribute to secondary non-targeted and delayed radiation effects. Work in the area is consistent, with a persistent tissue reaction to radiation injury characteristic of an inflammatory response. Importantly, the available data suggest that ionising radiation might contribute to tumorigenesis, and, in particular, the development of childhood leukaemia, by promoting initiated cells rather than being the initiating agent. A long-term inflammatory response

might also contribute to other radiation-associated pathologies (e.g. late normal tissue damage).

Professor Thomas Helleday (Gray Institute for Radiation Oncology and Biology, Oxford, UK) gave an overview of the genetics of genome instability associated with cancer cells. He described how DNA repair pathways can enable tumour cells to survive DNA damage induced by chemotherapy and discussed how they provide therapeutic targets to increase genomic instability and enhance tumour cell kill. Furthermore, he described how mutations can make some cancer cells reliant on a reduced set of DNA repair pathways for survival. There is evidence that drugs designed to inhibit one of these pathways in such tumours could prove useful as single-agent therapies, a process known as synthetic lethality, with the potential advantage that this approach could be selective for tumour cells and have fewer side effects. One such example is the treatment of *BRCA1/2*-deficient tumours, which are defective in homologous recombination (HR) repair, with poly-ADP-ribose polymerase (PARP) inhibitors. These agents induce single-strand breaks that can result in double-strand breaks (DSBs) as a result of stalled replication forks. Such lesions would normally be repaired by HR, but this is prohibited in *BRCA1*- or *BRCA2*-deficient cancer cells. This strategy is showing promise in clinical trials. Professor Helleday also suggested that PARP inhibitors could be useful in combination with radiation therapy, as fractionated radiotherapy forces cells into the G2 phase, where the cells become dependent on HR for repair. Finally, he suggested that inhibition of the protein kinase ataxia telangiectasia mutated (ATM) and DNA-dependent protein kinase (DNA-PK) would be useful in combination with radiation. DNA-PK is important in the

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process of non-homologous end-joining (NHEJ), which is required for the repair of radiation-induced DSBs. Cells defective in DNA-PK are highly sensitive to ionising radiation, suggesting that inhibition of DNA-PK might sensitise tumours to radiation treatment. Likewise, ATM is central to cellular responses to DSBs. The rationale behind the clinical use of ATM inhibitors is based on the premise that ATM inhibition should improve the therapeutic index by hypersensitising tumour cells to agents that cause DSBs, such as radiotherapy.

Dr Geoff Lawrence (Clatterbridge Centre for Oncology, UK) continued the theme of radiation-induced genomic instability by highlighting the risk of secondary cancers following radiotherapy. The risk was illustrated using findings from a US study that analysed 1319 patients with Hodgkin's lymphoma. The study reported that 181 patients developed secondary malignancies and showed that the risk was higher with increasing radiation field size. Dr Lawrence highlighted the potential for adaptive image-guided radiotherapy to reduce the risk of long-term secondary cancer by reducing radiation field sizes during treatment, while improving local control via dose escalation to smaller central tumour volumes.

Another approach being investigated to reduce radiation-induced normal tissue toxicity involves the exploitation of knowledge gained through sequencing the human genome and developments in high-throughput techniques for assessing genetic variation. Improvements in radiotherapy are increasing the number of cancer survivors, which makes it important to find ways to reduce the number of patients suffering from long-term side-effects of treatment. Dr Chris Talbot (University of Leicester, UK) described the potential of identifying genetic polymorphisms that predispose to or protect against radiation toxicity. The identification of these genetic variants might provide a way of predicting a patient's likelihood of developing side-effects and be exploited by developing a genetic test that could be used to personalise radiotherapy dose prescriptions. Work in this area requires detailed recording and analyses of radiation toxicity data, which will increase understanding of the relationships between different end points. A study carried out in Leicester showed a relationship between the development of telangiectasia and the long-term risk of developing cardiovascular disease in breast cancer patients, suggesting that a common aetiology might be involved. Genotyping studies require large numbers of patients, facilitated by local, national and international collaboration. Attention was drawn to the recent establishment of the International Radiogenomics Consortium for pooling genotyping data to enable adequately powered studies to be carried out.

Gill Barnett (University of Cambridge, UK) described some of the first results emerging from the national RAPPER (Radiogenomics: Assessment of Polymorphisms to Predict the Effects of Radiotherapy) study. RAPPER has collected more than 2500 samples from patients enrolled in radiotherapy trials across the UK. The first data analysed showed no relationship between polymorphisms in the *TGFB1* gene, currently the most widely studied gene in this area, and radiotherapy toxicity. Ongoing work is attempting to validate 68 single-nucleotide polymorphisms that have previously been reported to

be associated with radiation toxicity. With the exception of one or two of the genes, most have shown no association with toxicity. Thus, the findings to date highlight the need for confirmatory studies and large sample sizes.

The afternoon session centred on stem cell biology and started with a fascinating talk from Dr Robert Coppes (University of Groningen, the Netherlands). He provided compelling evidence that stem cells could be used to reduce the types of radiation-induced normal tissue damage introduced by the previous speakers. In particular, he focused on xerostomia (or dry mouth syndrome) resulting from radiation-induced hyposalivation — a problem encountered after radiotherapy for head and neck cancer. Using the salivary gland as a model, he demonstrated both *in vitro* and *in vivo* that stem cells isolated from salivary ducts could be used to restore damaged salivary ducts following exposure to radiation; increased saliva production was observed in mice after transplantation of donor stem cells. The ability to translate this into the clinic looks promising, as he demonstrated that stem cells could be effectively isolated from patients before radiotherapy. These cells self-renewed *in vitro* for up to seven passages, were able to form salivary gland structures and had the potential to reduce radiotherapy-induced salivary gland dysfunction.

Dr Susan Short (University College London, UK) went on to present data on the use of mesenchymal stem cells (MSCs) to study the genetics of tumorigenicity and its effect on radiosensitivity. In an elegant series of experiments she used a range of cell lines demonstrating the transition to tumorigenesis by stepwise transformation with human telomerase reverse transcriptase (hTERT), human papilloma virus 16 (HPV-16), simian virus 40 (SV40) and Harvey Ras (HRas). She showed that more tumorigenic cells had increased radiosensitivity, had more residual DSBs and exhibited increased apoptosis compared with immortalised parental cells or the less fully transformed MSCs. This study showed that, at least in this cell type, the fully transformed MSCs did not demonstrate the typical radioresistant phenotype often reported for cancer-derived stem cells.

Dr Rob Clarke (University of Manchester, UK) then presented evidence that breast cancer stem cells (CSCs) are resistant to radiotherapy. He demonstrated that CSCs derived from both primary cancer and breast cancer cell lines preferentially survive radiotherapy, showing a huge (50–90%) increase in survival compared with normal cells. However, Dr Clarke also presented data to suggest that targeting the Notch4 receptor, which has a key role in tumour initiation by CSC-enriched populations, might be effective in preventing radiation resistance. Notch inhibition decreased CSC activity *in vitro* and reduced the growth of breast tumours by up to 50%. The use of this inhibitor in combination with radiation therapy certainly suggests a way forward.

Two talks followed, which further interrogated the hypothesis that CSCs contribute to radioresistance in a range of cell lines/tissues of different tumour types. Dr Anthony Chalmers (Brighton and Sussex Medical School, UK) discussed similar features with respect to CSCs derived from glioblastoma cells. Glioblastoma multiforme are high-grade brain tumours that are refractory to radiotherapy, possibly because of a radio-resistant CSC population. He demonstrated that, under

certain conditions, CSCs derived from both primary and glioma cell lines enriched for CD133 expression were resistant to radiation. However, Dr Chalmers also highlighted the complexity of these experiments, as the cellular response appeared to be dependent on the microenvironment, and stressed that conditions needed to be carefully monitored to observe these effects. Dr Thomas Brunner (Gray Institute for Radiation Oncology and Biology, Oxford, UK) went on to assess the radio-resistant phenotype of CSCs derived from a whole series of cell lines used routinely. He demonstrated that CSCs derived from MDA-231 cells sorted for CD24⁻/CD44⁺/ESA⁺ were indeed radioresistant and had elevated Notch1 together with an increased ability to repair damage after radiation. After three weeks growth these cells developed radiosensitivity again as they became more heterogeneous; the radioresistant phenotype was better maintained in serum-free conditions. However, these effects were not observed in a second breast cancer cell line, MDA-MB-468, even though Notch1 levels were high, or in HCT-116 cells. Cell type will clearly be an important factor when analysing CSCs *in vitro*.

Finally, Dr John Hall (University of Manchester, UK) finished the day with a presentation on cervical cancer, stem cells and radiosensitivity. He used a microarray approach to derive a gene signature using pre-treatment formalin-fixed paraffin-embedded cervical

carcinoma specimens, for which intrinsic radiosensitivity (surviving fraction at 2 Gy, SF2) had previously been measured. The gene profiling identified a novel gene not previously implicated in tumour radiosensitivity. The gene was validated using immunohistochemical techniques and high expression strongly correlated with SF2 and a good outcome following radiotherapy.

Overall, the symposium provided a balanced overview of radiation-induced genomic instability and its association with tumorigenesis and normal tissue toxicity following radiotherapy. Our ability to target genomic instability with new DNA repair inhibitors and approaches to measure normal tissue and tumour radiosensitivity were major themes. The day also provided a fascinating insight into the role of stem cells in promoting radioresistance. We considered how stem cells could be targeted with novel Notch-targeted agents to improve therapeutic outcomes or harnessed to repair normal tissue damage following radiotherapy. This will be an exciting area to follow in the future.

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