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Identification and isolation of susceptibility genes involved in radiation-induced cancer in humans

(SUS GENES IN RAD CAR)

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(summary)

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Introduction

Humans are exposed to ionising radiation (IR) from natural (e.g. cosmic radiation, radon) as well as man-made (e.g. diagnostic X-rays, radiotherapy) sources. With nuclear power likely to assume increasing importance in EU countries during the coming decades as global oil reserves are depleted (for a recent review, see *Nature* **424**, 129, 2003), the precise mechanisms by which IR acts as a human carcinogen will become even more urgent, particularly with respect to the carcinogenicity of low-level radiation exposures. To be properly able to anticipate the risks of ionising radiation exposure and provide updated safety advice both to the public and to those whose work exposes them to IR, a detailed understanding of mechanisms is essential. For example, a great deal more by way of hard scientific information is needed about the nature of the molecular genetic alterations involved in radiation-induced cancer in humans. Identification of the key target genes and associated cellular pathways will furnish a basis for epidemiological studies, using novel molecular information, aimed at determining the precise role of ionising radiation in the development of human cancers. It may also lead to rapid screening procedures for predicting individual risk. This was the overall aim of the project SUS GENES IN RAD CAR.

Objectives of the project

The project was built on the considerable progress made in the Fourth Euratom Framework Programme (FP4) by a group of experienced European scientists. It was designed to provide mechanistic information outlined above and was founded on the following specific key objectives: (i) to develop human cell culture models for radiation-induced cancer based on clinically relevant cell types (e.g. breast epithelial cells); (ii) to pinpoint specific chromosomal regions in ionising radiation-induced malignant cells (obtained from radiation-induced cancers and malignant cells generated in culture) that may harbour target oncogenes (cancer genes) and tumour suppressor genes; (iii) to localise and isolate novel genes altered in human radiation carcinogenesis, using advanced cell genetic and molecular biological techniques already developed to a high level of sophistication by members of the consortium; and (iv) to provide an improved molecular description of the source of radiation-induced genetic instability, its relationship to altered telomere dynamics and telomerase, and a molecular characterisation of sites of genomic fragility in human chromosomes that are preferentially involved in IR-induced chromosome translocations. The project's key goals were reflected directly in four closely integrated work packages that formed the basis of the work programme of the project.

Research results

Two main approaches were adopted to study the process of malignant transformation of human cells in which a causal role for ionising radiation in the process could be confidently assumed. These were: (i) the study of human cancers associated with radiation exposure, namely thyroid cancers from Belarus and, from the clinic, secondary tumours that had developed in the radiation field following radiotherapy for an unrelated primary tumour, and (ii) the development and molecular characterisation of human cell-culture models responsive to malignant transformation by IR. With regard to the latter, we exploited the use of the gene (*hTERT*) encoding the catalytic sub-unit of the human telomerase (telomerase is activated during the development of 90 % of all human cancers) to generate immortalised human cell

clones, with normal chromosomes and growth characteristics, from clinically relevant tissues such as the human breast. Such cultures were then used as target systems for malignant conversion by ionising radiation.

The success of the project consortium in establishing cell-culture models for characterising early key steps in radiation-induced malignant transformation exceeded initial expectations. Two cell culture models were established, one based on human breast epithelial cells, in which additional transformation events could be induced following exposure to gamma and X-radiation. Interestingly, the most effective treatment regimes were those involving multiple fractionated radiation doses. Exhaustive characterisation of chromosome changes in cell lines transformed in this way revealed the presence of several common alterations associated with transformation (e.g. deletions involving chromosome-13 and gene amplifications involving chromosome-10) and a candidate novel oncogene was identified. Substantial progress was made in cloning two additional radiation-induced breakpoint-associated genes localised to chromosomes-17 and X, and in localising specific genes involved in two further aberrations (chromosomes 3 and 11) identified in radiation-transformed human breast epithelial cells. Similarly, in thyroid cancers from Belarus, breakpoint cloning resulted in the identification of the gene involved in a critical cancer-associated chromosome translocation. Cytogenetic and molecular analysis of secondary radiation-induced cancers (following radiotherapy of the eye orbit for an inherited eye cancer) produced a number of novel results, including direct demonstration of the involvement of two tumour suppressor gene-inactivating alterations and the identification of a possible radiation-specific molecular signature. Collectively, these results shed much new light on the mechanisms by which ionising radiation acts during the malignant transformation of human cells.

The identification of tumour suppressor genes central to the development of human breast cancer, an important tumour type in the context of ionising radiation, produced two sets of major findings. First, the gene responsible for the tumour-suppressive activity of human chromosome-1 in breast cancer cells (located in the frequently lost 1p35 region) was identified unequivocally as that encoding the epithelial-specific marker and growth suppressor 14-3-3 sigma. This gene was subsequently shown to be involved in our *in vitro* breast cell transformation system, suggesting a role in radiation-induced transformation. Second, major advances were made in understanding the genetics and molecular biology of telomerase repression in normal human cells (which must be overcome for human cancers to develop). We showed that the all-important repressor gene acts via transcriptional repression of the gene encoding a component of telomerase (known as *hTERT*) by a mechanism that involves repackaging of the gene. These results, recently published in high-impact journals, will be extremely valuable in facilitating the identification and isolation of the repressor gene itself, which we have recently been successful in locating to an extremely small region of normal human chromosome 3 (within band p21).

Telomerase activation is a necessary step in human cancer development as it is needed to maintain cancer cell immortality. The importance of telomerase activation in radiation-induced cancer was determined in both *in vitro* (cell culture) and *in vivo* (genetically manipulated mouse models). In the latter, clear experimental evidence was obtained (resulting in a number of high-impact publications) that telomerase substantially enhances susceptibility to cancer induction, and that its absence acts to suppress tumour development. The role of telomerase in human epithelial carcinogenesis was established using cell culture models based on breast epithelial cells, and with cultures derived from human head and neck cancers and premalignant conditions. The latter investigation identified loss of a key tumour suppressor

gene (known as *p16*) and telomerase activation as co-operating early events in the immortalisation process during cancer formation. In associated studies, additional molecular events that are likely to contribute to human cells immortalisation were identified.

The core objectives formulated at the start of SUS GENES IN RAD CAR were accomplished and, in some cases, exceeded. Several candidate genes and sub-chromosomal regions that may be important in human radiation carcinogenesis will require further characterisation and/or identification. Nevertheless, current understanding of how ionising radiation acts to promote malignant transformation in human systems has been significantly advanced by this project.

Implications of results for radiation protection

It has been emphasised above that a substantial improvement in our understanding of the molecular mechanisms by which ionising radiation acts as a carcinogen is needed to enable decisions on the potential hazards of repeated exposure to low doses of IR to be made with confidence. SUS GENES IN RAD CAR has generated new knowledge about the process of radiation-induced carcinogenesis, through the identification of novel gene and chromosomal alterations that appear to play a causal role in malignant transformation. In addition, technical advances in the development of human cell culture models of malignant transformation have permitted early events in radiation-induced carcinogenesis to be induced and studied in sequence in the laboratory. Such models will undoubtedly continue to make a major contribution towards our ultimate goal of achieving a complete molecular description of radiation-induced cancer in man.