Molecular and Cellular Mechanisms in Radiation Carcinogenesis

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Substantial epidemiological data exist which implicate ionising radiation as a causative agent for a broad range of human cancers (1). To date, epidemiology has been able to provide good evidence of increased cancer risk for single acute radiation doses greater than 100 mGy. Understanding and estimation of radiation cancer risks below this level remain problematic and controversial. Developing an in depth knowledge of the mechanisms by which ionising radiation causes cancer should help to strengthen low dose risk estimates.

In recent years there have been significant advances in the understanding of many basic cellular processes including the regulation of cell proliferation. Unrestrained cellular proliferation is the key characteristic of cancer. The causes of this unrestrained proliferation lie in alteration of the normal patterns of expression of genes involved in proliferative control. Powerful tools for molecular genetic analysis are now available to help elucidate the genetic alterations in cancers. Cell biological and biochemical methods can be used to define the cellular consequences of these genetic alterations.

The basis for much of the understanding of genetic mechanisms in carcinogenesis has its origins in the study of chromosomes. Two broad categories of chromosomal rearrangement have been identified in human and animal tumours: translocations and deletions. Chromosome deletion events lead to the loss of genetic information while translocations cause the rearrangement of genes but not gene loss. Translocations are largely, but not exclusively associated with, haematological malignancies (see reviews 2,3). Deletions are more commonly associated with solid tumours (see 2,4) although deletions have also been observed in some human haematological malignancies such as acute myeloid leukaemias (AMLs, eg. 5,6). The consistent association of a particular chromosomal rearrangement with a specific tumour is indicative of the importance of genes on the involved chromosomes in the development of the tumour.

There is now a very large number of consistent chromosomal translocations associated with specific tumours (eg. 2,3). Among those identified are the t(8;14) in Burkitts lymphoma (BL), t(10;14) in T cell acute lymphocytic leukaemia (T-ALL), t(9;22) in chronic myelogenous leukaemia (CML) and t(15;17) in acute promyelocytic leukaemia (APML). Molecular analysis of these translocations has led to the identification of the genes involved and given some indications of the consequences of the translocations. Essentially two classes of translocation have been identified, those that lead to the over expression of one of the partner genes and those in which a novel fusion gene is formed from which a fusion oncoprotein is produced. Translocations of the over expression type are common in lymphocytic leukaemias. In T-cell leukaemias one of the genes is commonly a T-cell receptor (*TCR*) gene while in B cell leukaemias immunoglobulin (*Ig*) loci are frequently involved. These genes are actively expressed in the corresponding normal cell type. Genes which are otherwise silent (ie. not expressed) can become active when translocated into the vicinity of one of the highly expressed genes. Thus in Burkitts lymphoma the *MYC* gene is over expressed due to its translocation close to an *Ig* gene locus and in the t(10;14) in T-ALL the expression of the *HOX11* gene is driven by a *TCR* gene. Examples of gene fusions include *BCR-ABL*, involved in the t(9;22) in CML and *PML-RARA* in APML. These fusions lead to the production of proteins with abnormal functions.

Both the over expression and gene fusion translocations can be considered 'gain of function' mutations, ie. normal genes are expressed inappropriately or novel fusion genes are expressed. These events are in general genetically dominant, that is only one such change is sufficient to give an effect. The products from this group of proto-oncogenes are frequently involved in the processes of signal reception and transduction from the cell membrane to the nucleus (signal transduction pathway genes) or more directly in the regulation of gene activity (transcription factor genes). Gain of function mutations can also be on a smaller scale as in the *RAS* gene base pair mutations associated with rodent skin, breast and liver tumours (7,8,9).

Deletion events involve a class of genes known as tumour suppressor genes. Frequently, these genes are involved in the control of cell proliferation, differentiation or transcription. Consequently, upon the loss or mutation of such genes, proliferation may be enhanced of differentiation blocked. The classic examples of deletions in cancers are the del(13) involving the *RB1* gene in retinoblastoma and the del(11) in Wilms tumours in which the *WT1* gene is lost. In general these events are genetically recessive and so loss or mutation of both copies of the gene is necessary for a phenotypic effect. The gene which has been found to be most commonly lost or mutated in human cancers, *p53* (10,11), also acts as a tumour suppressor although some dominant negative mutations exist.

A single genetic alteration is rarely, if ever, sufficient to give rise to a malignant tumour. In solid cancers it is likely that 5-10 genetic or epigenetic alterations are required. In haemopoietic system cancers it is probable that fewer events are necessary. Where a very consistent genetic alteration is associated with a specific cancer, it is a good candidate for an initiating event. However, many of the genetic changes in tumours have a less strong and specific association. A gene very consistently altered in one tumour may also be involved in other tumour types but in a less specific fashion. One of the best studied human cancers is colorectal cancer in which consistent and early changes to tumour suppressor genes is followed by an accumulation of secondary events (12).

These basic principles of carcinogenesis have in the main been derived from the analysis of human tumour material. The identification of human tumours which are known to be associated with radiation exposure is very difficult. Therefore much of the current research on radiation carcinogenesis exploits animal models. At NRPB there is a strong interest in mechanisms of radiation-induced AML in the mouse. This system will be described to give a flavour of how molecular and cytogenetic methods can be exploited to understand mechanisms of radiation carcinogenesis.

AML can be induced to a maximum frequency of ~25% of CBA/H mice following a single acute x-ray exposure (13,14). Radiations of other qualities such as α-particles and neutrons can also induce AMLs in this inbred mouse strain (16,17). Cytogenetic analysis of radiation-induced AMLs by G-banding revealed a consistent chromosomal aberration, deletion or translocation of one chromosome 2 homologue (18). Similar aberrations have been found in AMLs induced in other mouse strains (19-21). Chromosmoe 2 aberrations can also be detected at 5 days - 1 month post irradiation in bone marrow cells (18), well before the average 18 month latent period required for full tumour development. Not only do chromosome 2 aberrations form early but they form in excess by comparison with other chromosomes - an approximately 2.5 fold excess of chromosome 2 events was noted at sample times between 1 and 6 months post-irradiation (22). In this study (22) it was found that all irradiated animals carried chromosome 2 aberrations, so while these aberrations are very early and possibly initiating events, they do not determine which individuals will develop AML. Similarly this experiment indicated that secondary events, undetectable at the cytogenetic level, contribute to leukaemogenesis.

Further resolution of the DNA sequences involved in mouse leukaemogenesis has required the development of a system for molecular mapping. By exploiting differences in allele sizes between inbred strains for certain abundant and widely distributed molecular markers known as microsatellites, mapping of deletions in AMLs developing in irradiated F1 mice has been possible (23). Chromosome 2 deletions

were detected in about 65% of AMLs arising in x-irradiated F1 CBA/H x C57Bl Lia mice. While the breakpoints involved in the deletions were not entirely consistent, clustering was observed and it has been possible to define 5 deletion types (23). In addition to defining breakpoints at the molecular level it has been possible to identify a minimally deleted region of about 30 cM in size. In the absence of strong breakpoint specificity, a mechanism of tumorigenesis involving abnormalities in gene expression seems unlikely, gene losses are probably of greater importance.

The microsatellite markers associated with breakpoints and the minimal deleted region can be used to isolate larger DNA clones in these areas. Such clones can then be exploited for further molecular and cytogenetic resolution of the AML associated genetic alterations. Using the technique of fluorescence *in situ* hybridisation (FISH) with these clones, it is possible to quantitate more accurately the frequency of these AML associated aberrations at very early post irradiation times. Thus, the size of the pool of radiation-initiated cells can be defined in individual animals. Through further genomic-wide screening of AMLs by microsatellite analysis, additional target genes for acute myeloid leukaemogenesis may be identified. FISH experiments as described above should enable the assignment of a temporal position for such changes in the leukaemogenic process.

One further issue in radiation carcinogenesis which can now be addressed is that of genetic predisposition. Within human populations certain rare cancer prone individuals and families exist. Some of these cancer prone syndromes are associated with the inheritance of genes which encode elements of DNA damage processing systems. Examples of this category are xeroderma pigmentosum (24), hereditary non-polyposis colon cancer (25) and ataxia telangiectasia (26). Inheritance of classic tumour suppressors such as *RB1* and *WT1* underlie familial retinoblastoma and Wilms tumour respectively (see review, 4). Proto-oncogenes at present seem rarely to be associated with cancer predisposition. The only clear example here is the *RET* proto-oncogene involvement in multiple endocrine neoplasia type 2A (27). As yet there are no clear cases of a human gene or genes predisposing to radiation-induced cancer. However, with the recent identification of a gene mutated in ataxia telangiectasia (28) this situation may change. The differences in tumour incidence between strains of mice have been exploited to map tumour susceptibility loci (eg. 29). Such strategies can also be applied to radiation-induced cancers. It has proven possible to generate artificial mouse mutants susceptible to radiation carcinogenesis (30).

To summarise, the advances in the understanding of the process of carcinogenesis in general and radiation carcinogenesis in particular can benefit mechanistic modelling of radiation cancer risk. A knowledge of radiation cancer associated genetic alterations and their temporal sequence allow the construction of valid models which can be exploited in risk estimation. Furthermore, the target gene alterations identified can be used for screening of individuals for preneoplastic lesions and in some case suggest rational bases for therapy.

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