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Genetics of predisposition to radiation-induced cancer of the thyroid (GENRAD-T)

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Final report
(summary)

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Generic research in radiological sciences

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Objectives

The strategies available for estimating the risk of detrimental health effects after exposure to ionising radiation are changing rapidly. Research conducted by Euratom-funded laboratories and others has firmly established that genetic factors lay a role in influencing biological responses to ionising radiation. The conventional approach to risk estimations (at the population level) has been established by large-scale epidemiological studies of radiation-induced cancers in exposed populations. However, more recent evidence, showing the contribution of individual genetic background to this baseline risk, is now being considered as a significantly modifying influence of individual risk. Thus, the unique genetic composition of each individual will play a role in determining the extent of his or her biological response to ionising radiation. This phenotypic variation may be expressed both in a radiation-specific manner, i.e. in terms of the rate and extent of the repair of initial DNA and cellular damage, and in a non-radiation specific manner, such as the proliferative response to local cell losses, or one of many other general mechanisms promoting/modulating the progression of malignant disease such as neo-angiogenesis, apoptotic activity etc.

Thyroid cancer is a classical model of radiation-induced disease. Causality has been established in both epidemiological and animal experimental studies, demonstrating the link between both local external irradiation and internal contamination with radioiodine. Mechanistically, the principal genetic alterations underlying sporadic thyroid cancer are known, and appear to a large extent to be reflected also in the changes seen in radiation-induced tumours. However, despite the wealth of biological material and dosimetry data, progress towards an elucidation of the genetic basis of individual variability to thyroid cancer has been slow. It is unclear at the moment if population-based studies will ever be able to offer insight into the process.

Earlier studies performed in the Fifth Framework Programme have indicated that animal models of specific human radiologically induced cancers offer a powerful tool for identifying the genetic mechanisms of radiation-cancer susceptibility. Consequently, GENRAD-T has embarked on an ambitious study to generate thyroid cancers in inbred mouse strains, where both the tumours themselves and host genetic factors are amenable to detailed genetic analysis.

The inclusion of genetic risk factors as a component of overall individual risk requires both a basic mechanistic knowledge (how many genes, what processes do they modify, what is the effect of low dose, radiation quality, etc.) and a means for the quantitative evaluation of their contribution to the overall risk. The GENRAD-T consortium has conducted a set of experiments to identify both high- and low-penetrance genes modifying susceptibility to radiation-induced thyroid cancer using both mouse models and radiation-induced human thyroid cancers.

Brief description of the research performed and methods/approach adopted

Development of a mouse model of radiation-induced thyroid cancer

The initial phase of the project was focussed upon developing a mouse model capable of yielding genetic information. It has been known for generations that different inbred mouse strains show different phenotypic features, such as coat colour, size and immune response. More recently strain-dependent variability in the rates of both sporadic cancers and radiation-

induced cancers has been described, suggesting that genetic inheritance may be responsible for this phenotypic trait as well. By inducing thyroid tumours in different inbred strains, and in backcrosses between them, it will be possible to map and identify those genes modifying sensitivity of the thyroid gland to radiation. In this study, GENRAD-T elected to focus upon differences between the classical laboratory mouse *Mus musculus* (represented by the inbred strains BALB/c, C3H and C57B6) and the Asian mouse *Mus molossinus,* represented by the inbred strain JF1 (Japanese fancy No1). The reasoning here is to take advantage of the evolutionary distance between the mouse species, which results in a high degree of genetic polymorphism between the animals, and hence the density of informative genetic markers available for analysis. This strategy has already been used successfully in studies using another mouse species (*Mus spretus*), which has shown to be highly resistant to radiation-induced cancer, making it possible to rapidly identify genes involved in modifying sensitivity.

The induction of thyroid tumours in mice by radiation represented a major hurdle, as only a few studies have been reported in the literature, and there was a possibility that strain differences in latency times or indeed incidence may be dramatic. A second problem is the lack of a consistent histological classification scheme for radiation-induced tumours. Currently the human scheme follows the WHO classification used for non-radiation associated thyroid malignancy, but differs from that used in the mouse. To allow us to compare genetic information across species it was imperative to formulate a common frame of reference for tumour classification.

Genetic analysis

In the second phase of GENRAD-T the candidate genes modifying susceptibility were studied. This part of the project adopted both top-down and bottom-up strategies. In the top-down approach panels of human thyroid tumours with a radiation association were studied to identify which genes were altered in the tumours. The reasoning here is that any gene altered during thyroid carcinogenesis has the potential to be a modifier of risk. Two research objectives were followed here. The first compared tumour and non-tumourous tissue using high throughput expression array analysis, whilst the second adopted a candidate gene approach to screen known genes for involvement in radiation-induced thyroid cancer.

In the bottom-up approach the genetics of the mouse model were studied. This two-part study investigated firstly alterations in the genome of the mouse thyroid cancers using microsatellite-based allelotyping to identify regions of allelic imbalance that may harbour oncogenes or tumour suppressor genes. Secondly, linkage analysis was used to map low-penetrance genes exerting a modifying effect on thyroid cancer susceptibility in the different inbred mouse models.

Main achievements

The research of the GENRAD-T consortium has established a mouse model of radiation-induced thyroid cancer using a defined induction protocol. This system has proven effective in different inbred strains, making it a valuable tool for genetic studies. We have conducted a unique exercise to establish a set of diagnostic criteria for the histological evaluation of radiation-induced thyroid malignancies in the mouse. In a combined effort between human and veterinary pathologists we performed a rigorous examination of the tissues of radioiodine-treated mice and produced a classification based primarily upon the current human thyroid cancer classification.

Genetic analysis of the tumours and of the tumour-bearing mice was performed. It has established that genetic differences indeed do contribute to the risk of cancer in the animal model system. In particular, strain-dependent differences in both frequency and type of malignancy were observed. This has allowed us to initiate the quantitative analysis of the genes responsible for strain differences (QTL mapping). A set of gene loci was identified whose inheritance confers an increased risk of thyroid cancer. In parallel studies the genetic alterations within the tumour tissues were studied to give insight into the somatic gene alterations accompanying thyroid cancer development. A candidate gene strategy was initiated, using genetic analysis of radiation-associated thyroid cancers obtained after therapeutic irradiation or after exposure to radioiodine in the aftermath of the Chernobyl disaster.

Using high-throughput screening by expression array we were able to identify a set of expressed genes that discriminate between normal thyroid tissue and thyroid cancer. In an expansion of this study the method was used to define expression profiles unique to different histological forms of thyroid cancer. Finally the ability of this system to distinguish sporadic and radiation-induced thyroid cancer was examined, and again the assay proved capable of discriminating. From this study a set of thyroid-expressed genes were identified that are unique to malignant thyroid tissue. These are strong candidates for susceptibility modifier genes.

In a related project a candidate gene approach was applied to known thyroid cancer associated genes RET and B-Raf. Both genes are known to be involved in thyroid cancer, but the possibility that natural variants of these genes exist that can modify the risk of an individual developing either sporadic or radiation-induced thyroid cancer is unknown. We have conducted a series of genotyping studies to ascertain if polymorphisms of these, or a series of other thyroid cancer-associated genes, are associated with the risk of developing thyroid cancer. A set of candidate SNP markers showing association with the development of thyroid cancer has been identified.

These studies, together with the mouse model, place the consortium in a highly competitive position to challenge for leadership in the race to understand the genetics underlying thyroid cancer risk after irradiation. They have also given the group a lead in the development of a mouse model that is highly sensitive to radioiodine, where future studies at low doses will be possible.

Exploitation and dissemination

The primary dissemination methods for the studies of the GENRAD-T consortium have been oral and written communication of the results to the scientific community. These have been in the form of invited lectures, contributions to scientific conferences, publications and reports.

The findings from GENRAD-T are not yet incorporated into the body of scientific evidence that favours the inclusion of genetic parameters in risk assessment. When the project results are published it is expected to make a change in risk-assessment modelling.

The knowledge gained has already been incorporated in academia, being included in lecture programmes for advanced degree studies in a number of academic institutions, most notably

as course material for the EU-sponsored MSc in radiation biology.