Understanding the effects of radiation on health
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Ionising radiation is present at low doses and low dose rates in the natural environment, largely from radioactive atoms in minerals formed during the early history of the planet. The technological developments of the 20th century have resulted in the use of radioactive materials for military, industrial and medical purposes. The peaceful use of radiation includes power generation, industrial testing, bio-medical research, disease diagnosis and cancer therapy – all an integral part of our modern world.

These benefits to society need, however, to be weighed against the known potential of radiation to cause health effects in exposed people, principally – tissue injury, cancer and genetic effects that are passed to offspring. Much is known on the likelihood of these health effects after exposure to radiation at high doses and high dose rates. However, the vast majority of man-made radiation exposures to workers and the general public occur at low doses and low dose rates where risks cannot be reliably assessed by direct observation. The majority scientific view is that risks of excess cancer and genetic effects are likely to rise in simple proportion to radiation dose and that even the lowest of doses carries some risk, albeit vanishingly small. However, some suggest that there is a low dose region (the dose threshold) where there is no excess risk of any health effect; conversely, others claim that low dose risks are grossly underestimated. Because of scientific uncertainties, the low dose issue remains an important source of debate in radiation protection and more widely in policy/standard setting.

In order to weigh the socio-economic benefits of radiation against its potential effects on health, it is essential to gain a better understanding of this low dose and low dose rate issue. For this principal reason the European Commission has supported a programme of epidemiological and basic research on the health effects of radiation.

This brochure provides an outline of the basic research sponsored by the Commission in its 4th and 5th Framework Programmes. It will be of interest to a relatively broad audience with interests in radiation protection including the safety of nuclear power generation, medical aspects of radiation exposure, the application of new research technologies to environmental issues and new concepts concerning the biological action of radiation. The brochure contains two distinct parts. The first one provides a summary of the Commission supported research in the past decade. The main text of this part may be read at two levels with boxed notes acting to provide simplified explanations of scientifically complex issues and outcomes. The second part includes summaries of many of the projects that make up the relevant research portfolio of the Commission. Epidemiological research is outlined in a separate publication “Epidemiology and retrospective dosimetry” (EUR 19958).
Summary

This brochure summarises fundamental research on the health effects of ionising radiation supported by the European Commission in its 4th and 5th Framework Programmes. This outline is set in the context of the need for a detailed understanding of the mechanisms of radiation action on cells and tissues of the body in order to support judgements on the health risks after low dose and low dose-rate radiation. Also included is an indication of the main direction of research that will be pursued in the 6th Framework Programme.

Fundamental research supported by the Commission over many years has made a substantial contribution to the understanding of the mechanisms that underlie radiation-induced health effects. The overall objective is to provide a sound biological basis for radiation protection. The research areas considered to be of particular importance are DNA damage response processes in cells, the specific mechanisms of cancer induction, heritable susceptibility to induced cancer including individual risk, the modelling of low dose cancer risk, heritable effects, effects on the developing brain and the diagnosis/treatment of cancer and radiation injury (see notes 1, 2 and 3). Research in some of these areas included consideration of the health effects of the Chernobyl reactor accident.

The success of these research programmes has been promoted by involving many of the key European research laboratories and placing emphasis on interdisciplinary collaboration and communication. This success in achieving research objectives is well illustrated by the establishment of strong biological and biophysical links between the dose-dependent appearance of DNA damage in irradiated cells, the recognition and repair of these initial DNA lesions and the contribution made by residual DNA changes to cancer development in tissues. This experimentally derived information is providing an increasingly robust biological foundation for the development of improved judgements on cancer risk after radiation.

However, although much has been learned of radiation mechanisms and their modelling (see notes 4, 5, 6 and 7), it is clear that further research is needed in order to address remaining uncertainties on the risks to health after low dose and low dose rate radiation.

PART 1

1. Introduction

The damaging effects of ionising radiation on tissues in the body have been known for about 100 years. This knowledge was followed by evidence that high doses of radiation can cause cancer. These carcinogenic effects have been studied most closely by following the health (epidemiological study) of radiation-exposed survivors of the 1945 atomic (A) bomb explosions in Japan. These A-bomb studies provide much of the information available on the radiation dose-response of risk of leukaemia and solid tumours although further knowledge has come from other investigations of people exposed to radiation accidentally, in their work or through medical procedures. In addition, such studies have provided evidence on the more general aspects of radiation induced tissue injury and how it may be assessed and treated. Specific forms of non-cancer effects in tissues have also been considered, including effects on brain development following pre-natal exposures and damage to germ cells in reproductive organs which can result in heritable disease.

The achievement of an acceptable balance between the detrimental effects of radiation and its benefits to society requires a scientifically robust and acceptable system of radiation protection that is applicable to public and occupational exposures. The radiation doses that apply to these exposures are invariably below a level where direct epidemiological study can inform on cancer risks. At these low doses received at low dose rates the risks are very small when compared with the 20-25% chance of a typical individual developing fatal cancer from other causes. Nevertheless, for protection purposes, it is important to improve judgements on how the cancer risks known to apply at moderate to high doses may be projected downwards to these low doses and dose rates. To achieve this it is important to extend knowledge of the mechanisms through which radiation damage to cells in tissues allows a small number to enter a multi-stage process of mal-development which can lead to cancer (see note 3); this process can take tens of years to complete. Stated in a simple way, with good knowledge of the biology of radiation action within the cancer process it will be possible to construct well-founded mathematical models to describe radiation risk at the low doses that are most relevant to human exposures. These biologically validated models will be of substantial value in developing firm views on whether radiation risk increases as a simple function of dose (e.g. a linear dose-response) or alternatively whether there is a true low dose threshold where risk may be discounted.

An additional fundamental issue that has come to the fore in recent years is the question of variation in inter-individual risk due to the effects of genetic make-up on radiation response. A few rare genetic disorders that may substantially influence radiation risk are known but it is believed that more common genetic factors currently escape detection because their effects are relatively weak. An understanding of such common genetic factors will aid judgements on how radiation risks are distributed in the population and whether certain genetic sub-groups are under-protected.

For some time the Commission has recognised the importance of obtaining this fundamental information and to couple it with
epidemiological findings. Also, rapid advances in modern biology,
genetics and medicine are providing the background knowledge and
tools to seek answers to these outstanding questions in radiation
protection (see note 2). The research that the Commission has
identified and supported in these areas has made important progress
during the 4th and 5th Framework Programmes (FP). The research
strategies and scientific progress of these programmes are sum-
marised in the following sections of this brochure together with a
view of how work may be extended within the 6th FP.

When a track of ionising radiation passes through a cell in
the body it will deposit energy which can disrupt the organic
molecules and structures that make up the cell. A particu-
larly sensitive molecule within the cell is deoxyribonucleic
acid (DNA) which is commonly termed ‘the blueprint of life’.

These sets of helical double stranded DNA molecules are
very long, they are contained in microscopic structures
called chromosomes and provide a linear series of codes
for the production of cellular proteins. The structure and
activity of these proteins allow all cellular functions to
proceed. Each protein is coded by a specific stretch of DNA
called a gene; there are around 35 000 genes in each
human cell contained within 22 chromosome pairs and two
other chromosomes that determine gender. In this way the
DNA controls all cellular activity and, in reproductive (sperm
and egg) cells, acts to pass information from one genera-
tion to the next. The total DNA constitution of a cell is termed
the genome.

There is strong scientific evidence that damage to DNA from
ionising radiation, particularly DNA breakage, underlies many
of the health effects that are recognised from clinical studies
on tissue injury in individuals, from health studies (epidemi-
ology) of excess cancer and genetic effects in irradiated
human groups and from investigations with experimental
animals.

Cellular damage from radiation can lead to cell death. If the
radiation dose is high and enough cells are killed or func-
tionally impaired there will be clinically observable tissue
injury, e.g. in blood forming tissues and the intestines.
Severe tissue injury can be fatal but more subtle effects may
also occur, e.g. in the developing brain.

At low doses, tissue injury is not apparent but some cells
will survive radiation with changes (mutations) in their
DNA. Some of these mutations are visible at the chromo-
somal level and, in principle, all functional gene mutations
can alter cellular behaviour. Most importantly, certain spe-
cific mutations arising in normal cells of the body can result
in changes in cellular properties (e.g. in growth control) that
may contribute over many years to cancer development.
The full development of cancer is a multi-stage process
(see note 3) which is believed to require the acquisition of
a number of different mutations which are likely to have
different causes. Ionising radiation exposure may be most
simply viewed as one of these contributory causes of cancer.
One additional factor of growing importance is the influ-
ence of differences in genetic make-up on radiation cancer
risk – in this area our knowledge is very limited.

Radiation-induced mutations arising in reproductive (germ)
cells are not expressed as disease in the irradiated individual
but may be passed to the offspring and thereby to future
generations. Thus, radiation can contribute to the frequency
of heritable diseases of widely differing severity.

An important issue in the understanding of all the health
effects of radiation is the defence systems that cells possess
in order to protect against radiation damage. These include
cellular stress systems that act to recognise the damage that
has occurred, proteins that actively repair damage to chro-
mosomal DNA, systems that promote the death of heavily
damaged cells and various cellular and tissue processes that
serve to minimise the probability that appropriately mutated
cells will complete the multi-stage pathway to cancer.

The European Commission has sponsored research in radiation protection throughout the period since the EURATOM Treaty of 1957. In this way the Community, through the Commission, has made a substantial contribution to the world-wide development of knowledge on radiation effects/dosimetry and the application of that knowledge in radiation protection.

The recognition that further information was needed on the fundamental processes underlying radiation action on cells and the subsequent development of cancer was a major factor in the design of FP4 research in radiation protection. Previous research (note 4) had highlighted the critical importance of the damage caused by ionising radiation to the genetic machinery (chromosomal DNA) of cells and how DNA damage response systems within the cell can be mobilised to repair this damage. However, there is a certain probability that this repair will not always be wholly correct which can result in gene/chromosomal mutations and/or cell death. It was also becoming more clear that DNA damage response and repair/misrepair processes in cells were critical components in cancer risk. Thus, work on cellular responses had the potential to be coupled with research on tumorigenic mechanisms using selected animal models of human cancer. Cellular and animal models would also find use in studies on the heritable factors that influence radiation cancer risk. However of equal importance was the progressive increase in epidemiological knowledge of radiation cancer risk from studies on survivors of the Japanese A-bomb explosions; on groups exposed to natural radon gas; on medically irradiated people; on nuclear workers; and on children in the former Soviet Union (FSU) developing thyroid cancer following radiiodine exposure from the Chernobyl accident in 1986.

In respect of these advances the Commission sought to sponsor fundamental research on the mechanisms and predisposition to radiation associated cancer. The effects of radiation were investigated both at cellular/molecular and tissue/organism levels. The resulting gained fundamental knowledge was used to develop cancer risk modelling. Other research that was supported included diagnosis and treatment of post-Chernobyl thyroid cancer and the mechanisms of tissue injury. Non-cancer research projects concerning radiation effects on the developing brain and on biomarkers of damage to reproductive cells were also included. Because of the long-term nature of the research supported in this area, there has been no major discontinuity between FP4 and FP5. Therefore, the main findings of FP4 and FP5 research will be presented together in the following sections. More detailed information about individual contributions can be found in the second part of the brochure.

Note 2 – Research tools and approaches

Radiation-induced human cancer: For studies on cancer mechanisms there are few opportunities to obtain samples of human tumours that have a high probability of being induced by radiation; post-Chernobyl childhood thyroid cancer and cancer resulting from radiotherapy offer the best prospects for research.

Experimental cellular models: Many mechanistic studies of the effects of radiation utilise cells that are growing in culture. This approach has been particularly valuable for the identification of biologically important forms of DNA damage, cellular responses to this damage and how it is repaired. Cells with genetically altered sensitivity to radiation may be used to identify and characterise the genes that control different forms of cellular response and damage repair. Models based on cultured cells may also be used to study certain aspects of cancer development such as cell immortalization and DNA instability.

Experimental animal models: Cancer development is a complex process and at present cellular studies cannot adequately represent this complexity. For this reason certain problems in radiation induced cancer demand the use of mice or rats. These animal models allow the yield and characteristics of radiation-induced tumours to be determined; this information may be related to cellular responses. Genetically based experiments with mouse models also find use in investigations on the whole-body impact of DNA repair and on how heritable factors might influence individual cancer risk. In the past, radiation studies utilised very large numbers of experimental animals; new animal models coupled with cellular studies are far more efficient.

Experimental methods: Research on cells and animals benefit greatly from modern biological techniques. For example, the precise visualisation of altered chromosomes, the high-resolution analysis of DNA damage and the rapid determination of the structure and activity of genes involved in DNA repair, cancer development or altered brain function.

Cancer risk modelling: Cancer risk modelling involves the use of computer-based calculations using biological information on the cancer process to better describe epidemiological findings on excess cancer after radiation. A major aim is to assess the impact of low dose radiation on excess cancer in an irradiated human population.
I. Simplified schematic model of multi-stage radiation tumorigenesis

A. Cellular DNA damage from radiation

B. Mutant pre-cancerous cell

C. Multiplication of pre-cancerous cells

D. Growth selection of immortal/unstable cancerous cells

E. Development of fully malignant cancer

Incorrect DNA damage response/repair

Initial loss of growth control

Further mutational or non-mutational change

Final set of cellular changes and growth selection

II. Research strategies employed for different phases in the schematic model

Cancer mechanisms
A. Cellular and biophysical studies on DNA damage induction.
A→B. Cellular studies on DNA damage response/repair, bystander effects and genomic instability.
Studies with genetically altered (knock-out) mice.
B→C→D→E. Studies with normal and gene knock-out mouse models.
Studies with radiation-associated human tumours.

Cancer genetics
A→B. Studies on human/rodent cells and knock-out mice with defined deficiencies in DNA damage response or DNA instability.
B→E. Studies on mutant mice fully deficient in defined tumour-associated genes.
Studies on inter-bred mouse strains having natural variation in tumour susceptibility; identifying the responsible genes.
Cellular studies on DNA damage-response

The foundations for research on DNA damage response and repair were established in previous FPs and the new work sought to build upon the critical mass of EU expertise and knowledge that had been developed. At the level of initial radiation damage to DNA, the FP4 programme greatly strengthened the view that the appearance of excess gene/chromosomal mutations in cells was principally driven by the induction of double strand breaks in the helical DNA molecules that make up the chromosomes. Work was undertaken on the dependence of DNA double strand breaks induction by radiations of different qualities (different linear energy transfer – LET).

An important finding was that the complexity of this initial damage appeared to strongly influence the quality of DNA repair that was possible. During this period of research the biologically important DNA double strand breaks were suggested to be associated with multiply damaged sites which also contained local damage to other constituents of the molecule, i.e. clustered damage (see note 5). This feature seemed likely to be related to the types of gene and chromosomal mutations that arose after radiation.

In this general area of biophysics, technical developments allowed for the irradiation of single cells by single ionising particles of high LET radiation. This technique allowed study of the induction of cellular effects by the lowest possible dose of radiation and also revealed evidence of the potential for transfer of damage signals between cells – the bystander effect.

A number of projects addressed the specific mechanisms whereby radiation tracks caused initial DNA damage which was then mis-repaired to form chromosomal exchanges (translocations). These studies benefited greatly from a new technique, termed FISH, that allowed differential molecular staining of different chromosomes – using this technique and another that prematurely condenses chromosomes (PCC) it became possible to approach mechanistic problems of chromosome damage that had previously been inaccessible, for example the rate of repair of initial DNA double strand breaks in relation to chromosome damage. FISH techniques coupled with cellular/molecular analyses were also used to explore relationships between cellular radiation response, chromosomal instability and the specialised DNA sequences termed telomeres that cap the ends of chromosomes and occupy certain internal chromosomal sites. This and other work highlighted the potential importance of higher order DNA structure as a factor in chromosome stability and radiation response.

The response of cells to radiation include the imposition of checkpoints in the reproductive cycle in order to promote DNA repair – also as part of the mechanism of programmed cell death (apoptosis) that serves to eliminate heavily damaged cells. A number of projects succeeded in clarifying the above relationships and some of the genes/enzymes involved in the responses.

The potential role of telomeric sequences in radiation response, highlighted in studies on chromosomal instability in tumorigenesis, was investigated in mouse germ cells. These studies revealed up-regulation of the telomere elongating enzyme telomerase in response to radiation and evidence that some DNA double strand breaks can be healed by addition of telomeric sequences.

The study of mutant cells with altered radiation response had become a most important component of radiation biology and was used widely in many fundamental studies in FP4. Such mutants were selected from cell cultures or derived from carriers of radiosensitive human genetic disorders e.g. ataxia-telangiectasia. A major contribution was made to the characterisation of these mutants including the isolation of the responsible genes and others of note 4 – Important advances in knowledge during the period prior to FP4

- Knowledge on the extent to which damage to DNA and its repair determine the sensitivity of cells to ionising radiation.
- The strengthening of links between cellular radiosensitivity and cancer development.
- The use of molecular genetic techniques for chromosome analysis and genetic manipulation in radiation studies; advances in the development of animal models for studies on cancer mechanisms.
- General advances in cancer epidemiology relating to the Japanese A-bomb survivors, radon exposed miners and post-Chernobyl childhood thyroid cancer to support the development of biologically based models of cancer risk; also, for thyroid cancer, the availability of tumour samples for analysis.

Note 5 - Examples of some novel features of radiation response emerging during the period of FP4

- That a significant fraction of DNA double strand breaks induced by radiation are associated with a cluster of other damages and may be difficult to repair correctly.
- That in some circumstances radiation-induced cellular damage results in the expression of DNA instability over many cell divisions.
- That in some circumstances radiation damage to a cell can result in effects expressing in an unirradiated neighbouring cell (bystander effects).
- That minisatellite DNA sequences in human germ cells show increased mutation rates in a high dose exposed population.
related structure/function. Other advances included the elucidation of the relevant biochemical pathways and, latterly, the genetic manipulation of mice to carry the mutant genes. Progress in this whole area was most notable and the EU-supported work on DNA repair is acknowledged world-wide. The characterisation of the repair pathways for DNA double strand breaks repair and associated radiation responses has made a significant contribution to our understanding of the degree of error-prone DNA repair expected after radiation. In turn, this provides important input to the low dose cancer risk debate – stated simply, the error-prone repair of sometimes complex DNA double strand breaks may be used as one element in a scientific argument against the presence of a low dose threshold for cancer risk.

The characterisation of DNA damage response genes in FP4 has also provided information on candidate genes determining heritable sensitivity to radiation tumorigenesis. This theme appears in a number of cellular projects including studies on the potential association between heritable cellular radiosensitivity and breast cancer risk.

FP5 research in this area represents a more focused approach to key issues associated with initial DNA damage and the nature, control and consequences of the relevant DNA damage response pathways. The ongoing studies underway include detailed consideration of complex DNA lesions; post-irradiation protein-protein and protein-DNA interactions; DNA repair fidelity; and studies with recombinant (gene knock-out) mice to explore the consequences of DNA repair deficiency in whole animals. The induction of persistent genomic instability in cells and the transfer of damage signals from irradiated to unirradiated cells (bystander effects) are also being investigated.

Studies on the mechanisms and genetics of radiation tumorigenesis

The principal approaches used to investigate the mechanisms and genetics of radiation tumorigenesis were via animal models and radiation-associated human tumours. In some projects additional support came from cellular and chromosomal studies.

Animal models of radiation tumorigenesis were used to explore dose-response characteristics, tumour multiplicities, early cellular events and genetic factors in post-irradiation tumour development. The principal tumour types investigated in mice were leukaemia, lymphoma and cancer of bone, intestine and skin. Lung cancer after radon was investigated in rats in the broader context of a study which included the dosimetric modelling of risk. Much of the work on early radiation associated events in tumours centred on establishing consistent patterns of tumour-specific gene losses. The tumour gene categories of particular potential interest are those associated with the control of a) the cell reproductive cycle, b) DNA stability, c) apoptosis and d) cell growth/development. This approach was successful particularly for myeloid leukaemia and bone cancer and for intestinal tumorigenesis in a mouse model of a cancer-prone human genetic disorder. In these cases it was possible to lend support to the proposition that induced gene losses were critical early events in multi-stage tumour development after radiation.

Good evidence was also obtained on heritable genes that predispose animals to cancers of bone, skin, intestine and leukaemia/lymphoma. The picture that emerged from these FP4 studies was one of genetic complexity with different tumour types being influenced by multiple genes, some of which interact to determine tumour yield. Also, that the tumour-predisposing activity of strongly expressing genes can be modified significantly in different genetic backgrounds.

Human tumours associated with radiation exposure were obtained as sample material of second cancers after radiotherapy and of post-Chernobyl childhood thyroid cancers. Although a relatively small number of therapy-related cancers were available, chromosomal analyses proved possible and provided initial evidence of the expected chromosomal loss mechanism. Molecular studies on these tumours were initiated with emphasis on second cancers arising in cancer-prone retinoblastoma patients.

More attention was, however, given to cytogenetic and molecular investigations of the post-Chernobyl thyroid cancers where characteristic forms of chromosomal and RET gene-specific rearrangements were revealed. The specificity of RET rearrangement was suggested to be associated with age-related tumorigenic processes rather than being a particular molecular signature of radiation action. A number of projects included consideration of thyroid tumorigenesis in the context of cellular, histopathological and epidemiological investigation.

Overall, FP4 work on the mechanism and genetics of radiation tumorigenesis benefited greatly from recent advances in knowledge of mouse and human genomes. The animal studies provided valuable proof of principle evidence on tumour specific gene losses in radiation tumorigenesis and genetic modification of radiation risk. The human studies particularly those of thyroid tumorigenesis showed for the first time that direct molecular investigations can reveal much information that may be linked with parallel findings from studies on tumour pathology and epidemiology. In this way molecular studies, when coupled with findings from tumour pathology, can contribute to the clinical management of radiation-associated thyroid cancer. As information on the molecular pathology of other radiation-associated cancer types accumulate it may be that this additional benefit from fundamental research will become more general.

FP5 research in this area continues to be focused on the mechanisms and genetics of the complex post-irradiation processes that contribute specifically to cancer risk. For example, selected cellular and animal models are being utilised to explore radiation-associated gene/chromosomal mutations that characterise different tumour types; also the potential roles of telomerase and chromosomal telomerases. Mechanistic study of post-Chernobyl thyroid cancer and therapy-related cancer is also being extended; importantly, thyroid cancer studies include the establishment of a tissue bank and data base for collaborative follow-up. Finally, a number of projects are seeking further information on the genetic factors that influence radiation cancer risk.
Studies on the modelling of radiation cancer risk

FP4 research sought to promote a closer coupling of mechanistic studies and those centred upon the interpretation of human epidemiological data relating to radiation cancer risk. Work was initiated on the development of mathematical models of low dose cancer risk which incorporated a current understanding of the biological processes that underlie post-irradiation cancer development.

The models that were considered included mathematical approximations to describe cell mutation rates, the early post-irradiation growth of potentially malignant cells and the time dependence of their multistage development towards cancer; particular attention was given to two stage models and various points of radiation action were investigated. These cancer risk models were tested for their validity against epidemiological data on cancer risk e.g. A-bomb survivors, radon exposed uranium miners and in some cases animal data. This work was conducted in parallel with the biophysical modelling of DNA damage repair and the gene/chromosomal mutation induction noted earlier.

In FP5, cancer risk modelling is being refined and increased attention given to age- and time-related features of the expression of risk together with dosimetric and other uncertainties. The modelling of cancer-related chromosomal damage after radiation includes elegant computer-generated representations of the process at the molecular level.

The epidemiological input to this whole area is included in the brochure “Epidemiology and retrospective dosimetry” (EUR 19958).

Diagnosis and treatment of cancer and radiation injury

The follow-up of post Chernobyl thyroid cancer was an important component of FP4. Work in this area centred on the development of improved protocols for diagnosis and treatment of these cancers; also, consideration of preventative measures based on iodine supplementation in regions of iodine deficiency. Developments in FP4 included improved diagnostic imaging techniques, the utility of post-surgery suppressive therapy, improved dosimetry for radioiodine therapy of lung metastases and the provision of a map of iodine deficiency in contaminated regions. An important conclusion was that radiation induced thyroid cancer in children is in general a well treatable disease.

Studies on non-cancer effects

Although cancer risk at low doses dominates considerations in radiation protection, FP4 also gave attention to aspects of germ line damage and to pre-natal (in utero) effects on the developing brain. Radiation effects on in utero brain development are evident from the follow-up of the A-bomb survivors in Japan and a relatively large study was devoted to gaining a cellular/molecular understanding of the processes involved; animal behaviour was also considered. In brief, in FP4, in utero effects of radiation on the developing brain were revealed at relatively low doses but the general pattern of such effects were consistent with a low dose threshold of similar magnitude to that currently judged from the A-bomb data on humans.

A project sought and found evidence that hypermutable minisatellite sequences in the human germ line can act as biomarkers of radiation damage. This study which investigated generations of families from regions of Semipalatinsk (FSU) contaminated with fallout from nuclear tests provides the most convincing molecular evidence to date of such radiation-associated germ cell mutation in humans. Selected studies on the hypermutability of certain genomic sequences have been included in FP5 research.

Note 6 – Some features of biologically-based mathematical models of radiation cancer risk

With reference to the scheme of Note 3, these models generally consider:

- the different phases of multi-stage cancer development, e.g. a simple two stage model might include a first stage (A→B) including DNA damage to normal cells and the appearance of mutant pre-cancerous cells plus a second stage (C→D/E) involving a further mutation that drives a cell towards the fully malignant state
- biologically realistic mathematical values to describe the number of cells involved and rates of mutation, cell death/development and growth selection
- testing and validation of the models and their chosen mathematical values – how well does a given model fit available information on cancer risk in irradiated humans and experimental animals; what are the implications for low dose risk; how large are the uncertainties?

Work on the development and validation of these models also serves to identify aspects of cancer development and radiation risk where further information is critical. Accordingly, there is a need for ongoing dialogue, deep integration and cross-disciplinary research between modellers, epidemiologists and those undertaking experimental studies.

Recent review of current knowledge and remaining uncertainty by the Commission has served to identify research priorities in radiation protection for FP6. The main thrust of research in this area is to resolve uncertainties in the risk from exposures to radiation at low and protracted doses typical of those encountered in the environment and in workplaces. This remains a controversial science and policy issue with important health and economic implications for the use of radiation and radioactive materials in both medicine and industry.

Quantification of these risks will be achieved through epidemiological studies of exposed populations complemented by fundamental research on the interaction between radiation and DNA, cells and organs in the body.

One novel aspect of the research in FP6 is the integrated multi-disciplinary approach that should gather a critical mass of scientists to address ambitious research goals. Therefore, the studies in FP6 should be carried out within fewer projects (but with a broader perspective and possibly including more partners) involving, *inter alia*, epidemiology, radiobiology, medicine and dosimetry.

Cellular and molecular biology research in FP6 may be viewed as a more focused development of the advances made in FP4 and FP5, particularly in key areas where coherent scientific themes on low dose response have emerged.

All potentially important mechanisms should be addressed, from initial damage to health effects manifested in the organism. In particular, the following aspects could be included: the biological consequences of damage to DNA and other cellular macro-molecules and structures; interference of the various DNA repair pathways with other cellular processes (e.g. transcription, replication, apoptosis, chromatin remodelling); the role of intra/extra-cellular communication in tissue functions; unravelling the mechanisms of and susceptibility to radiation health effects; elucidation of the relevance of radiation-induced cellular endpoints (e.g. genomic instability, bystander effects, chromosomal aberrations and mutations) in the cancer process; further development of mechanistic models describing the multi-stage process of carcinogenesis.

The research should, in the first instance, be addressed through one or a few integrated multi-disciplinary projects involving, *inter alia*, radiobiology, genetics, molecular biology, biophysics and oncology.

The variability of radiation response within a human population and the identification of the molecular pathways contributing to susceptibility to radiation health effects will be important outcomes of this research.

Although it should not be expected that FP6 research would resolve all remaining uncertainties, the Commission regards FP6 as a most important phase of transition towards a much more complete understanding of the potential risk to health following exposure to low doses of ionising radiation.
Estimates of the risks of exposure to ionising radiations have to cover an extremely wide range of radiation types and exposure conditions. Current estimates of risk have mainly been derived empirically and their refinement depends in large part on improved mechanistic modelling based on a more complete understanding of radiation action at the molecular, cellular and tissue levels. The overall aim of this project was to provide experimental data that will input into the development of mechanistic models of radiation carcinogenesis. The development of more realistic and accurate models depends on an improved knowledge of the chain of events that leads from the earliest physical interactions through to the development of cancer. Such models, based on a mechanistic understanding of radiation effects at the cellular level, will aid the extrapolation of human risk data obtained following A-bomb and other high-dose exposures to the low levels generally occurring in the environment and the workplace.

Experimental data for the induction of cancer by radiation of different qualities (EDICAR)

Challenges to be met

The research was organised into six work packages which covered the interactions of radiation with DNA, double-strand break (dsb) induction and processing, the induction of chromosome aberrations and mutations and the effects of individual radiation tracks on cells. DNA dsb are believed to be particularly important initial lesions in the genotoxic actions of ionising radiations. Effects of radiation quality (linear energy transfer (LET)) are an important consideration in estimating risk and were an underlying theme of the work carried out during this project. One of the major challenges addressed during the contract was a critical appraisal of the methodology used to determine the induction and processing of DNA dsb. Another was the development of microbeam techniques to enable investigations to be carried out under conditions that mimic low-dose exposures, corresponding to the passage of a single radiation track through the cell.

Achievements

The project provided data that has contributed to the development of an improved understanding of the processes related to radiation quality that are involved at the molecular and cellular levels in cancer induction by ionising radiations. New data were obtained on the physico-chemical processes involved in the induction of DNA damage, especially dsb, on the yields and distributions of dsb in cells exposed to radiations of various types and how cells process these damages.

The combined expertise of the partners was able to produce a critical study of the experimental methods used to measure the induction and cellular processing of DNA dsb following exposure to a range of different types of radiation and this work was published by the consortium as a review. New data were also obtained on the formation of chromosomal aberrations following exposure to radiations of different qualities and the relation to dsb repair, as well as on the yields and patterns of mutations induced. Methods were developed to assess the fidelity with which cells repair dsb. A new aspect of mutation induction “non-contiguous deletion” was found and is specific to radiation quality, offering the prospect of a “signature” marking high-LET exposure. New microbeam and measured-track techniques were developed and initial data were obtained about how cells respond to the passage of a single radiation track, which is key to understanding responses at low doses.

Overall, the data provided by the project have contributed to the development of mechanistic models for the induction of cancer by radiation. Such models are needed to reduce the uncertainties in extrapolating known risks from acute high-dose exposures (mainly from atomic bomb survivor follow-up) down to the low doses and dose rates that generally apply to occupational, medical and environmental exposures. The achievements of the project are described in more detail in journal publications. The data obtained and the competence developed among partners in the various methodologies led to a further project, “RADNA”, being funded under FP5. In this project, the new understandings gained about DNA damage induction and processing and the induction of chromosomal aberrations and mutations are being pursued further to gain an improved understanding of the mechanistic basis of radiation risk. Also, the microbeam approaches developed by several partners under FP4 are being further exploited to study low-dose mechanisms, including the involvement of more newly recognised responses (bystander effect, genomic instability and adaptive response) that appear to have significant roles in the low-dose...
Yields of dicentric chromosomal aberrations per cell in human lymphocytes after exposure to different types of radiation during the G0 phase of the cell cycle. The aberrations were visualised using the technique of premature chromosome condensation (PCC) in which the human cells were fused with hamster CHO K1 inducer cells. The yields of aberrations, as revealed by PCC, are shown as functions of the delay time tD in hours between the end of irradiation and the start of fusion with the inducer cells. ○: 150 kVp X-rays (4 Gy); ▲: 3.45 MeV alpha-particles (2 Gy); ■: carbon-K (C2) characteristic X-rays (1 Gy).

region. A new project on genomic instability funded under FP5, “RADINSTAB”, benefits from the application of the microbeams developed under the FP4 “EDICAR” and FP5 “RADNA” contracts.

**Partnership**

Under this contract, it was possible to bring together much of Europe’s leading expertise in the field of cellular radiation biology and thereby establish a research cooperation spanning most of the cellular processes leading to the induction of cancer by ionising radiations. The contract also exploited the useful range of different radiation sources available at the various centres. Progress in a number of areas was made possible by collaborations that were supported by the project. Examples of these included studies of chromosomal aberrations induced by different qualities of radiation and the development of microbeam techniques at several centres. The development of microbeams required technology transfer between partners. These exchanges have substantially enhanced European competence to apply these new techniques to research into the effects of low-dose exposure.

**Selected references**


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The main objective of the RADNA project is to make experimental measurements of the actions of low doses of radiation on cellular systems. It is based on the concept that the low dose risks of cancer induction and genetic injury are the result of damage that takes place at the cellular level. There is emphasis on the role of DNA damage and repair in the production of chromosomal aberrations and mutations. The aim is to improve the quantification of radiation risk at low doses. The experimental work employs radiation types that cover an important range of LETs in respect of radiation quality factors. Studies using counted-particle irradiations are designed to provide new information about the form of dose-effect relationships, down to the ultimate low-dose of a single track per cell. These measurements relate to the development of mechanistic models that will improve the low dose extrapolation of high-dose epidemiological risk data, which come predominantly from the follow-up of A-bomb survivors.

**Challenges to be met**

The project focuses on the role of radiation-induced DNA damage, particularly double-strand breaks (dsb), in the production of chromosome aberrations and mutations as critical events in the induction of cancer. Underlying these studies are investigations into the role of DNA repair processes, particularly those involving dsb, in subsequent biological effects. The project utilizes conventional irradiation methods, and innovative microbeam technologies coupled with single-cell assays of response. The objective is to map out responses at the level of individual cells. The overall aim of the project is to provide data that will contribute to mechanistic modelling of radiation risk, particularly in respect of radiation quality factors at low doses.

**Achievements**

Following initial work carried out under FP4, charged-particle microbeam facilities have been developed at several of the participating centres; a unique focused soft x-ray microbeam has also been developed. Using these and other facilities, measurements have been made of low-dose direct and indirect (i.e., “bystander”) responses in Chinese hamster cells. These studies included the induced radioresistance for a range of radiation qualities extending from focused soft x-rays to alpha-particles. An assay developed under FP4 has been used to quantify correct rejoining of DNA double-strand breaks and compare this with total (correct and incorrect) rejoining. Pulsed-field gel electrophoresis (PFGE) assay for DNA dsb has been used to measure both dsb induction patterns and rejoining kinetics as functions of radiation quality, including studies to determine the influences of the level of packaging of DNA and proteins (chromatin organisation).

The cellular processing of DNA damage that is involved in the production of chromosomal aberrations has been studied using premature chromosome condensation (PCC) and fluorescence in situ hybridisation (FISH) techniques. The fast and slow temporal components of the formation of chromosomal aberrations have been studied successfully.

Radiation-sensitive cell mutants and DNA repair inhibitors have been used to correlate defined DNA damage processing pathways with the formation of chromosomal aberrations. Effects of the cell cycle status upon the formation of chromosomal aberrations by different radiations have been studied using wild-type cells. Treatments that modify chromatin structure have also been considered.

The formation kinetics and persistence of complex exchange aberrations have been studied, visualising all human chromosomes using FISH. The proportion of complex aberrations increased with LET and might be a signature for high-LET exposure. FISH has also been employed to estimate low- and high-LET induced complete and incomplete chromosome exchanges.

Analysis of mutations at the molecular level has been made using the polymerase chain reaction (PCR). Measurements have been made of the cross sections for the induction of mutations in the HPRT gene in Chinese hamster cells for a range of radiation qualities. Also the spectra of damage have been observed in terms of mutational type. A comparison of the cross sections for mutation dsb and cell killing as functions of radiation quality showed differences consistent with the functional importance of cellular processing of dsb. Measurements were also made of preferential deletion within the HPRT gene.
**Title:** Induction, repair and biological consequences of DNA damages caused by irradiations of various qualities (RADNA)

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**Microbeam apparatus used to target cells, or subcellular regions, individually with charged particles. Each cell is imaged and located automatically and is exposed to a programmed number of traversals by protons or by ions of helium-3 or helium-4 (α-particles). The ions are counted and “switched” so that strictly 0, 1, 2, 3 etc. can be delivered, cell by cell. This approach enables the effects of extreme low-dose exposures to be studied, also the effects of damage transfer between hit and non-hit cells (“bystander effect”) can be investigated precisely.**

**Partnership**

This consortium brings together much of EU expertise in the field of cellular radiation biology and exploits the useful ranges of techniques and the different radiation sources available at the various centres. Examples of the collaboration include the application of specialised techniques such as FISH and PCC and the development of microbeam techniques.

**Selected references**


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**RADNA Information Column**

**Title:** Induction, repair and biological consequences of DNA damages caused by irradiations of various qualities (RADNA)

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**Period:** Nuclear Energy 1998-2002  
**Status:** Ongoing
Chromatin structure and DNA repair in relation to ionizing radiation-induced chromosome aberration in mammalian cells

Challenges to be met

Though studies on radiation induced chromosome aberrations were initiated almost a century ago, the mechanisms underlying the formation of these aberrations are not fully understood. The results of investigations from the cooperative efforts of various groups with expertise in different areas should lead to a better understanding of some of the steps involved from the initial damage to the manifestation of radiation induced chromosomal aberrations.

Achievements

Techniques to identify and quantify chromosomal aberrations are primary requirements for understanding the mechanisms involved in this process. To achieve this, DNA libraries for human, mouse and Chinese hamster chromosomes were generated by flow sorting of chromosomes and micro-dissection of specific chromosomes, chromosome arms and chromosome regions. Fluorescence in situ hybridization (FISH) techniques were used to paint different chromosomes and chromosome arms and regions.

This allowed accurate identification of different classes of exchange aberrations, such as, exchanges between two different chromosomes (reciprocal translocations), exchanges between the two arms of a chromosome (pericentric inversions), exchanges within an arm of a chromosome (paracentric inversions). These important classes of aberrations, i.e., intrachanges, were found to be formed more frequently than exchanges between two different chromosomes. Heterogeneity in the involvement of aberrations was found between the chromosomes as well as between the arms or regions of a chromosome. An interesting factor contributing to this heterogeneity was the presence of intercalary telomeric sequences in Chinese hamster chromosomes. Interstitial translocations (insertions) contributed to about 10% of total translocations induced by high LET radiation (1 MeV neutrons) irrespective of the dose. Since these aberrations are stable, their frequencies can be used as a fingerprint for past exposures to high LET radiation.

Detailed analysis of patterns of aberrations recovered, gave some clues about...
the origin of complex exchanges (involving several chromosomes and breaks). These analyses may help to distinguish between two theories of exchange formation, namely, free interaction between contemporary open ends versus build-up from multiple simple cyclical interactions, (i.e., all being in reality sequential exchange complexes). The dose response curve for induction of simple reciprocal translocations was found to be linear, whereas for complex translocations it was quadratic. This observation was supported by data derived from dose fractionation experiments, in which it was found that simple translocations were induced in additive manner, whereas the complexes are formed in an interactive manner.

Frequencies of radiation induced chromosome aberrations in G2 phase of the cell cycle have been proposed to be a sensitive assay for determining the radiosensitivity and cancer proneness in human. The G2 sensitivity is very variable and the yield of aberrations depends on several factors including contaminating irradiated S phase cells. A technique to identify S phase cells was deployed which allowed accurate scoring of cells irradiated in G2, thus reducing inter-individual variability commonly observed in G2 radiosensitivity assay.

Investigations of radiosensitive Chinese hamster ovary mutant cells (Xrs) which are deficient in repair of DNA double strand breaks, showed that these cell lines have altered histone-DNA binding properties which in turn influence structural properties of the chromatin and chromosome organization. Significant differences in the activities of DNA topoisomerase I and topoisomerase II have also been observed between the radiosensitive Chinese hamster mutant cells and their wild type parental cells. These results indicated that in addition to the primary defect in DNA repair, other characteristics might also contribute to the increased radiosensitivity.

Human peripheral blood lymphocytes in G0 phase of cell cycle were found to undergo apoptosis at doses of 1 Gy and below. Induced proliferation led to reproductive cell death only for a fraction of the cell population leading to increased cell survival at the expense of genomic instability. In lymphoblastoid cell lines derived from radiosensitive ataxia telangiectasia (AT) patients, apoptotic response was found only after high LET radiation and not after gamma irradiation. This indicated that apoptosis is a complex process, since in normal human cells, apoptosis was induced by both high and low LET radiations.

**Partnership**

This project was a multinational one involving partners who have competence in different areas but with a common goal. This collaboration was mutually beneficial for the success of the project.

**Selected references**

Chromosomal aberrations have a major role in the development of neoplasia and hereditary defects in human. European epidemiological studies have shown that the chromosomal aberrations in human lymphocytes to be a good bio-indicator for future cancer risk reflecting both exposure to genotoxic agents and individual sensitivity. Though radiation induced DNA double strand breaks (DSBs) are important lesions leading to chromosomal aberrations, many factors influence the ultimate yield of aberrations. This project was aimed at improving our knowledge on (a) intra-genomic heterogeneity of radiation induced chromosomal aberrations, (b) the initial frequencies of chromosomal aberrations (as detected by premature chromosome condensation technique), (c) differential repair at the chromosomal level, (d) detection and quantification of aberrations at different stages of the cell cycle following irradiation, (e) the influence of chromatin packing, namely, heterochromatic versus euchromatic regions, on the yield of aberrations, (f) the role of enzymes involved in the detoxification of free radical damage (such as glutathione S transferase) as modifiers of radiation response of human cells and (g) differential sensitivity to low and high LET radiations.

**Challenges to be met**

Although DSB are primary lesions responsible for induced aberrations, the number of DSB induced are far more than the number of observed aberrations, indicating the existence of several intervening processes (including DNA repair) which operate. Utilizing the technique of fluorescence in situ hybridization (FISH) and premature chromosome condensation (PCC) this project attempted to assess low and high LET induced chromosomal damage, its repair and the kinetics of formation of aberrations at different stages of cell cycle.

**Achievements**

Ionizing radiation induced aberrations are supposed to occur at random among the chromosomes and this assumption has been used to estimate genomic frequencies of aberrations from the frequencies observed in two or three FISH painted chromosomes. By PCC technique, it is possible to visualize the breaks and exchanges induced immediately and after different times following irradiation of human lymphocytes. By combining PCC with FISH it was possible to study the process of exchange aberration formation with time. Some of the human chromosomes such as #1, #19 are rich in actively transcribing genes in comparison to chromosomes #4 and #18. The initial number of breaks induced in chromosomes #1 and #19 was more than those induced in chromosomes #4 and #18, indicating that the open structure due to transcriptional activity leads to more initial damage. However, these transcriptionally active chromosomes were also repaired very efficiently leading to no significant differences at the metaphases.

After X-ray or neutron irradiation, the initial frequencies of breaks, dicentrics and translocations increased linearly with the dose as assessed by PCC techniques. The RBE (Relative Biological Effectiveness) for 1 MeV neutrons was found to be in the range of 1.5-2. The initial frequencies of translocations were higher than dicentrics (about 2 to 3 times) both for X-rays and neutrons. The dose response curves for induction of exchange aberrations, generated at different times of recovery following irradiation, using the PCC technique were linear-quadratic for X-rays and linear for neutrons. For X-rays, the linear component was formed in the early phase (within an hour) whereas the quadratic component developed slowly during subsequent recovery time. Following neutron irradiation of lymphocytes, induced breaks rejoined slowly in comparison with X-rays. Early formed chromosome exchanges were incomplete, namely dicentric chromosomes were not accompanied by bicolour fragments and translocations were of one way (incomplete) type. However, with increasing recovery time incomplete forms were replaced by complete forms. There were marked differences in the extent of induction of breaks and kinetics of their repair between low and high LET radiations.

**Metaphase spread of an irradiated human lymphocyte containing a false incomplete one-way exchange between the painted chromosome 8 (green) and an unpainted chromosome. Both ends of the truncated painted chromosome 8 have telomeric signals, as well as the translocated painted terminal segment (arrows). The black and white images represent the DAPI counterstain (upper panel) and the telomeric signals (lower panel). * Interstitial fragment (without telomeres).**
In order to gain a better estimate of the initial frequencies of induced breaks following irradiation of human lymphocytes, an inhibitor of repair of DNA DSB, namely ara A, was employed before and during fusion with mitotic cells. In the presence of ara A, the frequencies of breaks induced by X-rays increased by about 2 fold, whereas for neutron irradiation, ara A had no influence on the yield of breaks, indicating the quality and repair of breaks induced by low and high LET radiation is different. PCC studies on human lymphocytes X-irradiated in G2 (post DNA synthesis) showed that chromatid exchanges formed rather rapidly and did not increase in frequencies, whereas the frequencies of breaks declined with time.

Whole chromosome painting was combined with FISH using pan-centromeric and telomeric probes in order to accurately estimate the true frequencies of incomplete (one way) and complete (two way and complexes) exchanges. When telomeric probing was not carried out, the frequencies of incomplete exchanges were 21% following 4 Gy of X-rays, whereas with telomeric probing this frequency was reduced to about 5%. A similar trend was also found after irradiation with neutrons. Since, the resolution of the employed FISH technique is limited, it may be concluded that in all probability, all exchanges are complete.

Attempts were made to study the individual susceptibility among humans for response to ionizing radiation. Apart from defects in the repair of DNA damage (such as ataxia telangiectasia patients) which bestow increased radiosensitivity, other factors are poorly defined. The enzymes involved in the detoxification of damage induced by free radicals may play a role in determining individual radiosensitivity. Mutations in the glutathione S transferase (GST) gene family is common in the general population. 57 individuals who have been earlier genotyped for their status in this gene family, namely GSTM1 and GSTT1 defective phenotypes were screened for their response to radiation. No differences were observed between these two groups in the background or induced response. A Poisson model was applied to test the contribution of the GSTM1 genotype, smoking status and age in the yield of radiation induced chromosomal aberrations. No trend was observed with respect to any of these parameters. It could be concluded that GSTM1 gene status does not affect the radiosensitivity of individuals as assessed by the induction of chromosomal aberrations.

**Partnership**

This project was bi-national and closely linked to the project “Chromatin structure and DNA repair in relation to ionizing radiation-induced chromosome aberration in mammalian cells”. The collaboration was mutually beneficial to both partners.

**Selected references**

Kinetics of the formation of chromosome aberrations in X-irradiated human lymphocytes, using PCC and FISH.

Low level of DNA repair in human chromosome 1 heterochromatin.

Distribution of radiation-induced exchange aberrations in human chromosomes 1, 2 and 4.
DNA is the carrier for the genetic information of almost all organisms and hence the 'blueprint of life'. It is of vital importance to all living organisms that the integrity of the DNA is well conserved and protected against internal and external factors. Injuries to the DNA may result from exposure to a variety of environmental factors including ionising radiation (IR). IR induces various types of DNA damage among which DNA double strand breaks (DSB) are the most harmful. IR induced damage can cause genetic alterations (mutations) that may ultimately lead to cancer and hereditary diseases. Cellular defence mechanisms including pathways directed towards repair of DNA damage and cell cycle regulation, have been evolved that can counteract the deleterious effects of IR. The importance of these defence mechanisms for humans is underscored by inherited disorders associated with defects in these pathways such as ataxia telangiectasia and Nijmegen breakage syndrome. At the cellular level these disorders are characterized by DNA instability and cancer proneness.

IR is very efficient in inducing chromosomal aberrations in cells both in vitro and in vivo and DSB are considered to be the most important lesion for the induction of these aberrations. In recent years, molecular cytogenetic techniques have significantly increased the resolution and accuracy of detection and quantification of different types of chromosomal aberrations. Along with the availability of mutant cell lines and knockout mouse models sensitive to radiation, this has offered new insights into the mechanisms of formation of chromosomal aberrations. The broad objectives of this project were to exploit these developments in order to gain a better understanding of radiation action.

Mechanisms of formation of ionizing radiation-induced chromosomal aberrations (CHROMOSOME STRUCTURE)

Challenges to be met

The present proposal is a multi-disciplinary approach aimed to unravel the mechanisms of radiation-induced chromosomal aberrations by studying the events occurring from the initial DNA damage, its repair or mis-repair and the biological factors influencing the ultimate yield of chromosomal aberrations.

The major challenge in the project is to connect the IR induced events in the interphase nucleus (induction of DSB, chromatin remodelling and repair) to the formation of chromosomal aberrations as observed in cells in metaphase. To reach this goal, different strategies will be undertaken including the visualisation of radiation-induced DSB in the cell nucleus, the assessment of effects of radiation on interphase nuclear architecture, the exploitation of mutant cell lines known to be deficient in specific repair pathways or in genes controlling cell cycle check-points. These studies are complemented by advanced high resolution detection of chromosomal aberrations in the whole genome by employing multi-colour FISH and image analysis systems (COBRA, SKY, Q-FISH).

Achievements

The resolution of detecting different classes of aberrations has improved by using the technique of fluorescence in situ hybridisation (FISH). Particularly, the introduction of centromeric probes along with whole chromosome painting has facilitated the discrimination between translocations and dicentrics. The introduction of peptide nucleic acid (PNA) probes for telomere detection has provided a valuable tool for detecting complete and incomplete exchange aberrations. Employing these tools, it appears that differences in chromosomal aberration frequencies among chromosomes with different level of heterochromatin or transcription are probably small. Moreover, incomplete aberrations (aberrations with open DNA ends) are either very rare or do not exist in first metaphase after IR suggesting that there is tight control of genomic integrity at this level.

Two factors appear to be crucial for the formation of chromosomal aberrations: spatial distribution of DSB and repair efficiency. Using chromosome arm specific probes (Fig 1) it became apparent that exchanges within a chromosome occur with much higher frequency than between chromosomes (Fig 2) indicating the crucial role of proximity (e.g. the distance between DSB) in the formation of chromosomal aberrations.

The relationship between the rate and type of DSB repair and chromosomal aberration formation has been studied using either inhibitors of DSB repair or employing cell lines deficient in the repair of DSB. Most of the results indicated that non-homologous end joining (NHEJ) is the most important pathway, though homologous recombinational repair (HRR) at some stages of the cell cycle can certainly not be ruled out. It is also evident that the longer the DSB remain un repaired, the higher the frequencies of aberration formation suggesting that chromatin/chromosome territories in the interphase nucleus are dynamic and capable to interact.
PART 2

CHROMOSOME STRUCTURE Information Column

Title: Mechanisms of formation of ionizing radiation-induced chromosomal aberrations: Impact of repair pathways and nuclear architecture (CHROMOSOME STRUCTURE)

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Status: Ongoing

Partnership

The partners were drawn from laboratories in seven EU universities. Future work aims at gaining much more insight into the organisation of the interphase nucleus by using newly developed multi-colour FISH to analyse higher order chromatin structure, chromosome territories using 4D microscopy and quantitative image processing, before and after irradiation of cells. Specific issues for the future include the importance of complex DSB, DSB repair in real time, the influence of telomere length on aberration formation and the exploitation of techniques for genome-wide aberration analysis.

Selected references


IR induced exchanges occur preferentially within chromosomes. The expected value is based on DNA content and known frequencies for the total genome.
The molecular basis of DNA damage response and radiosensitivity
(DNA DAMAGE RESPONSES)

Challenges to be met

The project has its focus on understanding the molecular basis of radiation sensitivity in humans. In particular: (i) the identification of radiation sensitive individuals, (ii) a mechanistic understanding of radiation sensitivity, and (iii) the consequences of radiation-induced DNA damage and repair for human health.

The various approaches include:
- The development of rapid methods to identify defects in repair of DNA breaks to be used for screening of individuals.
- Isolation and characterization of genes influencing radiosensitivity including those controlling DNA repair and the cell cycle response to IR.
- Analysis of gene functions and gene products from the molecular to the whole organism level.
- Construction of transgenic mice carrying mutations corresponding to those identified in human genetic disorders showing IR sensitivity.

Achievements

Repair pathways: To counteract the deleterious effects of DSB, several repair pathways have evolved. The non-homologous end-joining (NHEJ) pathway is an error prone pathway leading to loss of genetic material during repair of DSB. In contrast, homologous recombination repair (HRR) is an error free pathway for repairing DSB utilising an undamaged sister chromatid or homologous chromosomes. The relative importance of each pathway depends on the phase of the cell cycle, on the cell type and on the stage of development and possibly by the complexity of the DSB. All these damage response mechanisms are highly conserved in evolution. A complex consisting of proteins encoded by the genes RAD50, MRE11 and NBS1 participates in NHEJ and HRR probably as a damage sensor.

Non-homologous end joining (NHEJ). Cells affected in this pathway are sensitive to radiation and have reduced ability to repair DSB. In addition they are impaired in V(D)J recombination, demonstrating a link between repair of IR-induced DSB and breaks formed during the process of immunoglobulin rearrangement in lymphocytes. The recognition of DSB is affected by the DNA-end binding proteins Ku80 and Ku70 together with the large catalytic sub-unit DNA-PKcs. DNA-ligase IV and its partner protein XRCC4 carry out the joining step of the process.

Homologous recombination repair (HRR). At present in yeast ten genes have been identified to be involved in HRR and mutations in these genes lead to sensitivity to IR and defects in mitotic and/or meiotic recombination; human homologues of most of these genes have been identified. To address directly the role of HRR in mammals, mouse knockout strains have been generated.

Cell cycle checkpoints. Apart from their ability to repair radiation damage directly, cells have evolved additional responses as protection from radiation damage. Cell cycle checkpoints are important protective mechanisms, which arrest cell cycle progression to prevent cells containing damaged DNA from entering critical phases of the cell cycle, such as S phase and mitosis. At least ten genes involved in this process have been identified.

DNA damage response in man. The importance of the DNA damage response

Objectives

Information on human risk from exposure to ionising radiation (IR) comes from epidemiological studies, but is available largely for high doses and high dose rates of low LET radiation. To make rational judgements in radiation protection, it is necessary to extrapolate to low doses and low dose rates, and to have an appreciation of variation in response to radiation among the human population. This can be done with confidence only if we have a detailed knowledge of the mechanisms by which radiation induces cancer and genetic disorders. IR induces various types of DNA damage among which DNA double strand breaks (DSB) are the most harmful. If not processed correctly, IR induced damage can cause genetic alterations (mutations) that may ultimately lead to cancer and hereditary diseases. Cellular defence mechanisms including pathways directed towards repair of DNA damage and cell cycle regulation have been evolved that counteract the deleterious effects of IR. The importance of these defence mechanisms for humans is underscored by inherited disorders associated with defects in these pathways such as ataxia telangiectasia and Nijmegen breakage syndrome (NBS). In this project the major focus is on the elucidation of the molecular mechanisms underlying the various DNA repair pathways for IR induced DNA damage. This is achieved by cloning repair genes and by the characterization of the proteins encoded by these genes. This acquired knowledge has been used to construct mouse strains with defined deficiencies in these repair processes in order to study their roles in alleviating the deleterious effects of IR at the level of the whole organism. Moreover, a considerable effort has been made to identify radiosensitive individuals within the human population and to correlate their radiosensitivities with possible defects in repair pathways.
pathways in man is shown by the genetic disorder ataxia-telangiectasia (A-T), in which radiation hypersensitivity is associated with defects in repair of DSB and cell cycle checkpoint control following radiation damage. NBS is a related disorder with many similar features to A-T at both the clinical and cellular levels. The product of the NBS1 gene has been recently identified as a component of a repair complex. To identify radiation sensitive individuals, cells from cancer patients have been examined for radiation sensitivity and defects in repair of DNA. The data suggest that subgroups of patients are partially defective in repair of IR induced DNA damage. Marked overt radiosensitivity unlinked to recognised defects therefore does exist in the human population.

**Partnership**

The partnership brought together research groups with a broad range of expertise. Future work will provide major advances in the analysis of DNA repair pathways, and their involvement in human radiosensitivity, by combining the study of newly discovered human repair genes with the use of novel analytical techniques, model organisms, and in vitro biochemistry. Assessment of the dynamics of repair and the coordination between the different repair pathways is a focus of future studies.

**Selected references**


Challenges to be met

The main source of information on radiation-induced human cancer risk comes from epidemiological data on exposed populations. However, direct information is available only at relatively high doses (above 0.1 Gy), and mostly from low-LET radiations (X- and gamma rays). A linear extrapolation from this data is applied at lower doses and additional extrapolation is applied to other radiation types. The shape of the cancer dose response curve at the low doses is a matter of constant debate. Arguments range from a threshold or even beneficial effect of small radiation doses (hormesis) to non-threshold supralinear responses (implying that small doses are more hazardous than previously assumed).

Indirect radiation effects, such as genomic instability (novel mutations and cell death in the progeny of irradiated cells) and bystander effect (effects observed in the neighbouring cells not directly impacted by radiation), may be important early steps in the development of radiation-induced cancer. Gaining understanding of underlying mechanisms may have consequences for cancer risk assessment; these mechanisms could potentially be incorporated in biological modelling of tumorigenic responses in the future.

Achievements

It has been observed that the progeny of irradiated cells show occurrence of new mutations and/or new chromosomal aberrations or other genomic damage for many generations. Affected progeny also demonstrate high levels of lethal mutation, which may be measured as delayed reproductive cell death and/or delayed apoptosis. Participants in the project have developed an approach to determine the effects of the lowest possible dose of densely ionising alpha particles to cells, that of a single particle traversal. It was demonstrated that a single alpha particle is able to induce chromosomal instability in the progeny of cultured human cells.

The condition known as genomic instability has a strong dependence on the type of cell, genotype and radiation quality. Genetic susceptibility to genomic
instability induction is being studied using animal models. Genomic instability occurs in the progeny of irradiated cells at a frequency that is several orders of magnitude higher than would be expected for a mutation of a specific gene. Changes in gene expression are examined in order to find out whether an instability phenotype is associated with sustained alterations in the expression of genes, rather than specific locus alterations.

Existing data of radiation-induced genomic instability suggests that transmission involves non-traditional epigenetic inheritance (stable non-mutational changes). Work related to the epigenetic mechanisms of perpetuation of instability is in progress. Increased oxidative stress seems to be a long term characteristic of the progeny of irradiated cells. Participants in the project have demonstrated that the increased oxy-radical generation is actually maintained by a signal produced by the irradiated cells in the culture medium.

**Partnership**

The project brings together the major EU laboratories involved in the discovery, characterisation and mechanistic investigations of genomic instability and bystander effect. Pooled knowledge and resources are directed to answer key questions concerning physics, biology and implications for radiation protection. The multidisciplinary team has access to a wide range of specialised radiation sources, unique animal and cellular models and molecular biological techniques to address these questions.

**Selected references**


Telomeres are specialized structures at the end of chromosomes composed of short tandem DNA repeat sequences and proteins. The primary role of the telomeres is to protect chromosome ends from recombination. Telomeres, by means of TRF2 binding protein, allow the cell to distinguish the natural chromosome ends from DNA double strand breaks (DSB). These lesions are created by exogenous agents such as ionising radiations. Chromosomal DSB are particularly dangerous lesions for cells. DSB are usually stabilised through the acquisition of a pre-existing telomere by recombination, but it has been suggested that in some cells they can also be stabilized by the direct addition of simple telomeric repeats to the free ends of chromosomes by telomerase. Persistent DSB can lead to carcinogenesis through activation of oncogenes, loss of tumor-suppressor genes or loss of heterozygosity. If a DSB is stabilised in germ cells, they may lead to terminal deletions which are known to be associated with abortions and a wide variety of birth defects in humans. The overall objective of the project was to determine the extent to which telomere sequence processing influences radiation response.

**Challenges to be met**

Insight into the mechanisms of DSB repair and stabilisation in mammals is crucial in order to understand the biological consequences of exposure to ionising radiations. The challenge was to gain understanding of the role of telomere acquisition processes in the capping of radiation-induced DSB and how this molecular mechanism can modulate the formation and stabilisation of chromosome aberrations. To this end mouse germ and embryo cells have been examined. The partners investigated the expression of telomerase in these cells and their upregulation following in vivo exposure to X-rays, as well as the radiation-induced chromosomal rearrangements and their telomere status.

**Achievements**

Cells have been assayed for telomerase activity using a PCR based Telomerase Repeat Amplification Protocol (TRAP). High levels of telomerase activity were detected in mature female germ cells (oocytes) and in zygotes. Very low levels of telomerase activity were detected in mature spermatooza. Telomerase activity was also measured in male germ cell precursors. Cell extracts from adult mice testes containing mixed spermatogonial cell populations showed a relatively high activity. The cells responsible for the telomerase expression observed in these extracts were probably premeiotic spermatogonia.

A dose-dependent increase in the telomerase activity up to 3 Gy X-ray exposure of female mice was observed in mature oocytes. Increased levels of telomerase were also found in zygotes after in vivo irradiation of male mice. Again, these upregulations were found to be dose-dependent from 0 to 3 Gy, and were less marked at 4 Gy. The results of our experiments suggest a role of telomerase in DNA repair mechanisms. Chromosomal damage and telomere capping of radiation-induced chromosomal breaks in spermatogonial cells was assayed in spermatocytes at metaphase I. Adult male mice were given 3 Gy of X-rays to the caudal third of the body and were sacrificed at different intervals post-irradiation to sample spermatocytes irradiated at different spermatogonial stages. Exposure of pre-meiotic cells to ionizing radiation gave a considerably lower proportion of cells with aberrations (figure 1) as compared to the exposure of meiotic stages. Inter-stage variations in chromatin condensation and chromosome behaviour may contribute to differences in the response to irradiation.

When analysing the telomere status of radiation-induced chromosomal breaks, we observed that the majority of centric fragments had only the proximal telomere pair present. However, in two out of 378 spermatocytes analysed with a complete set of chromosomes and telomeres an extra pair of telomeres was observed at the distal end of the centric fragments (figure 1). We conclude that although formation of new telomeres on radiation-induced chromosome fragments does not occur regularly in mouse spermatocytes, in a small proportion of cells there is evidence of the generation of new telomeres.

Chromosomal damage and telomere capping of radiation-induced chromosomal breaks were also analysed in early embryos after in vivo irradiation of male germ cells at the stages of epididymal sperm, spermatocyte and spermatogonia. A total of 1282 zygote and 486 two-cell embryo chromosome complementes were analysed. The results showed a peak in the frequency of aberrant chromosome complementes in both stages when matings were carried out after spermatid irradiation. The analysis of the telomere status of embryo metaphases demonstrated that the acentric fragments associated with dicentric chromosomes...
always contained telomeres at each end. They were probably compound acen-
tric fragments with pre-existing telom-
eres resulting from the rejoining of the
free ends of the broken chromosomes by
illegitimate recombination (non homol-
ogous end joining, NHEJ).

Excess acentric fragments were also fre-
quently found in zygotes and embryos (figure 2). They originated from termi-
nal deletions, incomplete exchanges or
interstitial deletions. After irradiation of
spermatozoa at the epididymal stage,
about 40-50% of excess acentric frag-
ments had telomeres at both ends. This
percentage increased to about 100% in
embryos obtained after fertilisation with
spermatoza irradiated at the sperma-
tocyte and spermatogonial cell stages.
Assuming similar probabilities of cen-
tric and acentric fragments to rejoin,
the capped excess acentric fragments
observed in our study cannot be ex-
plained by NHEJ. Therefore, they rep-
resent the traces of telomere acquisition
events other than simple end-joining.

Overall, capping of fragments in sper-
matocyte I metaphases was very low
when prophase of the same cell stage
was irradiated. The proportion of capped
fragments was increased in embryo
metaphases when spermatoza and
spermatids were irradiated and was rel-
atively high when spermatogonial stages
were exposed to X-rays. This suggest
that the telomere acquisition events may
be dependent on DNA synthesis. In addi-
tion, our results show that telomerase is
active in oocytes, zygotes and male germ
cell precursors. The upregulation of
telomerase in the same cells after in vivo
irradiation points to the participation of
telomerase dependent mechanisms in
the capping process.

In summary, the origin of capped frag-
ments remains uncertain but, for some of
these, there are strong arguments that favour real capping events rather
than simple NHEJ. According to our
results, both chromosome healing by
telomerase and telomere capture by a
replication based mechanism can
explain the capping observed in sper-
matogenic and embryonic cells. It is
also possible that these two mechanisms
could be operating together following
DNA damage. Whatever the mecha-
nism, chromosome capping in cells may
influence the induction of terminal dele-
tions, and gene amplifications and may
reduce chromosome stability.

The ongoing FP5 project, TELORAD, is
expected to allow the determination of the
mechanisms underlying the stabi-
lization of radiation induced DNA breaks
as well as the mechanistic links between
telomerase, DNA repair genes and
telomerase binding proteins.

Partnership

The partners in this contract were EU
investigators with an appropriate range
of expertise in telomere structure and
function.

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Radiation induced chromosome breaks in mouse
early embryonic cells: telomere capping.
Ionising radiation damages DNA and induces mutations and chromosomal anomalies. Broken chromosomes are highly unstable (fusions, deletions) and are quickly repaired. Telomeres, the very ends of the chromosomes act as natural caps and prevent chromosomes from fusion with one another. The proteins involved in telomere maintenance play a role in the distinction between radiation-induced DNA breaks which are subject to repair and telomeres. TELORAD aims to determine the mechanistic link between telomeres, telomerase, telomere binding proteins and DNA repair genes and their respective roles in cellular radiosensitivity. Mechanisms of stabilisation of broken chromosome ends, a starting point in the transmission of radiation induced damage, will be studied. Short telomeric arrays are also present at intrachromosomal locations, probably reflecting species evolution. The stability of these Interstitial Telomeric Sequences (ITS) will be studied. Long term consequences, such as gene amplification will be investigated to identify the involvement of telomere maintenance in radiosensitivity of individuals.

Telomere instability and the formation and transmission of radiation induced DNA damage (TELORAD)

Challenges to be met

The aim of this proposal is to study telomere instability and the formation and transmission of radiation induced DNA damage. The work is divided into four workpackages (WP):

The work of WP1 aims to determine the mechanistic link between telomerase, telomeric proteins and DNA repair genes in radiosensitivity. WP2 is focused on the determination of the propensity of interstitial telomeric sequence (ITS) to breakage and on the determination of the role of telomerase and DNA repair genes in the sensitivity to breakage and in the efficiency of repair of ITS. The main challenge of WP2 is to answer the question : Are ITS hot spots for radiation breakage? WP3 deals with the characterisation of capping of radiation-induced DNA breaks. Two mechanisms of stabilization of radiation-induced breaks, recombination mediated telomere capture, and chromosome healing by telomerase, will be particularly studied. In WP4, the role of telomerase, telomeric proteins and DNA repair genes in the transmission of DNA damage in the progeny of irradiated cells will be questioned.

Achievements

Work on radiosensitivity of telomerase-, telomeric proteins- and DNA repair deficient mice and cells has provided different biological endpoints indicating that telomere length is one of the biological determinants of radiation sensitivity in mammals.

Mice with short telomeres are highly sensitive to split dose gamma radiation. Components of the non-homologous end joining pathway DNA repair process, Ku86 and DNA-PKcs, have an important role in protecting telomeres from fusions. Telomere length is a marker for chromosomal radiosensitivity. Tumor-derived cell lines with shorter telomeres are usually more radiosensitive.

Double mutant mice for telomerase and DNA repair and also telomeric proteins have been generated and the study of their radiosensitivity is ongoing. The effect of expression of telomeric protein is being analysed. Telomere dynamics were studied as a function of the targeting of telomere binding proteins to a specific telomere. The influence of cell irradiation on telomere binding by proteins will be analysed.

The radiosensitivity of interstitial telomeric sites seems different in hamster and human cells. Interstitial telomeric repeat sequences are detected as breakage hotspots in hamster cells. Up to now such results have failed to be observed in human cells. Further studies are ongoing to address the proneness of natural ITS to breakage. To identify why hamster ITTs are sensitive to ionizing radiation, it has been proposed that radiation-induced DNA DSBs located within ITTs may be preferentially targeted by telomerase.
because of their complementarity with the telomerase RNA template. The consequence of this may be chromosome breakage within ITs as a result of telomerase-mediated healing. Alternatively, preferential breakage may be the result of the hyper-recombination activity of ITs.

The analysis of the mechanisms of capping of radiation-induced DNA breaks are crucial for understanding the biological consequences of exposure to ionizing radiation.

Most of the preliminary studies, optimisation of the techniques, development of cell lines have been performed; work is in progress in mice and human cells.

Studies on transmission of DNA damage has shown that DNA repair gene defects increase the probability of gene amplification:

Interest is focused on gene amplification and chromosome imbalances as detected in human radiation-induced tumours. Variation in these processes may rely on the selection of specific chromosome imbalances in tumours due to abnormal telomere maintenance.

**Partnership**

The consortium is composed of seven partners, two of which are assistant contractors, and two subcontractors. TELORAD brings together partners from national research laboratories from cancer institutes and from university laboratories. This team has expertise in molecular biology, cell biology, genetics, radiation biology and animal sciences. Unique cellular and animal models are available in the team.

**Selected references**


Challenges to be met

The project focuses on the analyses of cell cycle regulating enzyme activities at the G2-phase/mitosis transition in relation to DNA damage, DNA repair and the development of chromosomal damage. The working hypothesis is that the higher the endogenous cdk1/cyclin B activity is in a cell, the higher will be the non-repaired damage in DNA at the time when the cell enters mitosis. To test this hypothesis different human cell lines and embryonic cells from different mouse strains with known differences in radiation sensitivity were analysed for cell cycle activity, chromosomal damage, and DNA repair capacity.

The project was separated in two major parts, one on adult somatic cells and a second on embryonic cells. The two major parts were further subdivided in 4 work packages each. The work package aims were, (I) to analyse changes in cdk/cyclin enzyme complexes in different human cell lines in response to irradiation, (II) to study the evolution of chromosomal damage after premature chromosome condensation (PCC) in these cell lines, (III) to measure the repair capacity in cells by using the comet assay, and (IV) to analyse intracellular signal transduction pathways in irradiated cells.

Achievements

Irradiation induces changes in phosphorylation of the central cell cycle driving enzyme complex at the G2/M transition point (cdk1/cyclin B protein kinase). In the cell lines analysed there was an about 50% decrease in the kinase activity in irradiated cells. At least up to an irradiation of 2 Gy, there was no difference in the amount of initial radiation-induced DNA damage for each particular dose in exponentially growing mammalian cells in vitro. Repair was comparable in all four cell lines.

The conversion of primary radiation-induced DNA damage into visible chromosomal damage under the influence of different levels of cdk1/cyclin B activity was analysed by premature chromosome condensation (PCC). The PCC breaks increased linearly with dose and the yield was dependend on the type of the mitotic cells used as PCC inducers. From these experiments it is concluded, that the differences in the conversion of radiation-induced initial DNA lesions for each particular dose into PCC breaks reflect differences in the cdk1/cyclin B activity levels during chromosome condensation in early mitosis.

Using the G2 PCC radiosensitivity assay, cancer patients (n=185, with different types of cancer) on the average showed an increased in vitro radiosensitivity of their peripheral lymphocytes when compared to controls (n=25). Because of our observation of a strong correlation between G2 chromosomal radiosensitivity and cdk1/cyclin B activity during the G2/M transition and of our failure...
to detect this strong correlation between sensitivity and DNA repair, we conclude that cell cycle regulation is a key player in determining human radiosensitivity.

To study cell cycle regulation in early mouse embryos and effects of irradiation on this regulation, the activity of the cdk1/cyclin B protein kinase was determined in single oocytes or embryos. After fertilisation, cdk1/cyclin B protein kinase activity in mouse embryos is low during interphase of the first cell cycle, increases during the first mitosis, and decreases again during the next interphase.

Embryos irradiated in the first cell cycle were arrested during G2. However, the dynamic of this radiation-induced G2-block in the first embryonic cell cycle is totally different between the mouse lines tested. This differential dynamic in the G2-block in one-cell embryos of the different strains is strictly paralleled by a similar dynamic in the cdk1/cyclin B activity.

Using the comet assay the amount of initial radiation induced DNA damage in embryos for a particular dose was comparable to the amount in the cell lines mentioned above. The repair in embryos of the mouse strains tested is extremely fast and almost complete after 30 minutes. This is significantly faster than in all cell lines and primary cells tested so far.

The results of these studies will allow a better understanding and assessment of risks from those effects which are characteristic consequences of exposure to small radiation doses, i.e. cancer, genetic damage and disturbance of development in utero. All results obtained so far are consistent with the proposed hypothesis that following exposure to ionising radiation, the onset and the efficiency of chromatin condensation-decondensation which is dependent on cdk1/cyclin B activity levels is the important determinant of the process that converts initial radiation-induced DNA damage into chromosomal breaks. This may, therefore, explain the differential radiosensitivity observed at the various stages of the cell cycle as well as among mutant cells and cells of different origin. More important we were able to show differences in in vitro chromosomal radiosensitivity of peripheral lymphocytes of cancer patients compared to lymphocytes from healthy control individuals.

**Partnership**

The project integrated the expertise of five laboratories all of which have made important contributions to progress of the project and the development of new methods needed. Their co-operation allowed the concerted approach to correlate key mechanisms in cell cycle control with damage to the DNA molecules and chromosomes at different stages of the cell cycle.

**Selected references**


Challenges to be met

Ionising radiation can activate a large number of responses in mammalian cells. The most important of these are the activation of a number of signal transduction pathways which in turn activate specific transcription factors, transient inhibition of cell division (cell cycle arrest), and induction of programmed cell death (apoptosis). The function of these reactions is to protect the organism as a whole against the consequences of radiation-induced damage. Elucidation of the mechanism by which these responses are activated by ionising radiation is a main objective of this study.

Two important transcription factors that are activated by radiation are NF-kB and cJun/ATF2. Two other consequences of exposure of cells to ionising radiation are a transient inhibition of cell division (cell-cycle arrest) and programmed cell death (apoptosis). The function of cell-cycle arrest is probably to provide sufficient time for the cells to repair the DNA damage in the absence of DNA replication (in this way preventing mutations). Programmed cell death is a suicide mechanism that is activated if the radiation damage is too extensive to be repaired. Both phenomena require the transcription factor and tumour-suppressor protein p53. The p53 protein is the product of a tumour suppressor gene, which is involved in the control of cell division. Its role in protecting genomic integrity is suggested by the finding that the protein accumulates upon irradiation and that cells lacking p53 do not show the radiation-induced cell-cycle arrest. The fact that apoptosis is also a function of p53 has important consequences for cancer therapy, since tumour cells containing mutant p53 are much less responsive to chemotherapy than tumours containing functional p53 (chemotherapeutic agents normally kill tumour cells by apoptosis). One of the genes that is activated by radiation-induced signalling is the gene coding for ornithine decarboxylase (ODC). Among others, ODC plays a role in the response of cells to radiation damage. Interestingly, it was found that certain human cells fail to activate ODC after radiation damage, and that these cells are derived from individuals who appear to be resistant to UV-induced cancer. This suggests that the activation of certain genes may increase the risk for oncogenic transformation, while at the same time contributing to cell survival.

Achievements

Radiation-activated signalling cascades

In this workpackage the activation by ionising radiation of two important transcription factors in the cellular stress response, NF-kB and cJun/ATF2, was studied. Activation of NF-kB depends on degradation of its inhibitor IkB. This is caused by phosphorylation of IkB by a mechanism different from that induced by ultraviolet (UV) light. Similarly, while UV light causes phosphorylation of both cJun and ATF2, ionising radiation activates only ATF2. Thus, ionising radiation activates two important transcription factors via mechanisms distinct from those used by UV light. Finally, evidence was found that not only nuclear DNA damage but also processes in the cytoplasm (plasma membrane) involving inactivation of tyrosine phosphatases play a role in activation of the signalling pathways by radiation.

Activation of p53 by ionising radiation

The tumour suppressor protein p53 plays a central role in the response to ionising radiation and for a large part determines the fate of the irradiated cell. It was shown that the stabilization (and thus increase) of p53, which is the key event in its activation, is probably independent of phosphorylation of the p53 protein, as was generally believed.
Rather, it seems likely that modulation of Mdm2, the degradation-inducing enzyme which binds to p53, is the important factor.

Constituents of protective responses
Ornithine decarboxylase (ODC) is the key enzyme in polyamine biosynthesis and is strongly activated by ionising radiation. Intracellular levels of polyamines are important factors in protecting cells from radiation damage. It was shown that in cells derived from certain “cancer-resistant” individuals, ODC was not activated after exposure to ionising radiation or UV light. Thus, the absence of ODC induction can possibly protect against carcinogenesis. This idea was tested in radiation-sensitive mice which were irradiated with UV-B and simultaneously treated with DFMO, a specific inhibitor of ODC. Indeed, it was found that application of inhibitor strongly diminished the frequency of skin tumours.

Novel ionising radiation-activated genes
Attempts were made to isolate genes that are specifically induced by ionising radiation in intact mice and in human cells. In the mouse studies a gene has been identified, named Scotin, which seems involved in the apoptotic response to ionising radiation. Induction of the gene is dependent on p53 since it is not activated in p53-null mice.

Surprisingly, in the experiments involving human cells no genes were isolated that were specifically induced by ionising radiation. In the same study, however, several genes were identified that were induced by the alkylating chemical agent methyl-methanesulfonate (MMS) (but not by radiation). One of these genes is a novel member of the signalling cascade from the endoplasmic reticulum to the nucleus, and is activated by the presence of unfolded proteins, the so called unfolded protein response (“protein stress”).

Overall these studies have shown that ionising radiation activates different mechanisms than other forms of stresses such as UV light. Unravelling these mechanisms will further help understanding what ionising radiation does to the cell and how it differs from other stresses. This knowledge may contribute to a better understanding of the harmful effects of ionising radiation.

Partnership
This work was dependant upon the complementary expertise of three EU laboratories with long standing interests in cellular stress responses.

Selected references


The aim of this contract was to assess the risk due to inhalation of radon and its decay products that escape from certain forms of rock. The central objective was the assessment of human risk that requires combination of several topics and a multidisciplinary approach. Five main topics were addressed: radioactive aerosol studies, modelling, human studies, animal studies and retrospective assessment of radon exposure studies. These tasks were distributed between five working groups.

**Challenges to be met**

**Aerosol Studies Group** sought to determine the properties and behaviour of the radon decay products in order to provide improved characterisation of the indoor atmosphere. Studies were focused on the size distribution of the unattached and aerosol-associated radon decay products and the amount of the unattached activity. These are the most important parameters in models used for calculating lung doses.

**Modelling Group** was acting to develop two complementary approaches. One used the model based on the new ICRP dosimetric model for the respiratory tract (ICRP Publication 66, 1994), and the other used a stochastic model that included the clearance process and radiation interaction at the cellular level.

**Human Studies Group** aimed to conduct inhalation studies on human volunteers in order to provide a better definition of radon progeny specific parameters, including the deposition pattern of the unattached fraction and the dependence of deposition on age and gender.

**Animal Studies Group** sought to assess the effect of exposure rate at low cumulative radon exposure for the induction of lung cancer in rats. The extent to which early biological dosimetric markers, such as nuclear aberrations and cell proliferation might be used to predict late effect from exposure to radon and its progeny was also to be investigated.

**RARE (Retrospective Assessment of Radon Exposure) Group** was acting to provide data for the retrospective reconstruction of indoor radon exposure. The studies were focused on techniques allowing measurement of long-lived radon decay products either by measuring $^{210}$Po in indoor glass surfaces (surface traps) or in porous materials (volume traps).

**Achievements**

Progress was made in improvement, calibration and automation of experimental techniques for continuous and integrated measurements of the unattached fraction $f_p$ and equilibrium factor $F$ values. Measurements were performed to determine the variation of size distributions of unattached and aerosol-associated radon decay products under typical living conditions and were considered for updated dose calculations. Controlled chamber studies to understand the basic behaviour of airborne activity concentrations were undertaken. Measurements were performed to determine neutralisation rates of $^{218}$Po, to understand the cluster growth with residence time and to understand the hygroscopic growth of aerosol particles.

The programme RADEP has been developed to calculate the weighted committed equivalent lung dose per unit exposure of radon progeny ($H_w/P_p$) which implements the ICRP Publication 66 Human Respiratory Tract Model (HRMT). The stochastic deposition model (IDEAL) was compared with the deposition model used by the HRTM; the agreement was good. A deterministic radon progeny dosimetry model (RADOS) has also been developed. Initial calculations with RADOS show that the basal and secretory cell doses are slightly smaller compared with that of the HRTM.

A sensitivity analysis has been performed that has identified those HRTM model parameters that most affect the $H_w/P_p$. 

### Risk assessment of exposure to radon decay products (RARAD)

#### Objectives

- **Comparison of bronchial and pulmonary deposition as functions of particle size for sedentary breathing conditions using the Human Respiratory Tract Model (ICRP Publication 66) and a stochastic model.**

#### Calculated average $^{222}$Rn concentration, for more than 20 years in the past, from retrospective measurements on 225 pairs of personal objects.

**Monte Carlo Model:**

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**ICRP Model:**

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A stochastic rat deposition model (RALMO) and a clearance model for the rat based on the HRTM have been developed to calculate equivalent dose.

Human studies were focused on the deposition patterns of the unattached fraction and the dependence of deposition on age and gender, the transfer of inhaled radon progeny to blood and the comparison of total deposition of radon progeny for adults and children in the domestic environment.

The results of experiments on the influence of exposure-rate on lung cancer induction results indicate that at relatively low cumulative exposures comparable to lifetime exposures in high-radon houses or current underground mining exposures, the risk of lung cancer in rats decreases with decreasing exposure-rates. These data suggest that in terms of risk of induction of lung cancer, there is a complex interplay between cumulative exposure and exposure rate. A positive dose rate response was seen for proliferating epithelial cells at relatively high exposure levels. The response of proliferating epithelial cells was found to depend on dose rate at higher doses, whereas at lower cumulative doses no significant elevations in proliferation were observed.

Protocols were also developed for selection of the most suitable surface and volume traps to be used for radon measurements. In addition a questionnaire was developed to obtain estimates of room parameters that control the deposition and build up of $^{210}$Po on surfaces. Computer models have been produced which make it possible to make a retrospective estimation of radon exposure in the past using surface trap measurements and questionnaire information.

**Partnership**

This contract has pooled the expertise of EU laboratories working on different fields of radiation protection. Collaborations were developed with other groups of the Nuclear Fission Safety Programme involved in research on alpha particle carcinogenesis, dosimetry of alpha-emitters and with the different epidemiology programmes.

**Selected references**


Radioactive materials play an important socio-economic role in most developed countries. In addition to their industrial uses, especially in energy generation, there is increasing utilization of ionising radiation in the therapeutic and diagnostic procedures of modern medicine. Ionising radiation is also present in the natural environment where it may also have a socio-economic impact. Since ionising radiation is a proven human carcinogen it is clearly important to improve our knowledge of the risks involved in its use – this applies particularly to health effects that result from low dose exposure of the public and workers. Since epidemiological studies have insufficient power to directly measure excess cancer after these low doses it is necessary to project risks from high dose studies. Establishing the biological validity of these projections is an increasingly important issue in radiological protection research. Accordingly, the experimental studies outlined here utilized a combination of experimental models of cancer induction and molecular biology techniques to investigate the mechanisms and genetics of radiation carcinogenesis. The work was initiated in FP4 and is currently being extended in FP5; there is close collaboration with the GENRAD consortium.

The mechanisms and genetics of radiation tumorigenesis (MAGELLANS)

Challenges to be met

The specific challenges to be met in the project are best illustrated by the following questions. First, radiation is a proven carcinogen but where does it act in the complex multi-stage process of cancer development operating over many years and what are the implications for low dose risk? Second, we know from radiotherapy observations that rare human genetic conditions pre-dispose to radiation-induced cancer but should we expect more common genetic variation that might distort population-based estimates of low dose risk? At this stage in knowledge it is necessary to seek proof-of-principle evidence on these questions using relevant experimental models of cancer induction. The project was designed ahead of FP4 to make full use of information expected from human and mouse genome research. In the project the partners are seeking detailed knowledge of radiation induced myeloid leukaemia, lymphoma, skin tumours and breast cancer in selected mouse models.

Achievements

Induction of myeloid leukaemia and lymphoma: Changes in the normal structure of chromosomes are frequent in myeloid leukaemia and lymphoma arising in humans and mice; both these tumour types are thought to originate in bone marrow cells. Chromosomal studies relating to radiation-induced myeloid leukaemia in the mouse have shown that the crucial radiation-induced damage during multi-stage tumour development occurs in primitive, normal bone marrow cells. This damage is consistently expressed in bone marrow cells and tumours as loss of a small segment of chromosomal DNA and the segment has been isolated in fragments and reconstructed. Using genome information and technology the likely target genes have been positioned in this chromosomal region and analysed further to determine their importance for tumour initiation. Similar progress is being made for lymphoma development but, here, the critical DNA loss occurs on a different chromosome.

The picture emerging from this sub-project, and indeed from others in the same EU programme, is that radiation is principally acting very early in multi-stage carcinogenesis as a DNA-deleting agent for tumour-specific genes in single cells. There is already good knowledge of this mechanism of DNA damage and, overall, the research is adding important support to the view that cancer induction by radiation does not occur through an unusual mechanism. On this basis, cancer risk will tend to rise with dose without a low dose-threshold where risk can be discounted.

Genetic susceptibility to myeloid leukaemia, skin tumours and breast cancer: Previous studies with mouse models had provided some evidence of common, genetic variation in radiation cancer risk but little specific information. As given below, work on this sub-project has added substantially to knowledge. In the case of myeloid leukaemia, a single heritable gene distributed amongst mouse strains appears to be the major determinant of risk. The evidence suggests that the variant gene acts to reduce the ability of bone-marrow cells to respond adequately to DNA damage in certain chromosomal regions; the gene appears to be tissue-specific in its action. The chromosomal location of the gene has been estimated and work on its identification is underway. The genetics of skin-tumour susceptibility is more complex involving the interaction of a number of common variant genes, some of which have been located. Importantly, there appears to be shared genetic mechanisms for susceptibility to radiation and chemical carcinogens. One susceptibility gene which produces an
altered hormone-related protein has been isolated and its mechanism of action is under investigation; studies on a new mouse genetic model are also underway. Finally, a third sub-project on genetic variation in the genes that control response to radiation damage in DNA led to an external collaboration on breast cancer risk. This work involved gene location, isolation and characterisation showed that partial deficiency in a single key gene was associated with tissue radiosensitivity, chromosomal instability and breast cancer risk. Further work on the origins and impact of this variant gene, which is not common, is being shared with GENRAD.

The principal outcome from work in this area is evidence that variant genes influencing radiation cancer risk can be, but are not always, common. They tend to be tissue-specific in their action and frequently work in concert; cross-sensitivity to other carcinogens will probably apply to some variant genes. Overall, the work adds support to the view that, for genetic reasons, tissue-specific cancer risk after radiation will not be uniformly distributed in the human population. Also, that we should expect much of the common genetic variation in low dose cancer risk to be inherited in a complex fashion – predictions on the response of most individuals are likely to remain most difficult.

Partnership

The partnership brought together EU expertise in animal radiobiology, tumour biology, chromosome analysis, molecular biology and genetics. Without this combination it would not have been possible to fully exploit the scientific potential of the mouse models; in this area collaborative studies are essential. The progress made in MAGELLANS and GENRAD illustrates the growing power of these models, coupled with genome research, to comment upon complex biological problems associated with low dose risk. The work is also providing guidance on the future development of new approaches based upon human cellular material; this applies particularly to the issue of cancer-susceptibility.

Selected references

A cancer modifier role for parathyroid hormone-related protein.

Use of intercross outbred mice and single nucleotide polymorphisms to map skin cancer modifier loci.

Analysis of loss of heterozygosity in lymphoma and leukaemia arising in F1 hybrid mice locates a common region of chromosome 4 loss.

Elevated breast cancer risk in irradiated BALB/c mice associates with unique functional polymorphism of the Prkdc (DNA-PKcs) gene.
One limitation to the recommendations for dose limits in radiation protection is the tacit assumption that all individuals receiving the same dose of ionising radiation are equally at risk of developing cancer. It is now apparent that the human genome is highly variable, and that inherited genetic information influences individual sensitivity to many environmental carcinogens. At the moment radiosensitive individuals are recognised post-hoc, i.e. after tumours appear. The GENRAD consortium, in partnership with the MAGELLANS group, is striving to identify the genes that are responsible for determining individual cancer risk after irradiation. This knowledge will ultimately be used to develop a genetic screening platform for use in determining individual cancer risk following or even prior to, an exposure to ionising radiation.

**Challenges to be met**

The risk of developing cancer after exposure to ionising radiation is currently extrapolated from epidemiological data derived from exposed human populations. Although these cohorts are well defined, exposure is primarily high-dose radiation applied at a high dose rate, and no allowance is made for inter-individual variability. At the low doses and low dose rates more conventionally encountered the contribution of genetic variability may be more significant.

The most readily identified source of genetic variability is the rarely encountered group of genes responsible for familial cancer syndromes or acute radiation sensitivity. An inherited mutated form of one of these genes greatly increases the risk of developing cancer after radiation exposure. A less dramatic, but equally important, source of genetic variation is gene polymorphisms arising through the natural variability of the human genome (current estimates suggest there may be over 4 million sequence differences between normal individuals). These polymorphisms are responsible for only small differences in gene function and individually make a minor contribution to overall risk. However, in individuals who by chance inherit several such polymorphisms the net effect on risk may be appreciable.

The ability to more accurately predict the outcome of radiation exposure will greatly change modern radiation protection. Not only can individuals with workplace exposure be more accurately evaluated prior to exposure, but also adverse effects of radiation therapy can be minimized. Following accidental exposure those individuals at greater risk can be identified and offered more comprehensive care when resources are limited or enrolled in more frequent follow-ups. An additional product of this research will be a firmer understanding of the mechanisms of radiation carcinogenesis, which will allow a more direct approach in the evaluation of the risk, and hopefully the development of targeted radioprotective therapies.

Before we can use genetic profiling to determine individual risk we must first establish which genes influence risk. There is very little chance of using human epidemiological data to retrospectively identify genetic risk factors. However, as an alternative, recent advances in molecular genetics make it possible to identify susceptibility genes present in the mouse and rat genomes. During FP4 the consortium has developed animal models of human radiation carcinogenesis, selected to reflect the most clinically relevant target tissues. These models were validated to establish proof of principle for the use of genetic screens designed to identify the relevant genes.

In FP5 we are deploying genetic screens covering the entire mouse and rat genomes aimed at identifying the genes

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**Objectives**

Inheritance of a set of 5 susceptibility genes (red curve) increases radiation-induced tumour formation and shortens latency.
responsible for determining radiation cancer risk. Two different screening strategies are used. In the first, a genome-wide scan for loci exhibiting allelic imbalance (AI-screen) is used to map and ultimately identify regions of the genome harbouring genes with the potential to act through the germ-line to modify risk of radiation carcinogenesis. This screen is based on the premise that such genes manifest themselves through a change in the number of gene copies in the tumour tissue. Such a change is detectable through an assay of allelic status.

In the second screen, linkage analysis is used to identify those genes that exert a more modest influence on individual susceptibility. In a cohort of genetically heterogeneous animals those that inherit predisposing genes will be more likely to develop cancer. Consequently these genes will be found to be inherited more frequently by tumour-bearing animals (such a gene is said to be linked to the risk of developing cancer) (see figure).

Achievements

Five animal models of radiation carcinogenesis, breast, intestine, lung, lymphoid tissue, and bone, have been established in genetically defined systems amenable to analysis. In all models, genome-wide screens for allelic imbalance have already been conducted. A number of known and novel gene loci have been identified. For example in bone tumours (alpha-particle-induced osteosarcoma) a set of 10 loci, each believed to harbour a gene capable of increasing tumour risk when present as a germ-line mutation, have been mapped in the mouse genome.

In the search for genetic polymorphisms influencing cancer risk the linkage analysis studies have been performed, and statistical evaluations are underway. In one tissue this has already led to the identification of a polymorphism in a gene controlling the rate of cell proliferation. A highly significant linkage between inheritance of this polymorphism and the development of cancer after irradiation has been demonstrated.

Partnership

Modern genetic analysis requires large-scale coordinated efforts from a multidisciplinary team, with contributions required in the fields of animal sciences, radiation biology, tumour pathology, molecular genetics, and statistics as well as a mastery of highly complex molecular biology technologies. Radiation protection research is encountering increased public awareness, but no single European laboratory can hope to maintain expertise in all of these fields. The specialties required for this research project are provided by the partners in the GENRAD consortium.

Selected references

objectives

There is strong evidence that some people have a high sensitivity to radiation. People with rare cancer-prone genetic disorders such as ataxia-telangiectasia (AT), if treated by radiotherapy suffer severe side effects such as burned skin. However there are also real differences in sensitivity between seemingly healthy people in the general population. There is an incentive to develop a reliable test that could identify radiosensitive individuals. It could, for example, be used to avoid unacceptable normal tissue damage if such a person developed a cancer and received radiotherapy. In addition, there is the socio-legal question of whether identified sensitive subjects should be employed to work with radiation. The question of whether such people are more liable to develop radiation induced cancer is still open. The broad objective of the project was to develop and validate cellular assays for human radiosensitivity.

Challenges to be met

A technically demanding assay was developed, using skin fibroblasts which were cultured, irradiated in the G2 stage of their cell cycle and analysed in the metaphase stage to measure the frequency of radiation induced chromosomal aberrations. Cells from sensitive subjects showed a higher level of damage. A slightly simpler test was then developed using blood lymphocytes also irradiated in the G2 stage. A more straightforward test was developed, also using lymphocytes, but irradiated in the G0 stage and scored for the frequency of micronuclei seen shortly after the following metaphase. All three tests have been applied to cells taken from known radiosensitive persons such as AT cases and their relatives and they scored sensitive compared with apparently healthy control people. There was also evidence that some cancer patients had a greater likelihood of being radiosensitive than healthy controls.

The three tests measure different types of chromosomal damage all of which could potentially cause gene rearrangements or losses that are important steps in cancer formation. Chromosomal damage is broadly classified into two types; stable and unstable. The former, as its name suggests, is able to pass through cell divisions into daughter cells. This type is therefore more likely to be associated with early steps in the cancer process. Analysing for randomly distributed stable aberrations – translocations – in large numbers of cells is now possible by using the fluorescence in situ hybridisation method (FISH).

The study had two distinct aims. First, to extend the database of subjects tested by the G2 and G0 lymphocyte assays to determine the extent to which they are comparable in distinguishing radiosensitivity. This work concentrated on sampling newly diagnosed breast cancer patients and healthy controls. Second to select four small groups from the data base, patients and controls who scored at the high or low extremities of the sensitivity scale by one of the tests; the G0 micronucleus test. Lymphocytes from these subjects were test irradiated in G0 with gamma rays and examined by FISH to determine whether any systematic trend could be found in induced translocation frequencies that correlated with their sensitivity status.

Achievements

Data were collected for the G2 test on 105 healthy persons and 135 patients and for the G0 test 68 and 130 respectively. Eleven controls and 80 patients were tested by both methods. In each test a significantly higher proportion, about 40%, of the breast cancer patients showed higher aberration levels compared with about 10% of the healthy subjects. Within each test group variability from repeatedly sampling the same people was quite small; variability between persons was more marked.

The question was asked whether the two tests correlate, i.e., does the same person score high or low in both. Of the 80 patients only 3 scored doubly high; 44 were sensitive in just one whilst 33 were doubly negative. This poor correlation indicates that different mechanisms are responsible for the types of damage measured by the two tests. The well documented radiosensitive syndromes, like AT, that exhibit sensitivity in both G2 and G0 tests are conditions that involve multi-system disorders. They are characteristic of inherited mutations in regulatory genes that influence the activity of several other genes. By contrast in breast cancer patients the latter genes themselves may be where the mutations arise, with typically only one mutation event per patient. These would include genes involved in different DNA damage repair pathways. A mutation of this type would

A FISH image of a human lymphocyte metaphase with chromosome pairs 2,3 and 5 highlighted in green, the remainder in blue and centromeres in red. This cell contains radiation induced unstable aberrations involving some of the green chromosomes; a dicentric, a ring and their associated fragments.
therefore tend to confer either G2 or G0 sensitivity but rarely both.

The FISH study concentrated on 16 subjects; 8 patients and 8 controls with 4 of each having tested high or low in the G0 sensitivity method. Various combinations of 11 chromosomes, together comprising 62% of the human genome, were highlighted and their involvements in translocations measured. Following a 2 Gy test dose given in G0 the induced translocations yields in all 16 study subjects were very similar and they fell generally within 15% of expected values based on previous dose response studies using a small pool of healthy donors. There was no significant variability between subjects and especially no trend could be discerned that correlated with the radiosensitivity scores. The relative involvements of the 11 chromosomes tested in different classes of aberrations was also examined and again no trend relative to sensitivity could be found.

The conclusion of this study is that it is possible to distinguish a spectrum of radiosensitivity among apparently normal healthy persons and that breast cancer patients tend to fall within the sensitive end of the range. The presumption can be made that this would also apply to some other cancers. More research is required on characterising cellular radiosensitivity and it is highly likely that several distinct molecular processes operate. Whatever these processes might be, it appears that they can not be distinguished by measuring stable chromosomal translocations which are final products of DNA misrepair.

**Partnership**

This EC funded study enabled two radiobiological disciplines to collaborate. One partner had a long term research objective to develop sensitivity tests that could assist with radiotherapy treatment planning. The other partners had developed FISH analysis for use in biological dosimetry but also had the brief to exploit it as a tool for more fundamental research. Much was learned about the sensitivity tests and the likelihood is that, with further research, they will prove of practical value.

**Selected references**


Challenges to be met

Radiation-induced tumorigenesis is potentially an emotive issue particularly when cancers develop in children. They are caused by exposure of individuals to environmental sources of radioactivity, to material released at accidents or following military use to exposure in the work place and to medical irradiation.

It would be useful to determine whether signatures of radiation-induced cancers could be identified. An improved understanding of the molecular mechanisms and chromosomal changes induced by radiation would lead to a better understanding of the process of cancer formation. It is also important to establish whether particular sub-groups of the population are at increased risk of developing cancer.

Achievements

Radiation-induced childhood cancers from Belarus.

The consortium have painstakingly developed a tumour tissue bank from childhood cancers of the thyroid induced by radioactive iodine. Using this resource, it was possible to grow cells in tissue culture and thus study the chromosomes in the tumours. Specific hot spots in specific chromosomes where breakpoints occurred have been identified. Using molecular techniques, the DNA from specific regions can be amplified and checked for differences between normal DNA and tumour DNA. Characteristic rearrangements of the DNA were identified in the childhood tumours where the chromosomes are broken then repaired inappropriately leading to defects in the cells.

Radiation-induced human tumours induced in cell culture.

An alternative approach is to develop a laboratory model where normal human cells can be exposed to radiation and the stepwise process of cancer development followed. Using this in vitro approach tumours have been produced following exposure of cell cultures to gamma irradiation and alpha particle irradiation. Studies of the resulting tumour cells were able to establish characteristic chromosome changes and changes in gene expression.

Structural organisation of chromosomal regions in radiation-induced human cancers.

A genetic map of chromosome 10 has been constructed around the region where breakpoints occur in thyroid tumours. This was undertaken to try and explain why breaks occur at specific regions on the chromosomes and link this with DNA structure.

The programme has formed a useful transition into FP5 where new and established collaborations have developed. Using improved models of human epithelial cell cultures we have further established molecular and chromosomal changes in human cancers and will be applying this model to human breast cancer.

Partnership

The laboratories taking part in this programme have been collaborating for a long period of time and the EC funding has been essential in allowing good links to be maintained. The range of expertise being applied to this important issue of cancer induction in man could only be achieved by collaboration between laboratories experienced in this field. Future research will build upon the advances made in this project.

Molecular mechanisms of radiation carcinogenesis in man

The project aimed at investigating radiation-induced cancer in man focusing on the molecular and chromosomal changes that are induced so that a better understanding of these mechanisms could be obtained. The approaches used compared these molecular and chromosomal changes in:

a) The cancers that developed in children in Belarus induced by irradiation of their thyroid glands by radioactive iodine released following the Chernobyl reactor accident.

b) Experimentally induced human tumours produced following irradiation of human thyroid epithelial cells in the laboratory in cell culture.

Using this integrated approach with the expertise of a number of laboratories across Europe, the aim was to identify key molecular and cytogenetic events, which were important in the development of radiation-induced cancers in man.
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Radiation-induced transformation of SV40-immortalised human thyroid epithelial cells by single exposure to plutonium alpha particles *in vitro*. 

Distinct frequency of *ret* rearrangements in papillary thyroid carcinomas of children and adults from Belarus. 

Cytogenetic changes in radiation-induced tumours of the thyroid. 

Clonal chromosomal aberrations in simian virus-40 transfected human thyroid cells and in derived tumours developed after *in vitro* irradiation. 
*Int. J. Cancer* 96: 166-177 (2001)
Identification and isolation of susceptibility genes involved in radiation-induced cancer in humans
(SUS GENES IN RAD CAR)

Challenges to be met

Sources of tissue from human cancers that can be reliably associated with radiation exposure are rare. However, members of the project consortium have collected panels of such materials from two sources: thyroid tumour samples from children from Belarus (associated with radioiodine contamination from the Chernobyl accident) and tumour samples from secondary cancers induced (in the irradiated field) following radiotherapy for an unrelated primary tumour. A major challenge in the project is to employ state-of-the-art molecular cytogenetic and genomics techniques to characterize the genetic changes that drive the process of IR-induced human cancer, including the identification of individual cancer susceptibility genes.

It has recently been discovered that activation of the enzyme telomerase which generates protective caps for chromosomes leads to the immortalization of normal human cells. This has provided a means for developing cell culture models of human cancer that permit early critical steps in the process to be induced and characterized, in the absence of confounding genetic instability typically found in many overt cancers. A second main challenge is to develop such systems and to compare key genetic changes involved in IR-induced malignant transformation in culture, with those found in radiation-induced tumour material. The means by which telomerase is induced during radiation carcinogenesis needs to be established, as well as the role of telomerases in radiation-induced genetic instability.

Achievements

Analysis of human radiation-induced cancers has revealed common chromosomal and genetic alterations. Progress towards cloning of translocation breakpoints present in cells from childhood thyroid tumours has exceeded expectations. Molecular cytogenetic analysis has identified a number of interesting translocations including seven involving a known oncogene (RET). Cytogenetic and molecular analysis of secondary IR-induced cancers (following radiotherapy) is also yielding novel results. Rearranged chromosomes have been observed in these cancers with several common rearrangements detected. Molecular studies on 7 tumours have provided evidence for the involvement of the known tumour suppressor genes p53 and RB1 in the cancer process. An abrupt change in chromatin structure in the chromosomal region carrying RET has also been identified.

Major advances have been made in establishing cell culture models for radiation carcinogenesis based on human cells. Repeated exposure of telomerase immortalized retinal epithelial cells to X-rays converted them into tumour-forming populations. A large panel of radiation-induced malignant cell clones has been assembled for molecular and cytogenetic analyses. Three out of seven such lines were found to have suffered characteristic chromosomal losses. Human ductal breast epithelial cells have been
immortalized by the same procedures, and induced to undergo malignant transformation following IR exposure. A panel of around 50 transformants are being isolated for study.

Substantial progress has been made in the fine mapping by chromosome fragment transfer of a putative telomerase ‘master regulator’ gene on the short arm of chromosome 3. Several candidate genes from the region are being studied for the presence of mutations in human cancers. In addition, transgenic mouse model systems have established conclusively that lack of telomerase confers resistance to carcinogenesis and point towards an important role for telomere length in radiation sensitivity. Conversely, experiments in which telomerase has been constitutively expressed in specific tissues clearly show that the enzyme substantially enhances susceptibility to cancer induction, and consequently that telomerase activation is likely to be a key event in radiation-induced cancer.

**Partnership**

The partnership brings together a group of 8 European laboratories that have considerable expertise directly related to the scientific goals of the project. The technical and scientific expertise of the partners provides a suitable balance without significant overlap. The collaborations established will be important for achieving the goals of the project.

**Selected references**


**Challenges to be met**

To develop a network which enabled the application of a number of highly specialised assay techniques to the material in order to maximise the information available. To correlate molecular biological marker with clinicopathological information.

**Achievements**

We studied the pathology of over 800 thyroid tumours from patients aged up to 20 years at operation who were exposed to fallout from the Chernobyl accident. The majority (96%) were a type of thyroid cancer called papillary carcinoma. In those aged under 14 at operation, nearly 75% were of a particular subtype of papillary carcinoma (called solid/follicular SF). In adolescents only just over half of papillary carcinomas were of this morphology. This subtype is also found in young children from a non-radiation exposed area (England and Wales), but the frequency was much higher in the Chernobyl-related cases.

Two other types of thyroid cancer (follicular and medullary carcinoma) were much less frequent in the study population. More detailed study combined with previous data suggests that the latency for the solid/follicular subtype may be shorter than that for other thyroid tumour types. If this is true, adjustments to the prediction of risk of development of thyroid cancer following exposure of young children may require revision.

We have also studied the involvement of certain genes causing thyroid cancer in 180 of these tumours. These genes have been shown to be broadly involved in non-radiation associated thyroid carcinogenesis; some of these genes are known to be involved in carcinogenesis in other tissues e.g. the ras and p53 genes, others appear to be specific to carcinogenesis in the thyroid, e.g. alterations in the TSH receptor and ret genes. The ret oncogene codes for a cell surface receptor that is not normally expressed in thyroid follicular cells. It is activated when a breakage of chromosome 10 occurs in such a position that splits the DNA coding for the ret gene product in two. When the break is repaired, in some cases the DNA coding for the intracellular part of the ret gene becomes fused to part of another gene which is normally expressed in thyroid follicular cells i.e. the gene is rearranged. This leads to inappropriate activation of a signalling pathway inside the cell. This has been shown to occur with ret being fused to a variety of genes.
different genes; however, the most common rearrangement appears to involve inversion of part of chromosome 10 to generate two different rearrangements called PTC1 and PTC3.

Rearrangements of the ret oncogene have been shown to be associated with papillary carcinoma. The results from this study show that only papillary carcinomas show ret gene rearrangement, follicular tumours from those exposed to fallout from Chernobyl do not show this alteration. In addition, we have shown that a particular type of rearrangement, PTC3, is associated with the solid/follicular type of papillary carcinoma (see figure 2). However, the frequency of involvement of the ret oncogene in post Chernobyl thyroid tumours does not differ from that of a non-exposed age matched population, indicating that ret rearrangement cannot be used as the sole marker for papillary carcinoma as a result of exposure to radiation.

We have shown that mutation in ras, TSH receptor and p53 is not found in papillary carcinomas, and that a similar frequency of ras mutation is observed in thyroid follicular tumours post Chernobyl to that observed in those from populations not exposed to radioactive fallout. There is also no evidence from our studies on loss of heterozygosity for involvement of any of the known tumour suppressor genes in post Chernobyl thyroid tumourigenesis or for genetic instability in these tumours. In summary, the molecular biology of post Chernobyl thyroid tumours does not seem to be markedly different from that observed in non radiation associated thyroid tumours, but there are differences in the frequency of tumour types. Because of the rarity of thyroid cancer in children and young adults exposed to fallout from the Chernobyl nuclear disaster.

**Selected references**


**Partnership**

The partnership involved European scientists with expertise in pathology and molecular biology plus collaboration with colleagues in the former Soviet Union. The data from this project indicate that further studies will be required to fully understand the consequences of the Chernobyl accident, and that we do not yet have sufficient data to make accurate risk assessments because of the changing frequencies in the morphological type of thyroid tumour.

**Information Column**

**Title:** Pathology and molecular biology of thyroid tumours in children and young adults exposed to fallout from the Chernobyl nuclear disaster

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**Period:** Nuclear Energy 1994-1998

**Status:** Completed
The key aims of this project are to (a) provide a better assessment of thyroid cancer risk posed by radioactive fallout from the Chernobyl reactor accident. This would help to decide appropriate population screening procedures and therefore improve healthcare economic planning in the event of a future accident, as well as informing on prophylactic measures, prognostic markers and health care surveillance; (b) indicate new therapeutic avenues, which may be of use in other human cancers; (c) lead to a better understanding of the relationship between radiation exposure, gene defects and DNA repair in carcinogenesis.

**Chernobyl, an integrated pan-European study: morphology, oncogenes, DNA repair and outcome in radiation carcinogenesis (CHIPS)**

**Objectives**

The key aims of this project are to (a) provide a better assessment of thyroid cancer risk posed by radioactive fallout from the Chernobyl reactor accident. This would help to decide appropriate population screening procedures and therefore improve healthcare economic planning in the event of a future accident, as well as informing on prophylactic measures, prognostic markers and health care surveillance; (b) indicate new therapeutic avenues, which may be of use in other human cancers; (c) lead to a better understanding of the relationship between radiation exposure, gene defects and DNA repair in carcinogenesis.

**Challenges to be met**

The consequences of the Chernobyl accident have provided a unique possibility to advance our understanding of thyroid carcinogenesis in general, and in particular the factors involved in the genesis of a tumour where the aetiological agent is known – exposure to ionising radiation in childhood. It is clear from our earlier results that alterations other than those in the ret oncogene must be involved in thyroid carcinogenesis, but that these factors are not those which have already been shown to be involved in non-radiation related thyroid carcinogenesis. This project will continue study of the involvement of the ret oncogene to see if the pattern of involvement changes with time; it also seeks to identify novel markers involved in thyroid carcinogenesis. It is essential that an integrated approach is applied, as it is increasingly likely that several different oncogenes will participate in the development of a tumour.

**Achievements**

Work package (WP 1) will obtain RNA and paraffin sections from post Chernobyl thyroid tumours from the NISCTB (EC project no. FIS5 2001 008). Thus far the majority of thyroid cancers post Chernobyl have been of the papillary type, but follicular tumours are becoming more frequent. We will document any change in the pathology of post Chernobyl tumours with time by comparing the results from these studies with those of our previous project.

We will also use a variety of different techniques to study the frequency of ret rearrangement (WP3 and 4), and to look for rearrangement involving other chromosomes using fluorescence in situ (FISH) analysis (an example is provided in figure 1). It is clear from our previous studies that ret is not the only gene that is involved in thyroid carcinogenesis.

We have already shown that other genes known to be involved in other types of thyroid cancer with different aetiologies are not involved in the post Chernobyl thyroid cancers. WP2 will use the new technology of cDNA array to identify other genes which may be altered in the carcinogenic pathway.

WP4 will develop epithelial cell cultures from both normal thyroid and from thyroid tumours and will investigate whether culturing the cells alters the ret rearrangement status or alters any other tumour related rearrangement identified using FISH. This could indicate that a rearrangement of the ret oncogene was present in a cell type other than the epithelial cells which compose the majority of the tumour. FISH is a very powerful technique which allows identification of chromosomal abnormalities in single cells and will be very useful in approaching problems of this type.

Control of cell division involves a complicated cascade of signals initially received at the cell surface (through proteins such as ret) which can trigger replication of the DNA. DNA replication is strictly controlled in normal cells; one hallmark of cancers is that DNA replication is dysregulated. There are many factors which control DNA replication; unwinding of the DNA to allow replication is one of these. We have already shown that other genes known to be involved in other types of thyroid cancer with different aetiologies are not involved in the post Chernobyl thyroid cancers. WP2 will use the new technology of cDNA array to identify other genes which may be altered in the carcinogenic pathway.

Control of cell division involves a complicated cascade of signals initially received at the cell surface (through proteins such as ret) which can trigger replication of the DNA. DNA replication is strictly controlled in normal cells; one hallmark of cancers is that DNA replication is dysregulated. There are many factors which control DNA replication; unwinding of the DNA to allow replication is one of these. There are a number of proteins which have recently been identified, called minichromosome maintenance (mcm) proteins, which form the origin recognition complex where DNA synthesis starts. The consortium involved in this project has developed an assay which allows assessment of the presence of these proteins in isolated nuclear and cytoplasmic cytosol.

MCM proteins can also be demonstrated in intact sections of tissue. Nuclei in cells which are primed to undergo DNA replication stain red in this system;
those that are at rest stain blue. In figure 2, the upper panel shows normal adult mouse thyroid which has a very low growth rate, and therefore few cells are positive (arrow). The lower panel shows actively growing mouse thyroid, where clearly many cells are in a state of readiness to undergo DNA replication. WP5 will compare the results obtained using mc m assay with those using conventional markers of dividing cells. We will relate cellular growth rate to the presence and type of ret rearrangement, clinical parameters which measure the invasiveness and the ability of the tumour to metastasise. A key aim is to investigate in vitro the factors which are involved in the regulation of DNA replication and compare these with results obtained on paraffin embedded material from the primary tumour. These studies will help us to assess whether in vitro cell cultures of thyroid tumours could be used as a good guide to the anomalies that are present in the primary tumour. The comparison of all these scientific data with information on the clinical aspects of the disease in the patient from which the tumour was removed will help us to assess which of the markers we have identified provide information on the prognosis for the patient. The identification of key factors involved in the generation of the tumour will also open new avenues for therapy.

Partnership

This project is a collaboration involving 5 main European centres. It is a development of a longstanding partnership between these centres to investigate the consequences of the Chernobyl accident with particular reference to thyroid cancer; it will extend information provided by our earlier project “Pathology and molecular biology of thyroid tumours in children and young adults exposed to fallout from the Chernobyl nuclear disaster”.

Selected references


Challenges to be met

This project aims to promote collaboration and avoid competition for a very valuable but limited resource; to properly document in a uniform manner pathological specimens and to collect blood samples from those exposed as children to radioactive fallout from the Chernobyl accident, who subsequently develop thyroid tumours; to provide aliquots of nucleic acid extracted from thyroid tumour, normal thyroid and blood from these cases to the worldwide scientific community for research; to collate and record data on a case by case basis from scientific projects that have access to material and to provide a database which permits later multifactorial analysis for each case.

Achievements

The project is governed by six important principles:

a) The interests of the patient must come first. b) Spare tissue of potential value should not be wasted, c) Blood and tissue samples should only be used if appropriate consent is given by the patient, and the laws of the country concerned are observed. d) A tissue bank and a nucleic acid bank should be established in the country in which the tissue was removed. e) International agencies should participate in such a project, providing financial support, training, equipment and expert advice. f) The relevant countries of the former Soviet Union (FSU) will be fully represented in all committees related to the implementation of the project. There should be full agreement within the committees of the thyroid tissue and data bank and the scientists intimately involved in the project regarding the protocols used for the collection of material and to maintain the archive.

The project is a cooperation between the governments of Belarus, Russia and Ukraine and the European Commission, the National Cancer Institute of the US, the Sasakawa Memorial Health Foundation and the World Health Organisation. This project is a unique response to a unique situation. The occurrence of such a large number of human tumours of a single tissue, the majority of which are of a single histological type, due to a known cause on a known date provides an opportunity to fully investigate the link between exposure to radiation and thyroid carcinogenesis. The study cohort is composed of all cases of thyroid carcinoma and cellular follicular adenoma from patients who were born after 26th April 1967 (i.e. under 19 years at the time of the Chernobyl accident) and who are or were at the time of the accident, resident in Belarus, Northern oblasts of Ukraine or 4 contaminated oblasts of Russia. Thyroid cancer relatively rare in this age group – therefore the majority of cases are due to exposure to radiiodine. Informed consent is obtained from the patient or his/her parent or guardian, a sample of blood
is taken pre-operatively for serum separation and extraction of DNA from blood. The patient is then operated on and the operative specimen is seen by the pathologist. The pathologist then records the location of the tumour or tumours on a simple diagram which is later scanned into a database, noting the positions from which he or she has taken blocks of tissue that will be processed to paraffin for diagnosis. When the material that is necessary for accurate diagnosis has been taken, small blocks of tissue from the remainder of the tumour and normal thyroid are snap frozen. The majority of these tumours are small, but where possible 3 blocks of tissue from tumour and three from normal thyroid are taken. The position of these blocks relative to those taken for paraffin are noted on the form.

It is very important to ensure that all the information relating to these specimens is stored in a way that can be accessed easily. Each of the three Institutes involved in the project has an identical Access database and a back-up integrated database is kept at the Coordinating Centre. All patient data is anonymised and is totally confidential. The identical format enables regular updates to be made to the Coordinating Centre and facilitates exchange of data between centres if so wished.

One of the major aims of the project is the release of nucleic acid (DNA and RNA) to researchers. Provision of pieces of tissue to individual researchers would result in wastage of a lot of nucleic acid. DNA and RNA are therefore extracted from the same piece of tumour or normal thyroid, and standard-sized aliquots are provided to researchers. Quality control is carried out on each extract by RT-PCR (reverse transcriptase polymerase chain reaction) for RNA and PCR for DNA. The quality control information is recorded on the database. DNA will also be extracted from blood and this will be available in the near future.

The documentation of the pathology of thyroid tumours and collection of frozen tissue from tumour and normal thyroid (where available) began on 1st October 1998, and collection of blood specimens began in 2000. The extraction of RNA and DNA from tissue commenced in March 2000. The current status of the bank is as follows: full pathology documentation on 1380 cases, (720 from Belarus, 447 from Ukraine and 213 from Russia); of these 913 are papillary carcinoma. A significant number of benign follicular tumours are also included in the NISCTB. Frozen material is available from 1139 cases (614 from Belarus, 361 from Ukraine and 164 from Russia). The majority of cases have samples of both normal tissue and tumour available. Aliquots of DNA and RNA are available from 421 cases with more than 4000 individual aliquots of RNA and DNA from tissue and tumour. In over half of these cases there are more than 5 standard aliquots for both tissue and tumour available. In addition serum and samples of blood from which DNA will be extracted are available from 171 of these cases.

Applications for access to materials and information are now invited. All applications are reviewed by an External Panel of experts nominated by the governments and the sponsors of the project. Further details of the project, together with information on how to make an application for access can be found on the project website (http://nisctb.swan.ac.uk).

**Partnership**

This is an experienced consortium with skills in clinical medicine, pathology, molecular biology and data maintenance; the success of the project is dependent upon ongoing collaboration.

**Selected references**


The aim of this project was to improve the radiation protection of humans and their environment by a better understanding of the mechanisms of radiation action at low doses and dose rates for different radiation qualities. The group is confident that it contributed substantially with this first internationally coordinated project on this topic to a more solid basis for such urgently needed, risk estimation models.

In particular the project aimed to integrate a maximum of present knowledge in radiation research into comprehensive mechanistic models for the induction by radiation of somatic late effects. These models included the formation of chromosome aberrations and of mutations in humans and the many physical, chemical and biological processes that determine these consequences. These models ultimately serve as a better basis for extrapolation of epidemiological data for human radiation risks to low doses and dose rates, other types of ionizing radiation and different exposed populations, and individual radio-sensitivities.

**Challenges to be met**

An important problem is the quantification of radiation cancer risks to humans at low doses and low dose rates of ionizing radiation of various qualities. Since this problem cannot be solved by biological investigations nor by epidemiological studies alone, the scientific knowledge from both areas have to be combined to develop and test quantitative mechanistic models for the dose-time-effect surfaces of radiation carcinogenesis. Existing data and theories have to be evaluated and used for the modelling of radiation transport and interaction, for the modelling of subsequent damage and repair of DNA, genes, cells and organs taking normal oxidative damage into account, and for the multi-step modelling of carcinogenesis. Conclusions have to be drawn for the general radiation protection of the worker and the public.

**Achievements**

The project contains six work packages (WP):

WP 1 “Mechanistic Models for Radiation Oncogenesis”: The two-stage clonal expansion model of Moolgavkar, Venzon and Knudson was selected as the basic model. The model can help in extrapolation to low dose rates: when applied to data on lung tumours in radon-exposed miners and rats, radiation was found to act on both initiation and promotion; the model predicts the possibility of a dose-rate effect at low dose rates, and an inverse dose-rate effect at high dose rates.

WP 2 “Mechanistic Models for Chromosome Aberrations”: A biophysical model for chromosome aberration induction by ionizing radiation was developed. The code allows the simulation of dose response curves for different kinds of aberrations, under the main assumption that a subclass of DNA double strand breaks (dsb), namely complex lesions, are responsible for chromosome aberrations, less severe damages being repaired systematically.

WP 3 “Mechanistic Models for Mutagenesis”: Mutation at the hprt gene locus has been selected as a paradigm for modelling radiation mutagenesis because this locus has been most broadly studied experimentally. For this purpose an extensive database of about 400 reference records has been compiled of hprt radiation mutagenesis. Measured patterns of exon-deletions in hprt-mutants after X-ray irradiation could be reproduced by simulations with mutants arising from pairs of dsb due to the non-random DNA fragmentation below 10 base pairs.
WP 4 “Mechanistic Models for DNA Damage and Repair”:
Computations of clustered and simple DNA damage have shown a relevant role of clustered DNA damage determining cell killing. Inclusion of chemical pathways for indirect effects in the models show that >50% of low LET induced dsb are complex. For high LET radiations, the spectrum of complex lesions shifts to more complexity and some of the very complex lesions may be unique to high LET radiation. Protons produce a more complex spectrum of dsb than alpha-particles for the some LET.

WP 5 “Chemical Pathways Involving Track Species in Cells”:
Comparisons of various Monte Carlo track structure codes show similarities in the distribution of damage at the chromatin level whereas differences are noticed at the level of naked DNA.

WP 6 “Production of Initial Track Species in Mammalian Cells”:
An atomic model of DNA able to simulate higher order DNA structures has been developed and coupled with the track structure modules in the PARTRAC code. A new set of cross section for electron transport in liquid water has been derived and implemented.

WP 7 “Transport of Radiation to Cells of Interest”:
Data from related GSF- and PSI- work provided the necessary start spectra.

The FP5 project Low Dose Risk Models
It is the main goal of this project to improve our knowledge on the risk estimates for somatic health effects in various groups of humans after exposures to low doses of various types of ionising radiation at low dose rates.

The project is organized in a "bottom up design" manner into five work packages:

1) Critical processes and data evaluation,
2) Primary damage models,
3) Repair consequences models,
4) Mechanistic cancer model development,
5) Conclusions for radiation protection.

The modelling work of the present project can consider results of experimental work done in other consortia and draw conclusions on the consequences for risk to humans exposed to radiation. The research concentrates on radiation induced cancer, but the mechanisms are likely to be to some extent universal for cancer induced by other environmental or nutritional agents. So the project may also help to estimate small additional cancer risks due to other reasons than radiation.

Partnership
The success of these projects has depended upon the combined multidisciplinary expertise of a total of eight EU laboratories.

Selected references
Challenges to be met

The Chernobyl nuclear reactor accident took place in April 26, 1986. The accident was unprecedented in scale and in size of the population exposed. Very large amounts of radioactivity, including isotopes of iodine were released from the reactor. The most apparent health consequence of the accident was the increase in the incidence of thyroid cancer in the following years. Thyroid carcinoma occurred mostly in children of southern Belarus, northern Ukraine, and, to a lesser extent in Russia. Western experts have independently confirmed the pathological diagnosis of the great majority of cases. Tumors are virtually all papillary carcinomas, relatively aggressive, with the majority showing invasion of extra-thyroid tissues, and lymph node metastases. Children have been shown to be more susceptible than adults to the carcinogenic affect of radiation to the thyroid. Radiation dose to the thyroid from isotopes of iodine was probably higher in areas of iodine deficiency, such as the regions surrounding Chernobyl. Thus, the most likely cause of the post-Chernobyl increase in childhood thyroid cancer found in Belarus and Ukraine is the release of radioactive isotopes of iodine from the reactor.

In addition to the basic question of the causal relationship between the post-Chernobyl outbreak of thyroid cancer and exposure to the radioactive isotopes of iodine released by the reactor, several questions have been raised by the "epidemic" of post-Chernobyl thyroid carcinomas:
1) Does thyroid cancer in young children differ in its clinical behavior from that in older individuals? 2) Is there evidence suggesting that thyroid cancer caused by radiation behaves differently from sporadic thyroid cancer? 3) What should be the guidelines in managing children with thyroid cancer, especially in regard to surgical treatment and post-operative medical treatment and follow-up procedures. 4) Is there a possible role for the prophylactic use of iodine and/or thyroxine to suppress pituitary TSH in the very large population exposed to the Chernobyl accident?

The present project was aimed to answer these questions, having in mind the concept that the outcome of differentiated thyroid carcinoma in children is largely dependent on the adequacy of the management, at the diagnostic, therapeutic and follow-up levels.

Achievements

The present project was aimed to improve the health status of the population exposed to the Chernobyl accident and to develop effective means for managing the radiobiological consequences of the radioactive contamination, with particular regard to the post-Chernobyl thyroid carcinoma. The project was designed as a natural continuation in the first collaborative project (JSP4) between CIS countries and European Union.

According to the project objectives, the definition of guidelines for diagnosis, treatment and follow-up of thyroid cancer, including the correct use of l-thyroxine suppressive therapy after surgery have been developed and validated in the affected territories. This resulted in a dramatic amelioration of the metabolic controls of hypothyroidism in thyroid cancer children and in the standardization of laboratory procedures to be used for the correct follow-up of thyroid carcinoma.
Development of new diagnostic imaging techniques and standardization of traditional techniques for the post-surgical monitoring of thyroid carcinoma have been developed and made available to the CIS colleagues.

New insights in the non-tumoral thyroid consequences of the Chernobyl accident have also been studied. The main achievement was the discovery of autoimmune phenomena in children and adolescents exposed to the radioactive fallout. This finding opens the important question of following exposed subjects in the future not only for the early diagnosis of thyroid nodules and cancer but also for the development of autoimmune thyroiditis with or without hypothyroidism.

The evaluation of the biological and clinical behavior of radiation-induced thyroid carcinoma, including the response to treatment, compared to that of naturally-occurring differentiated thyroid cancer in western European children and adolescents, has been accomplished through analysis of data on more than 500 children treated in western Europe in the past 20 years. The results of this study have shown that thyroid cancer in children is a very well treatable disease in general, and that the radiation-induced variant of papillary thyroid cancer has the same response to modern treatment as naturally occurring tumors. The assessment of iodine deficiency and the employment of iodine prophylaxis, has been the most difficult task to perform, due both to logistic difficulties and to lack of well-documented historical information. However, the project developed a detailed map of iodine deficiency in contaminated territories that will constitute the basis for future development of programs devoted to the correction of iodine deficiency disorders. Furthermore, a simple method for measurement of urinary iodine excretion has been developed and validated in a large number of patients.

All the information collected during the three years period of the project has, whenever possible, been described in written documentation in the form of books, flow-charts, guidelines and recommendations translated into Russian and distributed to a large number of specialists.

**Partnership**

The project brought together four European partners with complementary expertise in clinical medicine and tumour diagnosis and therapy.

**Selected references**


Challenges to be met

The principle challenges were:
To improve the measuring techniques for the determination of iodine kinetics and mass of thyroid carcinoma metastases particularly for lung tissue. To improve dosimetric methods and models. To determine cross sections for the interaction of electrons and photons with lung tissue for the purpose of Monte-Carlo simulations. To coordinate European efforts to treat patients with pulmonary metastases of thyroid cancer; to propose, test and apply an optimal protocol for the treatment of those patients. To follow-up the treated patients in order to observe late effects of radioiodine therapy.

Achievements

In the course of this project it could be shown that it is possible to measure the biokinetics of I-131 under therapeutic conditions, particularly during the first 24 hours after the application of radioiodine. Monte-Carlo calculations for the decay of I-131 in small spheres were simulated as this radionuclide might be superior for the treatment of small metastases. Further calculations were carried out simulating the two extreme cases, diffuse metastases and focal metases, in the lungs for a phantom (see figure). The results show that in the case of a focal metastasis the dose to the lung is more than ten-fold lower than in the case of diffuse metastases.

Applying all these data to the treatment of children it was shown that the achievable dose to the lung and/or tumor with one radioiodine therapy is limited to a few Gy. It may not be possible to reach the tumoricidal dose of 80 Gy with just one therapy as exceptionally high activities of I-131 would have to be administered. This might cause severe side effects as the blood dose may well exceed 2 Gy. Several courses of radioiodine treatment will probably reduce the dose delivered to the metastases per therapy.

An ongoing treatment project in Germany with children from Belarus shows a comparatively high rate of successful treatments without severe side effects. With comparatively low
cumulative mean doses to the lung of approximately 7 Gy (range: 0.1 Gy - 25 Gy) the secondary tumors could be eliminated in 52 out of 80 cases. In only one child the development of lung fibrosis was related to I-131 treatment.

As a result of this study the following recommendations can be given for the treatment of lung metastases:

a) Radioiodine therapy is a safe and efficient therapy in the case of lung metastases from thyroid carcinoma; a comparatively high rate of successful treatments without directly related severe side effects can be achieved.

b) The per therapy dose to the lung and/or tumor achievable with one radioiodine therapy is limited to a few Gy. Administering higher activities of I-131 to the patients in order to achieve higher doses might cause severe side effects.

c) Care has to be taken in the case of exceptionally high lung uptake as the lung doses can become excessive (more than 10 Gy).

d) Several courses of radioiodine treatment will probably reduce the dose delivered to the metastases per therapy.

e) The local dose to small foci is much higher than the lung dose and might be sufficiently high for ablation.

f) If the metatases are smaller than a few millimeter the dose to the surrounding tissue cannot be neglected.

g) Using other radioisotopes such as I-125, perhaps in combination with I-131 may enhance the efficacy of the treatment of lung metastases.

h) More research is necessary on the histology, size and shape of micrometastases from thyroid cancer for a better understanding of the therapeutic efficacy of radioiodine.

i) A long-term follow-up of the children with thyroid carcinoma from Belarus, the Ukraine and the Western part of Russia is mandatory.

**Partnership**

The success of the project was dependent upon the bringing together of European expertise in clinical and nuclear medicine and medical physics plus collaboration with colleagues in Belarus.

**Selected references**


This project aims to develop a novel, multidisciplinary and complementary approach for improving the management of the radiation injury of victims exposed to high doses of radiation. With the purpose of addressing this objective three main tissues, which determine the fate of irradiated victims, have been chosen: the hematopoietic, the epidermal and the vascular tissues. In addition, studies in endothelial cells, which play a critical role in the response of these two tissues to irradiation, have been considered. In particular, our study proposes the development of new indicators capable of predicting the radiation injury produced in both hematopoietic and the epithelial tissues. Our project also aims to improve the understanding of the damage within the most critical cells in both tissues and, finally, to offer new alternatives for ameliorating the hematopoietic cellular damage, for preventing the inflammatory reaction and for the therapy of the cutaneous syndrome produced after irradiation.

**Challenges to be met**

*Development of new indicators of radiation injury:*

Early intervention is of pivotal importance to limit the consequences of accidental radiation exposure. Unfortunately, quantitative indicators of radiation injury are largely lacking at present, which also makes it difficult to establish the optimal treatment at an early stage. The project seeks to establish such indicators for damage to blood cell production and to the skin. Procedures based on the quantification of hematopoietic stem cells (HSCs) mobilised to peripheral blood will be used to estimate the HSC reserve of accidentally exposed subjects. Using cDNA microarrays and proteomic analysis, genes that are modulated by radiation will be investigated in human skin, and more specifically in keratinocytes. It is expected to identify clusters of gene expression/suppression, which may elucidate the basis of the molecular events leading to the injury of the tissue.

*Better understanding of the radiation-induced damage in critical tissues:*

Optimal medical management of radiation injury requires profound understanding of the pathophysiology in critical tissues. Using in vivo repopulation assays, the response of the different subpopulations of human HSCs to radiation will be investigated. The pathophysiological mechanisms of radiation-induced eosinophilic inflammation will be investigated by characterising the role of the CC-chemokine receptor CCR3 and its ligand eotaxin. Since TGF-β1 represents a key molecule and a master switch for the general fibrotic programme, the role of TGF-β1 at the level of signal transduction will be further investigated.

*Better treatment of the radiation damage:*

Although much progress has been made in the treatment of the hematopoietic syndrome, current insights highlight the possibility of more efficacious treatment, while treatment of skin damage is still largely conservative and eventually based on surgery. Although it has been recognised that the acute inflammatory reaction to radiation is a key element in the generation of damage and eventually fibrosis, this knowledge has not yet resulted in clinically effective interventions. The direct effect of cytokines, such as the blood platelet stimulator thrombopoietin, on the survival of hematopoietic stem cells exposed to radiation will be investigated. The capability of synergistic combinations of growth factors to promote amplification of surviving progenitors and stem cells will be also determined. Novel therapeutic approaches will target endothelial cells (ECs) with cytokines and antithrombotic agents. The activation state of endothelial cells following radiation will be determined. Novel therapeutic approaches based on surgery. Although it has been recognised that the acute inflammatory reaction to radiation is a key element in the generation of damage and eventually fibrosis, this knowledge has not yet resulted in clinically effective interventions. The direct effect of cytokines, such as the blood platelet stimulator thrombopoietin, on the survival of hematopoietic stem cells exposed to radiation will be investigated. The capability of synergistic combinations of growth factors to promote amplification of surviving progenitors and stem cells will be also determined. Novel therapeutic approaches will target endothelial cells (ECs) with cytokines and antithrombotic agents. The activation state of endothelial cells following radiation will be investigated.

**Achievements**

The demonstration that thrombopoietin – if immediately administered after irradiation – potentiates the hematopoietic response to other growth factors and markedly prevents the leukopenia, thrombocytopenia and anemia associated to the HSC damage, makes this

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**Peripheral blood cell counts after 5 Gy TBI and growth factor treatment**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Group</th>
<th>Time (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytes</td>
<td>huTPO</td>
<td>10^11/L</td>
</tr>
<tr>
<td></td>
<td>huTPO + G-CSF</td>
<td>10^11/L</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>placebo</td>
<td>10^11/L</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>G-CSF</td>
<td>10^10/L</td>
</tr>
</tbody>
</table>

- huTPO: Human thrombopoietin
- G-CSF: Granulocyte colony-stimulating factor
- Placebo: Control group
agent a key in the future treatment of radiation accident patients. Hence, it is recommended to provide such patients immediately with thombopoietin and G-CSF (fig.1).

Improved harvesting of HSCs from peripheral blood and the isolation of these cells will greatly promote the chances of survival in radiation exposure dose ranges refractory to growth factor treatment. This may therefore replace the bone marrow transplantation approach and may serve as a back-up treatment for those patients which otherwise cannot be saved. In this context, the use of peripheral blood HSCs will enable transplantation of large numbers of these cells if sufficient additional immunosuppression is given to the recipients. Experimental evidence was obtained that the use of very high numbers of HSCs results in the further abrogation of recipient resistance to donor HSC engraftment, even at sublethal radiation doses in combination with immunosuppressive antibodies.

Understanding of the intercellular communication between cutaneous cells, blood vessels and peripheral blood cells after treatment with ionising radiation gives the opportunity for new therapeutic targets and treatment approaches of radiation accident patients. Concerning late radiation damage, the postulate of irreversibility of fibrosis has been challenged. The most impressive results were obtained with SOD, which could rapidly reduce fibrosis in patients. In combination with the antioxidant alpha-tocopherol, pentoxifylline treatment resulted in a striking regression of fibrosis as well. Understanding of the underlying mechanisms may open several effective treatment options.

**Partnership**

The consortium is based upon European scientists working on three critical tissues i.e. hematopoietic, epithelial and endothelial tissues. In addition, the activities of the consortium are multidisciplinary in nature and include molecular biology, cell biology and animal models of radiation response.

**Selected references**

Challenges to be met

Statistical analysis of the epidemiological data on the in utero irradiated A-bomb survivors revealed a dose dependent increase in the frequency of mental retardation. On the basis of some analyses it could be argued that there is no dose threshold and that prenatal irradiation of the brain produces an all or none impairment. On the other hand, the great complexity of the target tissue which has great compensatory abilities as well as the criteria used to measure multifunctional IQ make it more likely that deterministic effects with thresholds represent the underlying mechanisms for effects on the developing brain. If a threshold is to be determined, studies must move to lower doses. In animals, it is clear from previous brain studies, also supported by the EC, that dose levels as low as 10 mGy can cause a small (2%) but statistically significant atrophy of the rat brain. However, given the plasticity of the brain and its great capacity to compensate for loss of function, the question is, whether such small changes would impair brain physiology or function.

The specific objectives of the project included the determination of the effects of irradiation on the developing brain with respect to: apoptosis and its underlying molecular mechanisms; growth factor gene expression, cell formation and migration; gross anatomical parameters; cortical and commissural diameters, synaptogenesis and synaptic remodelling, as well as behaviour. The effects of different doses, acute or protracted irradiation, as well as the developmental stage at which the brain was irradiated were evaluated.

Achievements

It was shown both in vivo and in vitro that irradiation-induced cell death follows the apoptotic pathway. The transcription factor c-Jun, which binds to regulatory regions of DNA, and controls gene expression, was shown to be involved in irradiation-induced apoptosis. NMDA type glutamate receptors are known to be involved in neurodegeneration, by allowing increased calcium influx. Calcium, in turn, activates processes leading to cell death. Our results showed that a calcium-dependent protease, calpain, plays a role in irradiation-induced apoptosis. p53 protein which is known as the guardian of the genome, inducing either repair or apoptosis when DNA is damaged by irradiation, was increased in apoptotic cells. Both classical neurotrophins such as NGF, BDNF, NT-3 and other growth factors such as IGF-II were increased following irradiation, suggesting that the damaged tissue reacts in a compensatory way, producing substances that ameliorate the injury. Indeed it was shown that some growth factors had ameliorative effects on irradiation-induced damage in the in vitro systems. Prenatal irradiation also produced alteration in the patterns of cell proliferation and migration in the neuroepithelium, leading to long-term morphological changes such as reduction of brain weight, atrophy of the cingulum, and reduction of the hippocampal area. Even 60 mGy was shown to be effective in producing diminution of the diameter of the parietal cortex. The effect of prenatal irradiation on the animal’s adult behaviour, the final output of brain function was also determined. Spatial memory appeared to be the most vulnerable to prenatal irradiation effects.

These results provide an integrated view of the cellular processes that might underlie radiation-induced mental deficits: Irradiation, even at doses of 100-500 mGy, leads to DNA damage. If this damage is not repaired, cells enter the apoptotic pathway and are eventually eliminated. This leads to lower cell numbers in the respective brain areas and consequent smaller structures. If
In utero irradiation of the foetal rodent brain: early effects

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Status: Completed

the affected brain structures are involved in the regulation of a specific behaviour (e.g. the hippocampus in spatial learning), then the ability to express that particular behaviour is impaired.

Partnership

The participation of different laboratories, allowed the use of a variety of experimental techniques and in vivo/in vitro systems. This multidisciplinary approach resulted in the view that doses as low as 100-250 mGy can induce a variety of effects on the developing central nervous system. The coincidence of the threshold-levels for experimental and A-bomb epidemiological studies suggests that similar types of cellular damage might underlie the induction of structural cognitive and mental deficits.

Selected references


The objectives of the SEMIPALATINSK project were 1) To establish a bank of blood samples of three generations of families living close to the Semipalatinsk nuclear test site and control families from clean areas, 2) To reconstruct radiation doses by using biomarkers of radiation exposure, and 3) To determine the germline minisatellite mutation rates of exposed people and the control families of the same ethnic origin living in non-contaminated areas.

The overall objective was to provide information relevant to the genetic risk assessment of exposure to ionising radiation in chronically exposed human populations and to provide advice for local authorities in order to help them mitigate the effects of ionising radiation on the population around the Semipalatinsk nuclear test site.

Challenges to be met

Information on the exposure of the local population has been available to the international scientific community only since the 1990’s. Many of the people that received the highest doses after the surface tests performed in 1949-1956 are now old and soon no longer available for examinations. To learn more on radiation effects in the future using technologies not yet available, biological samples need to be stored.

Reconstruction of radiation doses is an essential step in order to obtain quantitative information on the health risk of radiation. The participants in the project have used biomarkers of radiation exposure to assess the exposure of people that have been living in the most contaminated villages since the first Soviet nuclear test in August 1949. Stable chromosomal translocations in blood lymphocytes were analysed for dose reconstruction. In addition, glycophorin A (GPA) mutations in erythrocytes were analysed by a US laboratory (University of Pittsburgh).

Genetic risk estimates for man from exposure to ionising radiation have been based mainly on the information from animal experiments and general knowledge of radiation biology. No significant elevation of heritable mutation rate has been observed in the children of A-bomb survivors in Hiroshima and Nagasaki (Japan) by using standard genetic methods. Tandem repeat minisatellite loci monitoring of germline mutation in man is a new and sensitive approach.

Achievements

A biological sample database with an accompanying registry of background information on the examined subjects has been established for long-term storage of frozen lymphocytes, blood cell DNA and EDTA blood. Biosamples from 361 individuals living near the Semipalatinsk test site and 251 controls from Taldy Kurgan area are available for future studies addressing genetic effects of ionising radiation.

Chromosomal translocation frequencies in the Semipalatinsk and the control groups were similar. This suggests that on average, the magnitude of exposure to the examined group in the Semipalatinsk area has been considerably smaller than that reported in the local registries. Previously reported doses of the order of 1-4.5 Gy (2.9 Gy on average among the grandparent generation that lived in the villages at the time of testing) cannot be confirmed by the
present data. A more likely dose estimate is below 0.5 Gy. A similar conclusion was drawn on the basis of the GPA mutation data.

Differences in hereditary minisatellite mutation rates between the two rural populations from the Semipalatinsk and Taldy Kurgan Districts of Kazakhstan were small in general. However, the minisatellite mutation rate in the P0 generation (grandparents) directly exposed to radioactive fallout from the surface and atmospheric nuclear tests was 1.7-fold higher than in the control non-exposed population from the Taldy Kurgan District. Moreover, the minisatellite mutation rate in the F1 generation (parents) from the affected area showed a significant negative correlation with the year of birth. The mutation rate among those that were born in the 1950’s (during atmospheric testing) was significantly elevated, whereas no increase in the germline minisatellite mutation rate was observed amongst those that were born in the 60’s and 70’s and thus were exposed to considerably smaller radiation doses.

**Partnership**

The project brought together five competent Kazakh, European and US laboratories having knowledge on radiation dose reconstruction, genetic effects of ionising radiation, epidemiology, molecular biology and radiation protection. Three Kazakh post doctoral scientists were trained in European laboratories on molecular cytogenetic and mutation analysis techniques.

**Selected references**


Annex 1: projects starting late 2002:

Genetic factors predisposing to radiation induction of mutation during early gestation: the role of DNA repair and cell cycle control (GEMRATE)

Recent reports in the mouse indicate considerable radiation sensitivity of particularly the early zygote and the postimplantation gastrula stages. Data on the chromatin stability of human sperm and chromosome stability of preimplantation embryos point at the genetic vulnerability of these stages in human. We propose to assess, for the mouse, the roles of single and double strand DNA break repair at the zygote and gastrula stages in response to radiation-caused DNA damage of male postmeiotic haplophase stages, the zygote stage and the gastrula stage. Various genetic, and some cell cycle control endpoints will be utilised to understand the importance of DNA break repair and cell cycle control for the transmission of genetic damage of sperm, zygote and gastrula stages, such that risk assessment and mechanistic insight are coupled.

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Radiation specific DNA non-double strand break lesions: repair mechanisms and biological effects (NON-DSB LESIONS)

Ionising radiation induces a plethora of DNA non-double strand break lesions such as oxidised bases, abasic sites and single strand breaks. Strong evidence exists that ionising radiation induces, besides isolated DNA lesions, an unique form of DNA damage termed clustered damage consisting of closely spaced lesions on opposite DNA strands. There is also evidence that repair of lesions within clustered damage sites is compromised compared with the effective repair of isolated lesions. We will investigate the following aspects of radiation-induced single and clustered DNA damage: (i) frequency and nature of the damage, (ii) mechanisms of repair, (iii) interference with transcription, replication and chromatin dynamics and (iv) biological consequences (stress responses, mutations, lethality). To achieve these goals we will exploit a variety of new technologies and utilise different organisms with defined mutations in damage response genes.

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

Carcinoma of the thyroid is a serious public health problem in populations at risk of exposure to radionuclides accumulating in thyroid tissue. However, due to genetically determined differences in individual susceptibility to ionising radiation, simple dose-response relationships do not exist. Identification of those subjects who are predisposed to developing thyroid tumours would be of value for setting individual exposure limits and for tailoring post-exposure monitoring. The GENRAD-T consortium aims to identify the gene loci that are responsible for determining individual susceptibility to thyroid cancer. To do this we will use a mouse model of radioiodine-induced thyroid cancer combined with molecular genetic screening methods (QTL-mapping, microsatellite allelotyping and CGH). The applicability of the gene loci thus identified for use in human populations will be confirmed by analysing human thyroid tumours.

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Genetic pathways for the prediction of the effects of irradiation - European normal and tumour tissue bank (GENEPI-ENTB)

There is strong evidence for a genetic basis for reaction to radiation in normal tissues as well as in tumours. The aim of GENEPI is to create a tissue bank linked to a detailed outcome-database of a large cohort of patients receiving radiotherapy as an essential infrastructure for present and future genetic research in the field of radiation protection and health. A central database will be established that provides a link to existing decentral databases and tissue banks, to foster optimal utilisation and access to data and material. Dummy runs with modern statistical tools will be applied to monitor the quality of this novel research infrastructure. Protocols for outcome assessment, tissue handling, and use and access of the infrastructure will be developed and ethical and legal issues addressed. The tissue bank will remain open for at least 20 years. In parallel, a cohort of large kindred French families will be recruited, and peripheral blood lymphocytes will be stored. These samples will be made available to study the genetics of individual radiosensitivity.

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