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Sensitizing and Protective Substances in Radiation Therapy and Predictive Assays

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Acta Oncologica Vol. 41, No. 7/8, pp. 615–622, 2002

In an investigation by the Swedish Cancer Society, an expert group described the present status, critical issues and future aspects and potentials for each of nine major areas of radiation therapy research. The present report deals with the use of sensitizing and protective substances in radiation therapy and predictive assays on normal tissues and tumour response.

Received 11 September 2002
Accepted 16 October 2002

PRESENT STATUS

Hypoxic and other radiosensitizers

The relative radiosensitivity of tumour tissue compared with normal tissue is a major area of interest in radiotherapy. In tumours of low or moderate sensitivity there may be a potential for increasing the control rate by manipulating the tumour sensitivity in relation to the surrounding normal tissues. In a clinical setting, the only reason for modifying radiation sensitivity in tissues is to widen the 'therapeutic window', i.e. change the radiation sensitivity between tumour and normal tissues, leading to higher cure rates, fewer severe side effects, or both. If the modifying effect is similar for all tissues, including the tumour, the result will only be a change of the radiation dose needed to achieve the same effect as before, possibly with the addition of extra toxicity from the compound used.

Over the past few decades, a large number of substances have been tested and have shown promising results in experimental systems. However, the clinical results have been considerably less successful. Different strategies have been pursued in the search for mechanisms to explore. A brief description of some of the strategies that are relevant in this context is given below.

Hypoxia

It is well known that hypoxia and anoxia can render tumours radioresistant. The main reasons for radioresistance are decreased number of formed free radicals and decreased or no oxygen-mediated fixation of damage. When oxygen is depleted there is also a better chance of protective proton donation from thiols (e.g. glutathione), which also means increased protection. The quantitative determination of the oxygen effect is given as the ratio OER (oxygen enhancement ratio) between the dose necessary to kill a certain number of hypoxic cells and the dose necessary to kill the same amount of normoxic cells. The OER value for low LET radiation is normally in the range of 2.5–3 after exposure to single radiation doses (1). In general, a radical radiation treatment is given in 2 Gy fractions. At this fractionation, the OER is substantially lower, in most cases probably due to reoxygenation, indicating that there is less oxygen dependency if tumours are treated in this manner (2).

A large number of substances show some form of interaction with radiation. So far, the major attempts to reach a differential effect on tumours versus normal tissues have been to 'attack' hypoxia, which is common in tumours but rare in normal tissues. However, the clinical results of such manipulations have yielded only moderate benefits. The methods have only partially reached routine clinical use owing to complex techniques, toxicity or other reasons.

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 ISSN 0284-186X

Experimental studies have clearly shown that hypoxia is mainly a problem when low-LET radiation is applied. High-LET radiation seems to cause such severe damage along the particle tracks that the chemical environment, e.g. the oxygen status, is of less importance (1). When applying low-LET external radiotherapy it is assumed that the often-used fractionation patterns (normally 1.8–2 Gy/day up to a total of 60–70 Gy) cause reoxygenation during the fractionation and that the hypoxia problem is thereby reduced or eliminated.

The aim of a number of studies has been to correlate the degree of hypoxia in human tumours to the response to radiotherapy. Invasive methods using the Eppendorff® polarographic oxygen electrode have shown volumes with low oxygen tension mainly in head and neck tumours and in cervical cancer (3). Other methods of identifying hypoxic cells include in vivo labelling using substances with a high affinity to hypoxic cells and subsequent specific staining of biopsy material (4, 5). In this context, hypoxic imaging by, for example, PET or spectroscopic MR, may be more informative and more 'user/patient friendly' than direct intratumoural measurements.

Some of the investigated methods to overcome hypoxia include hyperbaric oxygen, imidazole derivatives and blood transfusion. Although some methods have shown limited success in clinical studies, they have not been generally accepted owing to practical problems, toxicity or lack of effect. In a meta-analysis of studies that addressed the problem of reducing hypoxia, Overgaard (6) concluded that approximately a 10% absolute benefit in locoregional control was achieved, overall.

In a study conducted as early as the 1970s (7) it was shown that the sparing effect from hypoxia was reduced by small doses per fraction. Several studies of hyperfractionated radiotherapy have subsequently shown a benefit for hyperfractionated radiotherapy in comparison with conventional radiotherapy. However, it is not clear whether this benefit is a pure effect of overcoming the problem with hypoxia or if other mechanisms, such as less repopulation, are partly responsible.

In later years there has been an increased interest in different types of hypoxia in tumours. Two entities have been identified: acute and chronic hypoxia. The former is caused by cyclic closure of vessels (8) and there is evidence that using vasoactive substances, e.g. nicotinamide, can prevent this closure. To overcome chronic or diffusion-limited hypoxia, the strategy is to increase the concentration of oxygen dissolved in serum, and one way to do this is by allowing the patient to inhale carbogen (9, 10). A trial investigating the combination of these measures is presently going on (11). However, it has recently been indicated that cells suffering from chronic hypoxia might have a decreased repair capacity after irradiation and that these cells are therefore radiosensitive (12). If the latter is true, then mainly acute hypoxia will be of clinical relevance.

A low haemoglobin concentration is almost invariably a poor prognostic sign in patients undergoing radiotherapy. Efforts have been made to give blood transfusions before treatment but the results have not been encouraging. During treatment, there is often a period of decreasing haemoglobin concentration. This could lead to a suboptimal oxygen dissociation in tissues, since the mechanisms shifting the oxygen dissociation curve may not keep up with the continuous change. Another hypothesis that is presently being tested in clinical studies is slowly to increase the haemoglobin concentration during therapy and thus achieve both a higher haemoglobin concentration and a beneficial dissociation pattern of oxygen from haemoglobin. Erythropoietin is a drug that is presently being tested in this context (13).

Hypoxia may offer other ways to attack tumour cells. When tumour cells are beginning to grow and a tumour is being formed, angiogenesis starts as part of the process. Hypoxia will occur when the tumour 'outgrows' the vessels. The hypoxic situation induces the production of, e.g., VEGF (vascular endothelial growth factor) and GLUT I and II, to facilitate the formation of new vessels, and Glut I and II speed up the cell intake of glucose (14). The hypoxic condition seems to be associated with more aggressive tumours. Many studies show the negative effect of tumour angiogenesis on the prognosis of the patient (15, 16). There are two divergent aspects of this finding: first, production of new vessels could transport more oxygen, making the tumour less hypoxic, and secondly, new vessel formation may make the tumour more aggressive, more prone to metastasize and thus becoming less curable.

So-called bioreductive drugs have an obvious potential by being cytotoxic mainly in a hypoxic environment; tirapazamine is an example (17).

Radioprotective substances

Radioprotective substances generally contain thiols that act as radical scavengers. Substances of this group have, so far, failed to show a selective protection of normal tissues. In some clinical studies, salivary glands have been more protected than other tissues (18). The studies seem, however, too limited to allow for conclusions on loss of tumour control (19). From preclinical studies most of the results do not support a differential effect on tumours compared with normal cells. The degree of protection in tumours is therefore difficult to predict and establish clinically. Thus, the possible increase in radiation dose due to this protection is still unknown and may well be variable depending on the type of tumour.

Monoclonal antibodies

Monoclonal antibodies in combination with radiotherapy are discussed in a separate section of this report (see (20)). They have been explored on a very small scale in combination with external radiotherapy. The approach has an

obvious potential to increase the often-lacking specificity of effect on tumour tissue. The antibodies may be directed directly towards the tumour cells or certain factors such as growth factors, as discussed below.

Growth factors

Considerable interest is presently being focused on growth factors in relation to radiation response. One factor that has been studied is VEGF (15). In tumours rich in expression of EGFR, monoclonal antibodies against EGF or EGFR could increase the cell-killing effect of radiation (18, 19). The manipulation of EGFR seems to be promising when the receptor is highly expressed and when local control is the main problem. Moreover, manipulation of other growth factors has shown promising results. The techniques for manipulating growth factors may involve monoclonal antibodies and gene therapy. Early clinical studies in these areas are being carried out in collaboration with pharmaceutical companies.

Gene therapy

Gene therapy is presently mainly focused on two strategies to suppress the function of specific genes. One way is to use antisense oligonucleotides, which hybridize with mRNA, thus preventing translation. The other way is to overexpress a protein that competes with its wild type to prevent it from functioning. The aim of these manipulations is to attack certain processes connected with the radiation response (21). These include DNA damage recognition, cell cycle checkpoints, signal transduction, transcription factors and repair proteins. One major problem is to find a vehicle that will be able to deliver the gene or gene product to practically every clonogenic tumour cell. Present techniques are not yet optimal in this respect. Modification of genes might also lead to apoptosis after very low doses of radiation. Another example is that cells may be transduced with genes coding for certain enzymes that transform intracellular substances into sensitizers (16). Manipulation of apoptosis induction and protection is one possibility that is presently being explored. This area is still at an early stage in its development and a number of clinical applications will probably become available in the future.

Hormones

The interaction between hormone therapy and radiation is a poorly investigated area (see also (20), this issue). For example, tamoxifen increases the risk of lung fibrosis, mediated by TGF-β due to radiation, but the effect on tumour response is not fully known. A similar lack of information seems to be the case for gynaecological tumours. For treatment of prostatic cancer, a combination of hormone- and radiotherapy is common. There are some reports from animal experiments that castration before radiotherapy increases the frequency of apoptotic tumour cells by a factor of 2.

Hyperthermia

Hyperthermia has been investigated clinically for many years. It seems to have a differential effect on normal tissues and tumours. The potential usefulness of this method could be large but the technical problems associated with controlled heating of defined 'target volumes' have limited the usefulness of the method. So far, this method has been confined to superficial tumours. New technical solutions will probably be needed before widespread use can be considered.

Predictive assays

Normal tissues

Patient-to-patient variability in the response to radiotherapy. Even after a standardized course of radiotherapy, patients show wide patient-to-patient variability in the degree of normal tissue injury (22–24). Factors responsible for this variation can be categorized into two major components: 1) A stochastic (or random) component related to various phenomena, such as the random nature of radiation-induced cell killing, the random variations in dosimetry and dose delivery, the randomness of the pathogenic pathways of tissue damage, and stochastic variations in the clinical assay; and 2) a deterministic (or patient-related) component related to various patient characteristics such as the existence of genetic and epigenetic individual differences in the induction or expression of damage. Furthermore, depending on the endpoint, patient-related factors such as age, haemoglobin level, smoking, diabetes mellitus and collagen vascular disease have influenced radioresponsiveness.

Assessments of the relative importance of the stochastic and deterministic components have been made in the Gothenburg fractionation studies. These analyses showed that 90% of the variance in radioresponsiveness could be explained by patient-related factors, while stochastic effects explained the remaining 10% (25). This strikingly high dependence of patient-to-patient variability on deterministic factors should give impetus to further research exploring whether this variability is predictable and if it is likely to contribute towards improving the outcome through individualized radiotherapy.

The LQ formula has an implied assumption that the normal tissue response is controlled primarily by the radiosensitivity of the parenchymal (or target) cells in that tissue (26). It should be pointed out that no formal proof exists of this underlying mechanism. However, this assumption has some important implications: 1) knowledge of the radiosensitivity of the target cells allows prediction of tissue response, and 2) various post-treatment interventions will not be effective in preventing tissue damage.

The first implication has led to extensive studies of cellular radiosensitivity in individual patients, which, at best, have demonstrated a weak correlation with the late

treatment response of the normal tissue of that individual (27). Concerning the second implication, there is increasing evidence that it is possible to modify treatment response with post-treatment interventions. One approach is to utilize the recent finding indicating the important role of inflammatory cytokines. In particular, TGF-β1 is a mediator of late response to irradiation (28). This offers potentially new ways of addressing this long-sought-after goal for radiation oncology.

Testing the radiosensitivity of individual patients

The findings that patients with ataxia-telangiectasia (AT) had an unusually severe reaction to irradiation and that fibroblasts taken from these patients reflected this increased sensitivity were an early indication that cellular radiosensitivity could reflect tissue sensitivity (29). The further finding that cells from AT heterozygotes could also be more sensitive to radiation raised the possibility that the variation in radiation response observed between individual patients might be due to differences in cellular radiosensitivity. The concept that there was significant variability in the radiation sensitivity of cells from different individuals was further confirmed by analyses of survival curves obtained for fibroblasts from a large number of individuals.

In clinical practice, the treatment tolerance dose is based on the expectation of a low probability of severe normal tissue complications (often about 5%). Since patient-to-patient differences in radiosensitivity might be genetically based, this may result in significant underdosing of many patients. This might be so because the tolerance dose has been determined by the response of a small fraction of unusually sensitive patients. Modelling of these effects led to the prediction of significant gains in local tumour control provided accurate predictive assays of radiosensitivity were available.

Direct testing of a possible relationship between treatment outcome of radiotherapy and radiosensitivity of fibroblasts began more than 10 years ago, and there are numerous publications on this issue. A wide range of techniques has been used to assess radiosensitivity, including cell survival, DNA damage repair and chromosomal damage (30).

Most of the work done to date has focused on early and late skin reactions, including fibrosis, and the radiosensitivity of fibroblasts or lymphocytes taken from individual patients has been determined. The conclusion up until now is that lymphocyte radiation sensitivity does not (or only poorly) correlate with acute and late effects, whilst fibroblast radiation sensitivity does not correlate with acute, but generally with late, morbidity. However, the quality of the correlation must be regarded as insufficient for a reliable predictive test with currently available methods. Two large recently finished studies are consistent with this conclusion of the low predictive power of intrinsic fibroblast radiosensitivity for late fibrosis following breast radiotherapy (31, 32). Many explanations for the weakness of the correlations have been offered.

Predictive assays on tumour response

Radiotherapy today does not have the tools for taking individual tumour sensitivity into account. Different fractionation and total doses are used but only on the basis of major tumour groups with known differences in radiosensitivity, such as melanoma, lymphoma and seminoma. Today the main known prognostic factors that influence the therapy are tumour site, stage and histology. All predictive assays need to be compared with these factors for evaluation of predictive strength and/or independent information on prognosis. Furthermore, when evaluating a predictive test, other prognostic factors such as tumour stage, patient performance status, age and therapy given must be controlled. Many studies are performed retrospectively, without quality assurance of such factors. Thus, absence of uniformity in staging, etc., may confound the results.

There are a number of reasons for the different responses of individual tumours within these groups, but the majority are not fully understood. A lack of control may be the result of a geographic miss owing to poor target definition or lack of treatment routines. Inappropriate fractionation schedules with, for example, prolongation of treatment time, may lead to local failures. Intrinsic properties of the tumour may include a large proportion of resistant cells due to hypoxia or other tumour environmental factors. A highly proliferative tumour might 'outgrow' the effect of radiotherapy by rapid proliferation (33). Intrinsic radiosensitivity of tumours depending on differences in repair capacities and other genetic factors may be determinants of failure in some cases.

Predictive assays will only be of value as long as there are different treatment strategies to choose from. In clinical practice, predictive assays are still of minor importance in many groups of cancer patients. However, in a clinical study using different fractionation schedules, radiation response modifiers, or chemotherapy, it is increasingly important to know about predictive factors for individual patients if the study arm is aiming at modifying a specific property of tumour tissue. For example, to use oxygen-modifying substances without knowing the oxygenation status of the individual patient (e.g. smoking or not) and his/her tumour may lead to 'dilution' of the results. For proper design of clinical studies and progress in the field of understanding individual tumour response to radiotherapy, strong and reliable predictive assays are urgently needed.

Predictive assays govern the adjuvant treatment in, for example, breast cancer. The use of stage, hormone receptor status, proliferation status and tumour grade is important when deciding postoperative adjuvant treatment (34).

The knowledge that hormone receptor status is predictive of the response of hormone therapy is a good clinical example of a clinically relevant predictive assay. The adjuvant radiation of large groups of patients, for example breast cancer and rectal cancer, makes it obvious that there is a need for predictive assays that could spare large patient groups from unnecessary radiotherapy.

By measuring the metabolic rate of glucose in human tumour in vivo, it has been shown that a decrease in glucose metabolism after 1–2 weeks of radiotherapy can predict therapy outcome. This has been shown for tumours treated with radiotherapy as well as for tumours treated with cytotoxic drugs. PET has thus the potential not only for predicting therapy outcome but also for providing important information about tumour hypoxia and tumour spread (35).

CRITICAL ISSUES

Hypoxic and other radiosensitizers

- Identification of reliable methods for quantifying tumour oxygenation.
- Identification of tumours that contain a significant proportion of hypoxic cells.
- Identifying the clinical importance of chronic hypoxia versus acute hypoxia, and investigating whether the two types of hypoxia should be subject to different therapeutic approaches in order to minimize their tumour protection.
- Further exploration of ways to reduce chronic and/or acute hypoxia in tumour cells.
- Exploration of methods of sensitizing hypoxic cells or using bioreductive drugs.
- Identification of radioprotective substances that specifically protect the normal tissues.
- Exploring ways of increasing tumour response to radiotherapy by utilizing monoclonal antibodies, gene therapy or targeting of growth factors and their receptors.
- Further exploration of the interactions between hormones and radiotherapy.

Predictive assays

Normal tissues

- Investigate whether fibroblasts are relevant target cells for predicting the sensitivity of other cell types that contribute to tissue injury.
- Explore better ways of estimating intrinsic radiosensitivity, for example at low dose rates when repair differences play a greater role.
- Finding relevant scales for scoring clinical response. Continuous scales should preferably be sought.
- Accurately determine the dose delivered to the tissues under study for establishing reliable dose–response relationships.
- Investigate the impact of cytokines and other intercellular communications.
- Evaluate the relevance of the target theory since the failure of individual measurements of SF2 values to provide clear prediction indicate that the radiosensitivity of target cells within specific organs is only one of several factors determining radiation response.
- Investigate the role of cellular interactions and other factors, such as inflammatory cytokines in the response to radiation.
- Are the substantial differences in clinical radiosensitivity seen in unselected patients predictable?

Tumour response

- Exploring methods to establish individual tumour radiosensitivity aiming at individualizing treatment planning.
- Increasing knowledge of predictive factors that can be addressed in clinical trials.
- Evaluating predictive assays for decision-making about radiation or no radiation in the adjuvant setting.
- Exploring predictive assays for oxygenation status.
- Evaluating the possibilities of using PET imaging for predicting and monitoring treatment results.

FUTURE ASPECTS AND POTENTIALS

Hypoxia and radiosensitizers

The identification of hypoxia in human tumours is still an important area for future research. There are substances that bind covalently in hypoxic cell regions and which do not bind in tissues with good oxygenation (1, 35). Such substances can be labelled with radionuclides to allow for scintigraphic detection (PET or SPECT). However, although promising, the methods have only been applied to a limited extent and more preclinical and clinical research is needed to evaluate the reliability of various approaches. Using magnetic resonance imaging (MRI) to detect hypoxia is a further possibility (36). However, it is unclear how reliable this technique can be and much more research is necessary. Invasive methods such as oxygen probes positioned in vessels and microelectrode techniques directly penetrating tumour tissue are mainly of experimental value, but have still been considered as the 'gold standard' (37, 38) despite criticism being raised against them (36). Such methods might be used for calibration of non-invasive methods such as SPECT, PET and MRI (see (39), this issue).

Microvessel density has been a way of indirectly measuring oxygen tension and has given conflicting results. High scores of microvessel density are a prognostic sign of aggressiveness in many tumours, leading to poor prognoses. This might be interpreted as contradictory to the hypothesis that high scores lead to better oxygenation and thereby better results of radiotherapy. In a recent paper by

Chao et al. (40), Cu-ATSM-guided, intensity-modulated, radiation therapy using Cu-ATSM for the detection of hypoxia in tumours was presented. This new non-invasive method for measuring hypoxia might give the clinicians a tool to modify the dose to hypoxic areas via IMRT. This might give better clinical proof of the importance of hypoxia in human tumours.

Surprisingly, it is far from clear to what extent human tumours suffer from such severe hypoxia that the cells really are radioprotected. One question is whether hypoxic cells still have the capacity to proliferate and repair radiation-induced damage. As indicated in recent studies (12), it is possible that chronically hypoxic tumour cells are not protected, whereas acutely hypoxic cells are. This has to be analysed further. The methods mentioned above do not discriminate between the two forms. One indirect way to study this might be to disturb tumour growth and tumour blood flow by therapeutic or other measures and study the changes in hypoxia with a hypoxia marker. The challenge will then be to interpret fast and slow changes in hypoxia in terms of chronic (possibly slow changes) and acute (possibly fast changes) hypoxia. However, the techniques for doing this have not yet been developed and much more research is still needed.

The problem of attacking hypoxic tumour cells still provides the potential specifically to sensitize tumours. New methods with high efficiency and low toxicity are sought for. One possible way to decrease the number of hypoxic cells may be continuously to increase the haemoglobin concentration during radiotherapy. It is hoped that this can be obtained by erythropoietin, as has been indicated, but more studies are necessary. The evaluation of other methods of increasing tumour cell oxygenation is still an important task for clinical research. Bioreductive drugs have so far rarely been submitted for clinical evaluation. This class of drugs, at least in theory, offers an attractive way of being selectively toxic to hypoxic cells, thus compensating for the loss of effect of radiation or even sensitizing hypoxic cells to radiation.

Several possibilities are at hand for modifying radiation response by using monoclonal antibodies and gene therapy. The area has considerable potential for improving the results of radiotherapy. Further preclinical studies and improvement of delivery techniques as well as well-designed clinical studies are important.

The value of further studies of hyperthermia in combination with radiotherapy is mainly dependent on the occurrence of new and safe techniques for controlling tissue heating.

Predictive assays of normal tissue and tumour response

Normal tissues

Determination of radiation-induced changes in the production of collagen and growth factors will help to identify the cellular and molecular mechanisms of radiation-induced fibrosis. The patient-individual differentiation pattern of the fibroblast fibrocyte cell system may prove to be a predictive test for identifying patients at risk to develop fibrosis. Among the various fibrogenic cytokines and growth factors, TGF-β1 is the major component in the development of fibrosis. TGF-β1 can induce collagen gene expression, enhance collagen production and inhibit its catabolism. Improved understanding of the importance of fibroblast differentiation in the development of radiation-induced fibrosis may lead to new, more specific, assays of clinical radioresponsiveness.

In vitro studies of normal human lung fibroblasts have shown that radiation induces immediate production of active TGF-β1 and at the same time causes the stimulation of terminal differentiation of progenitor fibroblasts into postmitotic fibrocytes (41). The assay time can be reduced to 3–5 days by using short-term primary cultures of fibroblasts from skin biopsies. For predictive testing, it should be possible to discriminate highly sensitive from averagely sensitive patients (42). Importantly, both TGF-β1-induced differentiation and collagen production might be prevented by a neutralizing antibody directed against TGF-β1.

Another promising predictive assay is the serum level of TGF-β as a measure of damage progression. Also, the overexpression of TGF-β found in many human tumours and irradiated normal tissues, suggests that normal-tissue injury after radiotherapy may depend on autocrine, paracrine and endocrine effects of various cytokines. This will be an important field for investigation in the future.

Other developments could be the establishment of combinations of molecular markers of radiosensitivity and damage progression markers to characterize the phenotype of each patient. Micro-arrays may then be helpful in revealing the most important gene expressions involved in development of acute and late normal tissue responses to radiotherapy.

In summary, there is need to develop robust assays that target the functionality of specific proteins identified in basic research of DNA damage checkpoints and of DNA repair pathways and, probably also, cell cycle control and proliferation and its cytokine regulation. This knowledge has to be integrated with clinical studies of patient samples and patient response. Finally, this may allow stratification of patients according to expected response.

Tumours. New knowledge in molecular biology and new techniques have opened the way for new possibilities. The DNA molecule is the target for radiation cell kill. The cellular response to the damage is governed by the cell's ability to deal with the damage and repair it. During recent years, interest has focused on DNA profiling of normal tissues and relating the outcome to radiation responsiveness. The work has so far focused on normal tissues but genetic characterization of tumour tissues as well as relating this to treatment outcome has been stud-

ied. With molecular biology, we can characterize genes that are important for DNA repair, apoptosis and cell differentiation. Molecular biology also makes it possible to map genes that are relevant for normal tissue and tumour sensitivity. The ideal marker should pick up tumours that are likely to respond to therapy regardless of tumour type, location and stage.

It is clear that deficiencies in DNA-damage signalling and repair pathways are fundamental to the aetiology and radiation response of most human cancers. Ionizing radiation induces DNA double-strand breaks (DSB) (41), and cells respond to this DNA damage by activating DNA-damage-response pathways. These include cell cycle arrest and triggering of programmed cell death and activation of genes on both a transcriptional and post-transcriptional level.

DSBs are repaired by both homologous recombination and non-homologous end-joining mechanisms. These are induced by checkpoint factors and protein kinases and involve chromatin remodelling and proper telomere structures. It would appear that the cell's ability to respond properly to, and to repair, DSBs relies on the integrity of a complex signalling network. Hence, the study of radiation-induced cellular changes should take into account the analysis of a broad set of genes and pathways (43, 44).

The human genome sequence has recently been published, and novel techniques have been developed for large-scale mapping of gene expression and function, which offers a new paradigm for solving problems within the life sciences. Array-based transcript profiling provides one of the fundaments of this new biology. Microarray technology is based on classical hybridization chemistry, and the novelty lies in the scale. Currently, tens of thousands of DNA fragments (oligomers, or PCR products from cDNA clones), representing individual genes, can be immobilized on single arrays (chips, or microscope glass slides), which gives the researcher a truly global view of the transcriptome in a single experiment. RNA extracts from the tissues or cells of interest are labelled and converted into cDNA that is hybridized to the arrays. Arrays are scanned for fluorescence intensity and data collected for further analysis.

Microarray technology is demanding with regard to means of data collection and storage (hybridization data and images), data formatting (normalization, filtering and clustering). The technology involves cross-referencing to public databases and exporting to statistical programs and bio-informatics expertise, with the focus on data interpretation (data analysis, visualization) and modelling of complex biological systems. Moreover, to significantly contribute to a deeper understanding of complex biological processes, it is crucial that the experimental design allows a sharp biological or clinical question to be addressed. Such an increased resolution may be achieved, for example, by a well-defined genetic make-up in transgenic, congenic and/or cell ablation models, carefully defined experimental conditions and carefully selected biological material representing well-defined temporal/cell cycle stage and type of cell, as well as sorted or micro-dissected cells and tissues.

The genome programs have provided powerful tools for transcript analysis of virtually all the human genes. However, for a more complete view of gene function in relation to radiation effects, transcript analysis must be complemented by analysis of protein levels and cellular functions.

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