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The genetics of radiation-induced and sporadic osteosarcoma: a unifying theory?

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Abstract

Cancer is a disease of the genome, with the neoplastic phenotype being passed from one cell generation to the other. Radiation-induced cancer has often been considered to represent a unique entity amongst neoplasia, with the energy deposition being held responsible for both direct (gene mutations) and indirect (bystander effects, induced instability etc) alterations to the cellular genome. However, radiogenic tumours in man and experimental animals appear to be physiologically and genetically indistinguishable from their sporadic counterparts, suggesting that the aetiologies of these two tumour types are in fact closely related.

We have conducted a general screen of the genetic alterations in radiation-induced mouse osteosarcoma, a tumour that is histopathologically indistinguishable from human sporadic osteosarcoma. Comparison of the two tumour types indicates the existence of a common set of genetic changes, providing additional evidence to support the concept that the molecular pathology of radiation-induced malignancy is no different to that of sporadic cancers.

1. Cancer is a disease of the genome

The cancer phenotype of a cell results in uncontrolled malignant cell growth, and is transmitted via the genome [1–4]. Thus after each division of a malignant cell both progeny tumour cells inherit the malignant phenotype [1]. This malignant capacity can be artificially transferred to a hitherto normal cell through the injection of purified tumour cell DNA. The transforming principle of oncogenic (transforming) viruses resides with specific genes encoded from the viral nucleic acid genomes. Indeed, expression of the transforming viral genes on their own can induce a malignant phenotype in a transfected cell [3, 4]. Final evidence for the genetic nature of cancer is furnished by the genetic analysis of the inheritance of the phenotype in

numerous cancer prone families, where simple Mendelian patterns of inheritance indicates a single gene mutation to be responsible for the inherited cancer [2].

2. Are there specific radiation-induced genetic changes in cancer?

Ionising radiation is a potent inducer of macromolecular damage in mammalian cells, and several studies have demonstrated that ionising radiation induces a highly reproducible set of mutations in DNA [5,6]. These mutations are potentially transmissible, and in the absence of efficient DNA repair processes will become established. Clonal expansion of cells retaining such damage can lead to malignant changes. Given the genetic nature of cancer, and the ability of ionising radiation to generate genetic alterations, it is not surprising that considerable effort has been put into identifying radiation-specific gene mutations in cancerous tissues arising after irradiation.

Two concepts of radiation-induced alterations are currently propounded. Firstly, a specific radiation-induced pattern of genetic alterations induced in the tumour cells, and secondly, a non-specific radiation-induced genomic instability passed on during cell division to succeeding generations. Both phenomena have been well characterised using *in vitro* model systems, but very little evidence has been accumulated to demonstrate their existence in tumours that have arisen as a result of exposure to ionising radiation.

A number of small-scale studies have succeeded in identifying gene mutations in radition-induced malignancies. Although no direct comparisons have been made between the radiation-induced tumour and its sporadic counterpart, it is possible to compare the two tumour types using contemporaneous studies of spontaneous cancers. The most convincing evidence for a radiation-specific effect has been presented in alpha-particle-induced lung tumours in man. An initial report of the genetic analysis of lung tumours in radon-exposed subjects described a very high incidence 16/53 (31%) of one specific p53 mutation, affecting codon 249 [7]. Despite great effort, this potential radiation fingerprint has never been seen in similar, but not identical, populations exposed to inhaled alpha-emitting irradiation [8,9]. The presence of a specific point mutation in alpha-particle-induced tumours is contrary to *in vitro* evidence of mutations induced by alpha particles, and suggests there is no causality of the mutation by the alpha-particle irradiation. The lack of reproducibility of this potentially significant finding also suggests that there is no fingerprint in these tissues.

A number of other studies have suggested the existence of other radiation-specific gene mutations in the radiation-induced tumours. These include Ki-*ras* mutations in rat lung tumours [10,11] and thorotrast-induced liver tumours in man [12] and in leukaemia in A-bomb survivors. In all of these studies a subsequent comparison with non-radiation-induced tumours of the same histological type and stage of cancer revealed similar genetic alterations in both materials [13]. In papillary thyroid cancers found amongst children exposed to the fallout from Chernobyl there have been a number of reports describing translocation events of the RET oncogene. Initial evidence from these studies suggests that there may indeed be a unique form of translocation present in the PTC of young children after iodine-131 ingestion. However, the frequency of this type of translocation in tumours of the same histological type developing in such young subjects has not been determined, and it is quite possible that all such tumours have this form of RET translocation, irrespective of possible radiation aetiology.

Radiation-induced acute myeloid leukaemia of the mouse is induced by several different radiation qualities, from x-rays through to neutrons. Whilst a tumour-specific interstitial deletion of chromosome 2 has been found, it is not dependent upon the LET of the inducing radiation [14]. Thus, both high and low LET are effective in causing large interstitial deletions, suggesting the event is associated with AML formation and not the initial irradiation event. As

a detailed molecular genetic analysis of sporadic AML in the mouse has not been published, the final confirmation of the radiation specificity of the chromosome 2 effect remains undecided.

A causal relationship between ionising radiation and specific tumour gene mutations in malignant tissue has not yet been established. In the cases reviewed above, both the mutation type and frequency seen in radiation-induced malignancies was comparable to that seen in the appropriate control tumours. In cases where doubt remains, the lack of appropriate control samples precludes a firm conclusion.

3. A common set of genes is involved in radiation-induced and sporadic osteosarcoma

In mice sporadic osteosarcoma is a rare disease, but an incidence of almost 100% can be achieved by exposure to alpha-particle-emitting bone-seeking radionuclides, such as thorium-227. The radiation-induced osteosarcoma is histopathologically indistinguishable from sporadic mouse and human tumours, allowing a direct comparison of the genetic changes in radiation-induced mouse and sporadic human osteosarcoma. A characteristic of both tumour suppressors and oncogenes is that the number of copies (alleles) of the gene in the genome is frequently altered during oncogenesis. The resulting allelic imbalance (AI) can be quantified and used as a means of locating the gene(s) involved. We have conducted a genome-wide screen for AI in osteosarcomas induced in (BALB/c × CBA) F1 hybrid mice by parenteral application of the alpha-emitting radionuclide thorium-227. AI was detected by DNA microsatellite allelotyping. Ten loci showed AI in more than 50% of tumours.

Seven of the ten loci were found to correspond to sites of known oncogene/suppressor genes that have been previously implicated in human osteosarcomagenesis. We have mapped the syntenic regions of the three novel loci to the human genome, and established that they too are all involved in human osteosarcoma. We conclude that the genetic alterations, and hence carcinogenic mechanism, in sporadic human osteosarcoma and radiation-induced osteosarcoma in the mouse are astoundingly similar.

4. If there is a common mechanism, does this exclude a unique radiation mechanism of cancer?

Study of the genetic mechanism of radiation-induced osteosarcoma has revealed the involvement of a set of loci harbouring both tumour suppressors and oncogenes. These loci are indistinguishable from those involved in sporadic human osteosarcoma. Thus, osteosarcomagenesis *per se* is initiated by a single set of genes, and, irrespective of aetiology, there are no additional genetic events required. The lack of a radiation fingerprint strongly suggests that both tumour entities arise by the same mechanism.

Given the suggestion that genetic instability induced by ionising radiation *in vitro* is a random agent for genetic alteration, it is hard to suggest instability as the causal agent for the ordered and highly selective events seen in osteosarcoma. Indeed, examination of the entire osteosarcoma genome has failed to provide any evidence for an instable genome. We conclude that, at least for osteosarcoma, there is no evidence to suggest the existence of a unique pathway of genetic alteration in radiation-induced cancer. The involvement of genetic instability in the formation of a radiation-induced tumour *in vivo* is not evident in osteosarcoma.

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