Biological Response to Radiation Therapy

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In an investigation by the Swedish Cancer Society, the present status, critical issues and future aspects and potentials were described by an expert group for each of nine major areas of radiation therapy research. This article deals with biological response to radiation. Separate sections deal with molecular responses to radiation, the stem cell and clonogenic cell concepts and the importance of cell proliferation, cell and tissue responses to doses above and below 1 Gy, respectively, the potential role of intercellular signalling pathways, the so-called bystander effect and radiation biology-based therapy planning and treatment optimization.

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MOLECULAR RESPONSES

Present status

Response to DNA damage. The avalanche of knowledge in cellular biochemistry and molecular biology has had a great impact on the current perspective on studies of DNA damage and cell cycle response after ionizing radiation (IR) and its application in radiation oncology. Mammalian cells can respond to DNA damaged by IR with immediate activation of two important cellular functions: cell cycle regulation and DNA repair. The repair pathways are constitutively active in most cells and are influenced by cell cycle checkpoints. Upon recognition of damaged DNA and a period of damage assessment, the cell has to decide upon DNA repair or cell death. The goal is to preserve the integrity of the genome (1, 2).

Individuals who exhibit either repair or checkpoint defects are at a much higher risk for cancer, as demonstrated by cancer-prone syndromes. In general, they are also more radiosensitive to IR. Upon IR exposure, cells normally react in three distinct ways: arrest of cell cycle progression and repair of the DNA lesions or triggering of the apoptotic response. IR results in significant cell cycle arrest even at doses well below the typical fractionated daily dose given clinically. This will allow cells sufficient time for DNA repair. In fact, a single double-strand break (dsb) can trigger arrest of the cell cycle.

Several genes are required for the accurate detection of DNA damage (e.g. *ATM, ATR, RAD1, RAD9, RAD17*). Furthermore, several mechanisms are responsible for arrest of the cell cycle. These involve mainly regulation of cyclins and cyclin-dependent kinase inhibitors (cyclin D, E, A, B, p21, p27, p15, p16). The ATM protein appears to function at the very early stage of damage surveillance by sensing and initiating the DNA-damage checkpoint signals. The activated ATM protein kinase phosphorylates a number of critical targets involved in cell cycle arrest, DNA repair and apoptosis. So far, immediate downstream targets of importance are the tumour suppressor genes *TP53, NBS1, RAD9 and BRCA1*, and others such as *CHK1* and *CHK2*. The proto-oncogene *C-abl* is also activated. Another phosphorylation target of ATM is the chromatin component H2AX and its phosphorylated form γ-H2AX is a very early response to dsb (3). Several of the phosphorylation targets function in a complex for repair of DNA damage, e.g. the BRCA1-RAD51-BRCA2 complex, the NBS1-

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MRE11-RAD50 complex. Also NF-Kβ is activated upon DNA dsbs.

RAD9 phosphorylation is required for activation of the DNA-damaged induced G_1/S-phase checkpoint. However, further downstream, effectors are unknown. The checkpoint kinase CHK2 has a key role in delaying cell cycle progression after DNA damage. Upon low doses of IR, CHK2, phosphorylation induces both G_1 arrest (via TP53) and G_2 arrest (via Cdc 25c). Of importance is that ATM-dependent activation of CHK2 requires NBS1. NBS1 is also essential for the formation of IR-induced nuclear foci at sites of dsb repair.

TP53 is one key protein that coordinates DNA repair with cell cycle progression and apoptosis. Upon IR exposure, TP53 is stabilized by phosphorylation at different sites. ATM kinase activity is crucial for the rapid stabilization of TP53 following IR. TP53 interacts with TP53 binding protein 1 (53BP1), which rapidly forms strong foci with hundreds of phosphorylated molecules per dsb in response to IR. 53BP1 is also regulated by ATM.

BRCA1 functions in the transcription-coupled repair and BRCA2 in the dsb repair pathway. Of immediate clinical importance are the findings linking *BRCA1* and *BRCA2* genes to radiosensitivity. Another finding of importance is that lack of C-abl means the absence of cell cycle arrest and no induced apoptosis by IR. On the other hand, NF-Kβ plays a key role in protecting cells from proapoptotic stimuli, including dsbs. Inhibition of NF-Kβ leads to radiosensitization through enhanced apoptosis, as is also seen with other agents that induce dsbs.

The final decision to initiate apoptosis rather than cell cycle arrest is likely dependent on the magnitude and duration of the damage stimulus. There is also evidence that cell cycle entry is necessary to initiate apoptosis in cells bearing DNA damage.

DNA repair. Effective DNA repair is fundamental in preserving the stability of the genome. In response to IR, the most important DNA damage related to reproductive cell death is the DNA-dsb. In addition to IR, dsb can also be formed by normal endogenous processes such as oxygen free radicals, DNA replication, or topoisomerase failure. Persistent damage is related to misrejoining events such as dicentric chromosomes and ring chromosomes, both leading to a mitotic catastrophe within 1–2 turns of the cell cycle. The dsb might also lead to critical translocations or insertion of genetic material outwith the original site. These critical events are generally stable over the next cell divisions but may include activation of oncogenes and deletion or down-regulation of housekeeping genes. Biophysical assays show a correlation between radiosensitivity and both fractions of unrejoined dsb and the kinetics of rejoining. However, even if most cells that rejoin the breaks slowly appear to be sensitive to IR, some radiosensitive cell lines rejoin the breaks normally.

There are at least two main repair pathways of IR-induced DNA-dsb in mammalian cells. The non-homologous end-joining (NHEJ) pathway is the dominant one in human cells and it is initiated by rapid localization and binding to the broken ends of DNA by the DNA-dependent protein kinase complex (DNA-PK). The downstream signalling and repair mechanisms are less clear but several proteins and enzymes are involved (Table 1, Figs. 1, 2). Disruption or inhibition of DNA-PK renders the cells extremely sensitive to IR and the repair becomes very slow. Knockouts of several other genes/proteins may produce non-viable phenotypes. Homologous recombination (HR) is the major high repair fidelity pathway of the dsb repair system. HR is particularly efficient in the S- and G2-M-phases of the cell cycle because of the availability of sister chromatids as repair templates. Many of the proteins involved in DNA repair move to, and accumulate, at the site of DNA damage and immunofluorescent detection of such foci has become an important way to study repair proteins and their interactions. A striking example is the phosphorylated form of histone H2AX, γ-H2AX, which rapidly forms strong foci with hundreds of molecules per dsb. The area of DNA repair is still unexplored regarding the understanding of the interaction with other cellular processes in normal cells and tumour tissue. Because tumour cells are often impaired in their DNA damage response, it follows that understanding the role of genes in DNA repair will also lead to insights into the aetiology of cancer (4).

Apoptotic versus clonogenic cell killing. The mode of cell killing due to DNA damage induced by anticancer treatment including radiotherapy has been highlighted in recent years. In particular, the understanding of the function of wild-type TP53 to induce apoptosis after DNA damage has led to a tremendous focus on programmed cell death. There is no doubt that lymphoid tissues and lymphoma cells mainly die a rapid apoptotic death after DNA damage that is dependent on wild-type TP53. In non-haematological normal and malignant tissues the ability of cells to undergo unlimited proliferation tested by a clonogenic assay has become the 'gold standard' for assessment of radiosensitivity and cellular sensitivity to other cytotoxic treatments.

The fact that apoptosis is considered to be related to genetically defined pathways has given rise to new concepts. One of these concepts is that the genotype of normal or tumour cells will predict the sensitivity of DNA-damaging agents. Another predominant tenet is that new therapies based on apoptotic cell killing will be superior to the existing cancer treatments, which mainly result in reproductive or necrotic cell death. Recently, it was established in a critical review that non-clonogenic short-term assays may be misleading for the overall cell kill induced by cytotoxic treatments (5). Short-term assays predominantly reflect the degree of apoptotic cell kill and exclude the late occurring

Table 1

Some major human dnb (double-strand break) repair genes[a]

Gene[b]	Properties of phenotype with defect gene[c]	Interaction proteins
Non-homologous end-joining (NHEJ)		
XRCC4	Radiosensitive, low V(D)J[d] recombination	DNA ligase IV
KU80/XRCC5	Radiosensitive, defective dsb rejoining, low V(D)J recombination	KU70 and DNA-PKcs
KU70/XRCC6	Radiosensitive, low V(D)J recombination	KU80 and DNA-PKcs
DNA-PKcs/XRCC7/PRKDC	Radiosensitive, defective dsb rejoining, low V(D)J recombination	KU70/80
LIG4 (encoding DNA ligase IV)	Radiosensitive, defective dsb rejoining, low V(D)J recombination	XRCC4
Homologous recombination (HR)		
RAD51	Not viable/chromosomal aberrations	Co-localize with BRCA1 and BRCA2
RAD52	Slightly decreased HR	
RAD54	Radiosensitive, defective dsb rejoining, decreased HR	
XRCC2	Radiosensitive	
BRCA1	Not viable	Co-localize with RAD51 and BRCA2
BRCA2	Not viable	Co-localize with RAD51 and BRCA1
Both pathways		
MRE11	Not viable/chromosomal aberrations	
RAD50	Not viable	All three form a complex
NBS1	Radiosensitive, radioresistant DNA synthesis	(MRE11-RAD50-NBS1)

[a] Modified from van Gent et al. (4). This is a selection of genes directly involved in the repair of radiation-induced dsb. The exact role for many of these genes is still unclear and several other gene products directly/indirectly indicated in the dsb-repair/signalling are not included in the table (e.g. BLM, γ-H2AX, RAD1, RAD9, RAD17, RAD51B-D, XRCC3, ATR).
[b] BRCA: Breast cancer susceptibility; DNA-PKcs: catalytic subunit of DNA-dependent kinase; MRE11: meiotic recombination 11; NBS1: Nijmegen breakage syndrome 1; XRCC: x-ray cross-complementation group.
[c] The phenotype of the same mutation in different cell types may vary.
[d] V(D)J recombination: a process critical for the generation of a functional immune system. This process involves enzyme-induced dsb.

reproductive cell death. This, for example, will result in a bias when comparing cytotoxic response of wild-type *TP53* cells with *TP53*-mutated or *TP53* −/− cells using the short-term assay. This assay will, in the first place, reveal the rapid apoptosis of *TP53* +/+ cells, which is not seen in *TP53* −/− cells, whereas a clonogenic assay may show similar cell survival for both genotypes. The conclusion in this review was that if clonogenic survival is used as the endpoint for cell killing, neither TP53 status nor the ability of the cells to undergo apoptosis appears to play a significant role in the sensitivity of these cells to DNA-damaging agents (5).

The equivocal role of apoptosis in non-lymphoid normal tissues for maintenance of the balance between cell production and cell loss was reviewed recently (6). With a few exceptions, apoptosis contributes to a limited extent to cell turnover in mammalian normal tissues. Cell loss from tumours is governed by several mechanisms, e.g. necrosis caused by insufficient angiogenesis. So far, there is little evidence to suggest that apoptosis is the major route of cell loss from carcinomas. In the light of this, the therapeutic significance of apoptosis might be questioned, and various new treatment approaches that only focus on increased apoptotic cell killing might also be disappointing (6). Still, there is no evidence to suggest the withdrawal of consensus from the bulk of the experimental data on cytotoxic tumour response indicating that loss of colony-forming ability is the key event in destroying tumour cells.

Critical issues

In the near future new pathways involved in cell cycle checkpoints and DNA repair will probably be identified. However, there are many obstacles in translating this knowledge into clinically useful therapies.

- The relevance of recent knowledge, exemplified above, for the in vivo and human situation is still almost completely unexplored.
- The relative importance of defects in the various signalling pathways involved in cell cycle checkpoints and of DNA repair for radiosensitivity has to be established.
- Individuals who are heterozygous or with polymorphisms for some of the genes in the complex machinery of cell cycle checkpoints and DNA repair differ in their radiosensitivity from the normal population. Improved knowledge about how prevalent these defects are among cancer patients is needed.
- What is the predictive significance of the tumour response to radiotherapy of different defects in molecu-

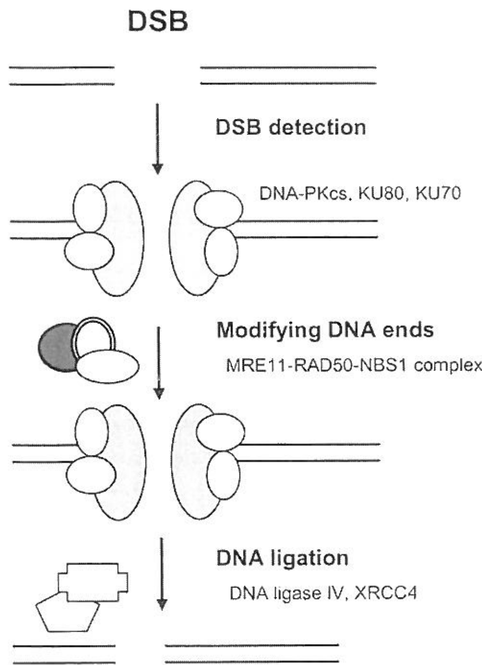

Fig. 1. Repair of double-strand breaks (dsbs) using the of non-homologous end-joining (NHEJ) pathway. The DNA-dependent protein kinase (DNA-PK) rapidly binds the free DNA ends. DNA-PK consists of the heterodimer KU70/KU80 and the catalytic subunit of DNA-PK, DNA-PK_{CS}. After modification of DNA ends by the MRE11-RAD50-NBS1 complex, XRCC4 and ligase IV finally bring the ends together. Short DNA sequences have been deleted.

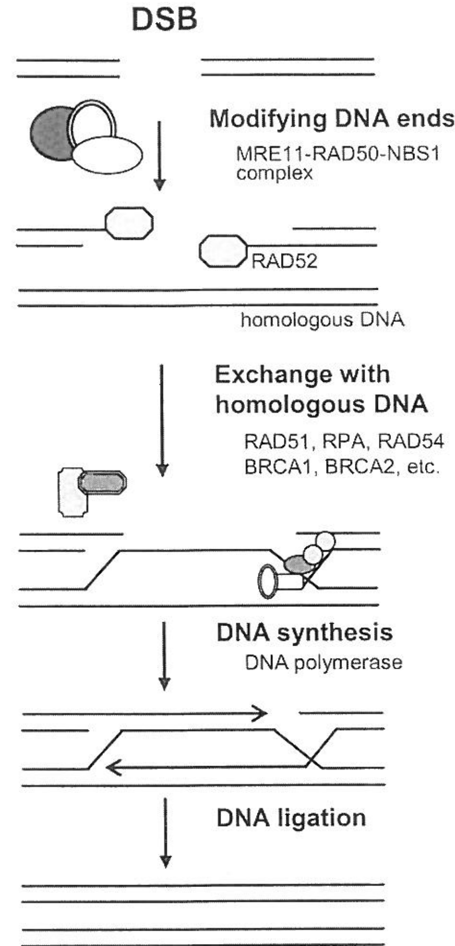

Fig. 2. One model of double-strand break (dsb) repair by the homologous recombination (HR) pathway. After possible modification of the DNA ends by the MRE11-RAD50-NBS1 complex, RAD52 binds to DNA and interacts with replication protein A (RPA). The strand-exchange protein RAD51 then initiates the recombination process with homologous DNA. This process involves the proteins RAD54, BRCA1 and BRCA2, but additional components might be involved. New DNA is synthesized and the remaining gaps are ligated. In general, HR is accurate and the original DNA sequence is restored.

lar signalling pathways regulating cell cycle checkpoints and DNA repair?

- Can modulators of DNA repair be developed that lead to a therapeutic gain?
- Further research is needed to obtain a deeper understanding of the importance of apoptotic cell kill versus necrosis and clonogenic cell kill, particularly with regard to the therapeutic potential of increased tumour cell kill by apoptosis.

Future aspects and clinical potentials

An overall aim of future research is to find out how DNA repair can be quantified and modulated to contribute towards improving radiation therapy, by both rapid and sensitive predictive assays and specific sensitization of tumour cells by knockout of critical pathways for repair. Since DNA is the primary target for therapeutic doses of radiation, basic knowledge about repair pathways in normal and tumour cells is highly important. The first gene known to play a major role in dsb repair in mammalians was identified in 1994 (7) and this area has a high potential for research. The significant progress in DNA repair research in the past few years has shifted emphasis from prokaryots and yeast to mammalian or human cells. Many aspects are, however, still unclear, including identification of additional key proteins, signalling pathways, alternative mechanisms of repair, activation/inhibition of pathways and proteins, the role of the cell cycle and proliferation, relocalization of proteins, the role of the 3D nuclear architecture, effects of high-LET (linear energy transfer) and multiple damage sites, heterogeneity within individuals and cell populations.

The knowledge gained could lead to future development in the prediction and prognosis of tumour and normal tissue responses. Similarly, the identification of important targets, preferably down- or up-regulated in tumour cells, may have the potential to improve tumour therapy via modulation of the repair. The research area includes complex interactions between molecular targets and there

is a need for innovative biochemical studies and to adopt the recent advances in genomics and proteomics (8).

What are the clinical implications of genetic determinants?. Several aspects of the biochemistry and molecular biology of DNA damage and repair may offer insight or clinical advantage to radiation oncology in the near future. However, the most important aim is to establish differentials in response between the tumour and critical normal tissues in order to gain a therapeutic advantage.

Laboratory studies of the subtle role of alterations in DNA repair capacity. This is of importance for cancer proneness, but may also have clinical therapeutic implications. Recent studies have indicated that individuals with modest reductions in DNA repair capacity may be susceptible to certain cancers, e.g. skin, lung, breast and colon cancers. These individuals might also have more adverse side effects to radiotherapy, including induction of genetic instability and later development of radiation-induced cancers (9).

Target gene inactivation strategies. There are many strategies under development in the laboratory with the potential for clinical use by either knocking out or suppressing repair genes in tumour cells such that radiation and chemotherapy strategies are more effective.

One important task for the near future is translational investigations of patient samples concerning aberrations in critical genes and gene function versus patient response to IR (10). New approaches in studying DNA repair and radiosensitivity by assessment of DNA repair foci should be encouraged. Such analyses may probably be performed on tissue sections after small test doses of IR and the response of each specific cell type might be determined. This information can also be coupled to specific expressions of various molecular markers in each cell type. This procedure will likely give reliable information of the patient's phenotype and individual radiosensitivity.

STEM CELLS, CLONOGENIC CELLS AND PROLIFERATION

Present status

Both tumours and normal tissues are composed of heterogeneous combinations of cells, with different phenotypic characteristics and different proliferative potentials. Tumour cells show a variable expression of normal differentiation, which indicates that some of the heterogeneity in tumours arises as a result of an anomalous differentiation. Control of cell proliferation and differentiation in renewing tissues depend both on control of cell cycle entry and on the probability of stem cell self-renewal.

Self-renewal is the hallmark property of stem cells in normal and neoplastic tissues. Therefore stem cell biology could provide new insights into the tumour biology profile (11, 12). Important issues are 1) the possibility that tumour cells arise from normal stem cells, 2) potential similarities in the mechanisms that regulate self-renewal of normal stem cells and tumour cells, and 3) the possibility of existing tumour stem cells. Therefore, the heterogeneity of proliferative behaviour that forms the basis of the stem cell model and the picture of the tumour architecture as a 'caricature' of its normal tissue renewal counterpart may have important implications for identification of the gene expression profiles of tumour cells. There are likely to be differences in gene expression between tumour stem cells and tumour cells with limited proliferative potential. Micro-array assessment on gross tumour biopsies results in a composite gene expression profile of a mixture of tumour cells and various normal cells. Therefore, the true gene expression of the highly proliferative tumour cells that drive tumour growth may be obscured, as these cells often represent a minority of the tumour cells. One way to overcome this problem may be efficient micro-dissection for cell-specific determination of the genetic characteristics of the tumour.

Definitions

Stem cells. Cells capable of self-renewal and of differentiation to produce all the various types of cells in a lineage, and sometimes pluripotent to regenerate all cell lineages in a tissue or tumour.

Transit amplifying cells. Proliferating cells that have low self-renewal capacity and a high probability of terminal differentiation.

Clonogenic cells. Cells characterized in assays by their ability to form a colony exceeding 50 cells within a defined growth environment.

Differentiated cells. Cells that have undergone qualitative changes in the cellular phenotype because of the onset of synthesis of new gene products that ultimately lead to functional competence.

Understanding how the stem cell is regulated is of considerable importance in cancer. Stem cell research provides a foundation for therapeutic advancement in oncology. Oncogenic lesions acquired by cells that are undergoing terminal differentiation have relatively little impact on a tissue, because the cells are rapidly lost. In contrast, stem cells are permanent tissue residents and therefore have the potential to acquire deleterious changes over time and to form a tumour. Knowledge of the pathways governing proliferative regulation and differentiation within various cellular systems will result in new medical strategies aimed at the root cause of cancer. Examples of tumour histologies where the stem cell concept is established are squamous cell carcinomas and colorectal carcinoma (13).

The study of stem cells is important for several reasons: 1) Regulation mechanisms of stem cell proliferation are the same processes that become disregulated in cancer development. 2) Rapidly dividing tissues of bone marrow, gut and skin are the first to be affected by cancer treatment and

are often dose limiting in radiotherapy as well as in using other cytotoxic agents. Isolation of viable stem cells could be used for repopulation of such tissues, or rescued by growth factor manipulation. 3) Identification of stem cells by reliable markers is necessary for stem cell gene therapy.

The stem cell is a relatively undifferentiated cell with a number of properties or options making it capable of proliferation and preserving its own population. It has been shown, e.g. for skin and colon, that the stem cell can produce a variety of cell lineages and also that they are capable of tissue regeneration following injury. Under normal conditions stem cells undergo asymmetric divisions, i.e. mitosis results in a single daughter stem cell and one daughter cell that differentiates. After tissue damage, stem cells may undergo symmetric divisions, i.e. mitosis results in two daughter stem cells and the stem cell population increases. In the small intestine, the early transient amplifying cells, which are comparatively radioresistant, retain stem cell properties after cell killing of the radiosensitive stem cells. The stem cells undergo apoptosis already after small doses, from 0.01 Gy with a dose-dependent increase up to 1 Gy.

There is indirect evidence that stem cells comprise only a small subset of the proliferating cells in normal tissues, and may also comprise only a small proportion of cells within a tumour. However, there is clear evidence that only a few surviving stem/clonogeneic cells are enough for complete regeneration of a tissue after injury, and also enough to generate tumour recurrence. Presently, there are no specific molecular markers that define the stem cell compartment. However, both intrinsic and extrinsic signals regulate the stem cell behaviour and some of these signals have now been identified. Interestingly, certain aspects of the stem cell microenvironment are conserved between tissues.

Over the years it has been debated whether the hierarchical stem cell architecture seen in normal epithelium is more or less preserved in epithelial malignant tumours. If this is the case, a minority of cells have the proliferative capacity to maintain the tumour and the majority of cells demonstrate some degree of differentiation and have only limited proliferative potential. In contrast, it has been suggested that a carcinogenic event occurring in a differentiated cell could render that cell an unlimited proliferative potential, but might still have the ability to organize tissue-specific differentiation.

With recent knowledge about cell cycle regulation, the stem cell architecture seems accurate although it needs modifications to allow for varying cell cycle times caused by factors such as high intratumoural pressure, hypoxia and lack of nutrients (14, 15). Such phenomena create subpopulations of tumour stem/clonogenic cells with much slower proliferation rates. These might be the key populations for entry and exit to the cell cycle, depending on the environmental circumstances. After IR, these cells might be radioresistant at one time but later on cause accelerated repopulation, thereby being critical for the outcome of the treatment.

The observation of macroscopic changes in tumours yields little information on the intratumoural processes and tumour cell kinetics. It is generally accepted that when cell death is accelerated after IR, a compensatory proliferation starts after some delay in the tumour or acutely responding normal tissues. The reason for this may be that previously growth-depriving factors such as hypoxia, malnutrition and contact inhibition decrease. Especially for normal tissues, but probably also for some tumours, the release of different growth factors will play an important role. As a consequence cells are recruited into the cell cycle and stem cells may go from asymmetric to symmetric division.

The cell proliferation rate during therapy has several effects. Cell repopulation means that the radiation dose is 'lost' because the number of cells left to kill will not decrease at the expected rate. As a rule of thumb, about 50% of the cells are killed by one fraction of 2 Gy. This means that the number of fractions that has to be delivered to eradicate a tumour with perhaps 10^9 cells must be large. A significant regrowth of tumour cells between fractions means that the tumour will not be cured by a tolerable radiation dose.

For normal, acutely reacting, tissues, regrowth during radiotherapy has been thought to increase the acute tolerance to the treatment. However, recent clinical data have shown that this is not always the case (16) (and unpublished data). The reason is that during accelerated repopulation the relative number of cycling cells is increased and during the cell cycle most cells are more sensitive to radiation than quiescent, non-cycling cells in the G_0 phase.

The potential of accelerated proliferation in tumours during radiotherapy plays an important part in the construction of novel radiotherapy fractionation schedules for clinical trials. It has been established that prolongation of overall treatment time reduces the tumour control probability, especially for squamous cell carcinoma. There are some data that are suggestive of a beneficial effect of shortening the treatment time, in particular for head and neck cancer.

There is an ongoing discussion of the prognostic importance of proliferative activity in tumour tissue measured before radiation therapy. Measurements of proliferative activity by labelling methods such as labelling index (LI) or potential tumour doubling time (T_{pot}) have been studied, sometimes with conflicting results (15, 17–20). Furthermore, a number of proliferation markers such as Ki-67, MIB-1, Cyclines A and B1, have been identified and are presently being studied with respect to their prognostic significance. One important question for these studies is whether patients that are likely to benefit from accelerated

radiotherapy can be identified through use of these factors. For tumours with slow proliferation, hypofractionation might be beneficial (21, 22).

Critical issues

- The identification, location and number of stem cells in normal tissues. This knowledge is important for appropriate radiobiological modelling.
- Intracellular repair capacity after radiation damage, and determination of DNA damage checkpoints and cell cycle checkpoints for stem cells and clonogenic cells.
- Type of cell death after radiation damage. How important is apoptosis in relation to clonogenic cell death?
- Regulation of proliferation after radiation damage, especially mechanisms of accelerated repopulation and the timing of this in the radiotherapy course.
- The capacity of early transient amplifying cells to retain stem cell properties in normal tissues and tumours.
- To establish reproducible methods for pretreatment assessment of predictive markers of proliferative capacity in tumours, and to establish the prognostic significance of such markers and their relevance for proliferation during radiation therapy.
- To establish whether rapidly proliferating tumours are best treated with accelerated fractionation or other treatment interventions.
- To further investigate the balance between the loss of efficiency of radiation due to accelerated proliferation, and the gain in efficiency owing to a larger proportion of actively cycling cells that are more radiosensitive.

Future prospects and clinical potential

It is clear that, in the near future, we can expect several advances in our understanding of the characteristics of stem cells for various tissues. The use of proteomics, micro-arrays and tissue arrays will probably be helpful in identifying intracellular and cell surface markers of the stem cells and the transit amplifying compartments. There will also be increasing exploitation of transgenic mice to study stem cell renewal and differentiation. One very central issue in radiation oncology (and also for other cytotoxic treatments) is to investigate the proliferation characteristics and regulation of accelerated repopulation of stem cells and early progenitor cells as a function of various dose-fractionation schedules. This should be done for various normal tissues, and also for various carcinomas in the first place.

Evaluation of present and new methods of identifying proliferation characteristics of tumours and normal tissues is a major task for effectively taking advantage of this information in clinical radiotherapy. Depending on the nature of the method, some parts of such studies may be performed on retrospective clinical material. In principle, the best information could be collected by using material from properly designed randomized trials. In other words, it would be interesting to use, for example, tumour material from patients in trials of accelerated and conventional fractionation to verify the clinical relevance of one or several proliferation markers. The ultimate aim of such studies would be to 'tailor'the radiotherapy schedules to the tumour characteristics. Obviously, there is a similar need for studies of normal tissues (16). Some of these questions probably need to be answered by studying the clinical outcome of different fractionations with respect to side effects. Experimental models are important in these situations to support the interpretation of clinical data.

It is likely that a balance exists between a loss of efficiency of the radiation dose due to accelerated proliferation and a gain in efficiency due to a larger proportion of the more radiosensitive cycling cells. The implications of these two opposite phenomena may directly influence the outcome of a particular fractionation schedule. It will be a future challenge to explore differences between tumours and acutely reacting tissues in order to choose the optimal fractionation schedule. It has been postulated that this is mainly a problem of tumours and normal tissues that proliferate slowly or moderately rapidly since they may have a larger capacity of recruitment of cells into the cycle than those that have a very rapid proliferation where most cells are already cycling. Further preclinical studies on the balance between the effect of accelerated proliferation and increased radiation sensitivity of the cycling cells are thus needed. The results then need to be translated into clinical studies.

CELL AND TISSUE RESPONSE TO DOSES ABOVE AND AROUND 1 GY

Present status

Radiation kills cells by producing a variety of types of damage in DNA. There is fairly strong evidence to show that damage to DNA is the primary cause of IR cell killing and mutations. Each 1 Gy dose of low-LET radiation produces about 1000 initial single-strand breaks (ssbs) and 25–50 initial dsbs in addition to a considerable amount of base damage. Cell killing correlates most significantly with the number of unrepaired or misrepaired dsbs breaks, which are therefore thought to be the most important type of cellular damage. However, only about 1–2% of the dsbs are really lethal. Most ssbs and dsbs are faithfully repaired.

In order to describe the dose-response relationship for cell killing, various models have been proposed based upon assumptions about target (a sensitive region of the genome) inactivation. Such models are the exponential and multi-target single hit survival curves. In fact, a combination of these two is more appropriate for most experimental cell survival data derived by clonogenic assays. An even better description of radiation cell killing to single dose fractions of a size for clinical use is shown by the linear quadratic

(LQ) model. The shape of the survival curve is determined by the α/β ratio, where α and β are cell specific under defined conditions, but may vary with radiation quality, dose rate and other various dose modifiers. Alternative repair models have been proposed, which are in close agreement with the LQ model in the clinical relevant dose region (23).

Besides the importance of describing the cell-survival curve to single doses under defined circumstances there is also a need for formalism in describing dose-fractionation and dose-time relationships for various tissue effects. Today, the most commonly used tool is the LQ model to which a generalized time factor accounting for the DNA repair rate is added. This formalism describes the fractionation and dose-protraction effects for the tissue specific α/β ratio and repair half-time for DNA damage, $T_{1/2}$ (24).

Recently, it was established that most other radiobiological models, including the repair models, predict very similar isoeffect relationships for alternate fractionated or low-dose-rate regimens compared with the LQ model (25). In order to account for cell proliferation and accelerated repopulation, another tissue-specific time factor $T_{proliferation}$ has to be added to the LQ formalism.

Comprehensive radiobiological studies were performed during the 1980s in order to determine cell- and tissue-specific parameters for α/β, $T_{1/2}$ and $T_{proliferation}$. The α/β ratio is a measure of the fractionation sensitivity and $T_{1/2}$ represents the half-time of repair of sublethal DNA damage of the cell type or tissue. Importantly, it could be established that acutely responding normal cell populations and tissues have significantly less fractionation sensitivity, with α/β around 10 Gy, compared with late-responding tissues, with α/β around 3 Gy (26). The α/β ratio was also determined for many human tumour cell lines, and the majority had values of about 10 Gy or higher, i.e. similar fractionation sensitivity to that of acutely responding normal tissues. There were a few exceptions: melanoma and some sarcomas showed a low α/β ratio (23, 27).

There is more or less consensus at the moment that the α/β ratio is roughly determined by the proportion of cycling cells, i.e. the growth fraction. This is the reason behind the different ratios between acutely and late-responding tissues. This also means that rapidly growing tumours generally have a low fractionation sensitivity, while slowly growing tumours may have a higher fractionation sensitivity. The other two parameters, $T_{1/2}$ and $T_{proliferation}$, are much less well established than the α/β ratio for both normal and malignant tissues.

In order to optimize and individualize the fractionation schedule for radiotherapy, and also for the best choice between high dose-rate or low-dose-rate techniques, we need accurate information on parameter values for the particular tumour and the surrounding normal tissues. One recent example of this is new knowledge about the fractionation sensitivity of prostate carcinoma, which was determined to be quite high (i.e. α/β is low and about 1.5 Gy). The implication is that instead of giving daily fractions of about 2 Gy, dose fractions above 4–5 Gy would increase the therapeutic benefit. Likewise, the low-dose-rate treatment with seeds ought to be less optimal than high dose-rate techniques with large dose fractions for prostate cancer (22).

A volume effect may be defined as the alteration of the dose-response function with changes in irradiated volume. It is known that if the dose-response function is steep, there will be very little volume effect, e.g. spinal cord injury. Otherwise, for most organs and for most endpoints, there is a volume effect. A threshold for the volume effect might exist, e.g. radiation hepatitis, radiation nephritis and radiation pneumonitis are endpoints for which this is likely the case.

The organ and tissue architecture and the existence of functional subunits will also determine the dose-volume effect. Tissue architecture is divided into the serial and parallel type according to the arrangement of the functional subunits.

Critical issues

- Uncertainty in the parameters of the LQ model overall. In particular, there is little knowledge on whether changes occur in the parameters during the radiotherapy course, especially for acutely responding tissues and for tumours.
- Lack of knowledge for the individual tumour concerning fractionation sensitivity, repair kinetics and the capacity of accelerated repopulation.
- To collect large enough databases in order to establish dose-volume-effect models and derive organ/tissue and endpoint-specific parameter values.

Future aspects and clinical potentials

Since the mid-90s, highly conformal radiotherapy using intensity-modulated radiation therapy (IMRT) has become accessible in routine clinical practice (28, 29). In order to determine the optimal shape of the dose distributions, we need information about the dose-volume fractionation responsiveness of the normal tissues at risk. Today, this information is not complete and research must involve collecting the dose-response data and to establish appropriate models. The dose-volume effect depends on the nature of the volume effect, which in turn is organ or tissue specific and also specific to a given endpoint. Most organs experience a spectrum of radiation-induced complications.

A key issue for the near future is to collect appropriate and large enough databases to make it possible to establish dose-volume effect models and to derive tissue- and endpoint-specific parameter estimates. The objective of new models should be to include inhomogeneous dose distribu-

tions. When modelling, the late tissue effects of IR censoring must be taken into consideration. There are several survival models that correct for censoring, but the problem is that dose-volume-response modelling cannot be accomplished using survival models. One way to circumvent the problem with censoring is to find early-appearing surrogate endpoints for the true late and mainly irreversible morbidity (30, 31).

In order to find the right balance between cure and complications, radiobiological models that can quantify as accurately as possible the response of heterogeneous tumours and organized normal tissues to non-uniform dose delivery, are required for accurate optimization of the dose delivery and treatment outcome. With such models it is possible to derive the intensity-modulated dose delivery that can maximize the tumour cure and minimize the risk of inducing severe normal tissue side effects.

Another important question to consider when reporting the result of clinical trials of radiation therapy for various tumour sites is whether there is a correlation and covariance between the responses of the tumour and the surrounding normal tissues. Reporting tumour response and complications separately is insufficient, since it is important to consider the correlations between these two endpoints in order to estimate the probability of a truly positive outcome.

CELL AND TISSUE RESPONSE TO LOW RADIATION DOSES—HRS/IRR

Present status

Improvements in the methodology of clonogenic assays within the past decade have made it possible to study cell survival with sufficient accuracy to resolve changes in radiosensitivity at doses below 1 Gy. When cell survival is close to 100% the number of cells 'at risk' in a colony-forming assay has to be determined exactly. This is achieved using either a fluorescence-activated cell sorter (FACS) to plate an exact number of cells, or microscopic scanning to identify an exact number of cells after plating.

By means of these methods the phenomenon of low-dose *hyperradiosensitivity* (HRS) was found to be a common feature of radiation cell survival below 0.5 Gy. HRS precedes the occurrence of a relative resistance per unit of dose to cell killing over the dose range 0.5–1 Gy, a phenomenon known as *increased radioresistance* (IRR) (32–34). The HRS/IRR features are seen following low-LET radiation but not following high LET.

HRS/IRR has been detected in about 80% of the 45 cell lines characterized so far. No relationship between the existence of HRS and histological type, p53 status, apoptosis, cell cycle time or G1 arrest has been seen (Joiner pers. comm.). Generally, the cell lines most radioresistant to 2 Gy demonstrate the most marked low-dose HRS. The cell lines assessed in vitro consist of colorectal adenocarcinoma, bladder carcinoma, prostate carcinoma, cervix squamous cell carcinoma, lung adenocarcinoma, neuroblastoma, glioma and melanoma (35). A non-malignant lung epithelial cell line has also been studied.

Importantly, the radiosensitivity in the HRS region of the cell survival curve was similar for all cell lines regardless of their response above 1 Gy. Recently, it was also shown for T98G cells that HRS occurred in fractionated irradiation using 3 doses of 0.4 Gy per day 4 h apart, which resulted in significantly increased cell kill compared with 1.2 Gy once daily (36).

The establishment of HRS/IRR suggests that both cancer cells and normal cells respond to radiation injury by triggering repair processes and that the activation is dictated by the amount of DNA damage received. However, the exact mechanisms for this are not known. The HRS of cell survival curves obtained with high dose rates might also be translated into the 'reversed dose rate' response seen following very low dose rates, i.e. an increase in radiosensitivity, as the dose rate is reduced below a critical value.

There is evidence that HRS in vitro translates into additional effectiveness of fractionated radiotherapy given in very small doses per fraction in vivo. This has been confirmed for mice in skin, kidney and lung (37, 38). In fact, these tissues showed a 'reverse fractionation' effect below 1 Gy. There is now also clinical data with convincing evidence of the existence of the HRS/IRR phenomenon in skin (16) and also an indication of HRS/IRR for the salivary function.

Critical issues

- Increased understanding of the mechanisms for HRS/IRR. This could have broad implications in areas from risk assessment to optimal cancer treatment.
- Lack of normal tissue and tumour studies in vivo of the existence of HRS/IRR. This ought to be done using tumour xenografts or other animal experimental models.
- Lack of clinical evidence concerning normal tissue and tumour response related to the HRS/IRR.
- Validated mathematical models for HRS/IRR response. It is important to establish models in order to predict the potential outcome of various treatment plans, assuming different HRS/IRR features for various tissues.
- The impact of HRS/IRR on low-dose-rate response. How does HRS/IRR, following acute exposure to IR, reflect the response to decreasing dose rates, and in particular the low-dose-rate range in clinical use? An example is ^{125}I or ^{103}Pd used in radioactive seeds in the treatment of prostate carcinoma.

Future aspects and clinical potentials

It is a matter of some urgency that we identify critical individual components involved in HRS and IRR that can be targeted to prevent IRR selectively for tumour cells. It is

also worthwhile investigating whether the HRS feature can be utilized in the clinic to increase the therapeutic gain for radioresistant tumours by producing enhanced cell kill in using very many low-dose fractions (ultrafractionation). To proceed with this matter, we need clinical data to establish the dose response below 1 Gy for various acute and late-responding tissues.

The implications of HRS/IRR for cancer risk related to radiation therapy are an important issue to consider. The existence of HRS in the cell survival response implies that cancer risk from small, acute exposures to IR might be lower than the current estimates. The result of HRS is protection of the cell population from mutations and malignant transformation by eliminating those cells with DNA damage from the population.

Another important issue for radiotherapy treatment planning related to the HRS/IRR phenomenon is the fundamental difference in IMRT plans versus the standard conformal plans concerning irradiation volumes. This raises the question of whether it is better to give a small dose to a large volume or a large dose to a small volume (28, 29, 39). This knowledge is critical for the acute and late morbidity, including the risk of IR-induced malignancies (40, 41), in order to be able to select the optimal treatment plan for the individual patient.

BYSTANDER EFFECTS

Present status

It is possible that a cell/target that has not been irradiated (bystander) responds as if it had been irradiated. This phenomenon, mainly identified in vitro in various assays using different endpoints, is generally referred to as 'bystander' effects, but sometimes also as 'untargeted' effects or intercellular communication. The term 'bystander' has already been used in other research fields, and in gene therapy bystander effects are described as occurring when the gene product of a transfected DNA travels from the transfected cell into neighbouring cells. The response of cells to the bystander signal may include induction of apoptosis, genomic instability or delayed death, enhanced cell growth, DNA damage, chromosomal aberrations, altered gene expression or mutations (42).

The history of radiation-induced bystander effects starts in the 1950s and several indirect effects have been reported, e.g. plasma from irradiated patients caused chromosome breaks in normal unirradiated lymphocytes in short-term culture, and plasma from irradiated animals induced tumours when injected into unirradiated animals.

In more recent experiments in vitro, non-irradiated cells have been exposed to culture medium (filtered) taken from cells exposed to low-LET ionizing radiation. The effect seems to be relatively constant and appears to saturate already at doses in the range of 0.03–0.05 Gy when clonogenic survival was used as the endpoint. The effect is still present several hours after exposure to radiation. Furthermore, it seems likely that this phenomenon does not require cell–cell contacts. The effect is, however, highly dependent on both cell type and cell density.

Other investigations have utilized mainly charged-particle microbeams or low fluences of high-LET alpha particles. Localized irradiation of cytoplasm with alpha particles did not change survival rates but gave rise to mutations within the same cell. The mutations induced by cytoplasmic irradiation were of the same type as spontaneous mutations and totally distinct from those that arose from irradiation of the nucleus. The effects were modified by radical scavengers as DMSO, which indicates action by reactive oxygen species (43).

Irradiation of the cell nucleus with alpha particles was found to increase the stress response (e.g. $P21^{waf1}$ expression, induction of DNA damage and increased phosphorylation of p53) in neighbouring, unirradiated cells. The exact mechanisms for these effects are not fully understood but there are indications of a gap junction-mediated intercellular communication (44) (Fig. 3).

Critical issues

The most important critical issue is whether the bystander effect is relevant to radiation therapy in vivo. If present, several questions arise:

- What are the mechanisms involved?
- How cell-type dependent is the effect?
- How much does it depend upon radiation quality?
- What uncertainties does it introduce into the dosimetry?

Future aspects and potentials

Bystander effects may be highly relevant to radiation therapy and many questions remain unanswered. The research has been intensified in the past few years but ongoing studies are mainly performed on tissue explant cultures. In order to resolve the most critical issues, animal and human data are needed. The research could include well-designed animal studies. Clinical follow-up and analysis of past clinical documentation may yield valuable information.

Ongoing research has resulted in clear controversies and differences of opinion about the mechanisms involved. The observations also challenge the conventional idea that DNA is the main, although not the sole, target for radiation-induced cell death. This statement might still hold but the bystander effect suggests that initial DNA damage may, in addition, be produced by signals transmitted from neighbouring cells.

Future in vitro studies should try to glean details about bystander signals and responses in different cells systems.

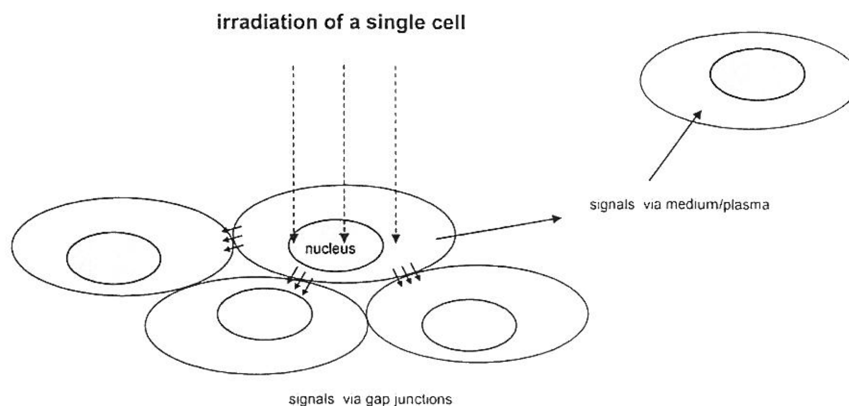

Fig. 3. Schematic illustration of bystander effects where an irradiated cell affects unirradiated cells. The signals could be transmitted by gap junctions in the cellular membranes but also mediated via culture medium or plasma.

Research tools such as low- and high-LET microbeams and new advances in gene and protein technology could be employed to understand the underlying mechanisms (which might be different for different biological endpoints) of bystander effects and possible influence of radiation quality.

Killing of bystander cells might be an advantage in radionuclide therapy and other situations where the dose distribution is heterogeneous. The increased cell killing may be modest or totally absent but it appears as if the effect is cell type dependent: in cultures with keratinocytes and some other cell lines, up to 50% of the cells were killed without being directly irradiated (45). A more critical implication of the bystander effect could be the induction of stable genomic changes within unirradiated cells, and these mutagenic properties, which might be specific for high-LET radiation, should be considered.

Much of the recent data are from high-LET radiation sources. Since this type of radiation induces highly complex and clustered damage, differences in results and controversies about the mechanisms might also depend on radiation quality.

Irrespective of the causes, if present, the bystander effect introduces uncertainty into the biological response. It may then be necessary to define a biological dose instead of a physical dose. It may actually be necessary to account for bystander effects when calculating dose to both normal tissue and tumour. The relative effects on normal and tumour tissue could differ and these differences may not always be predictable.

Finally, An important aspect for consideration is how the bystander effect may influence low-dose-rate situations, e.g. targeted therapy with radionuclides. If bystander effects are of significance, calculations and simulations of radiation doses to disseminated cell clusters should account for the biological dose and consider that a group of cells may respond as a single unit.

RADIATION BIOLOGY-BASED THERAPY PLANNING AND TREATMENT OPTIMIZATION

Present status

During past decades radiation therapy equipment has undergone significant development thus permitting advanced dose delivery to tumour volumes anywhere in the human body. The clinical problem of radiation therapy may therefore potentially shift from sheer dose planning to genuine treatment planning and treatment optimisation, taking the radiobiological properties of the tumour and normal tissues into account (29, 39, 46–48). To make radiation therapy curative with a high probability, the target volume has to be prescribed a sufficiently high radiation dose. However, the endpoint of highest relevance is the normal tissue complication probability (NTCP). Doses in radiotherapy are generally not limited by their effect on tumours but by their effects on adjacent normal tissues (49). Furthermore, targets frequently overlap with critical radiation-sensitive normal tissues, necessitating a local reduction of the dose delivery to ensure low risks of late adverse effects. Thus, to find the right balance between tumour cure (tumour control probability (TCP)) and the probability of NTCP, radiobiological models that as accurately as possible can quantify the response of heterogeneous tumours and normal tissues to non-uniform dose delivery are required for accurate optimization of the dose delivery and for prediction of treatment outcome. The complication-free cure (P_{+}, approximately TCP–NTCP) is an example of such modelling. With such models, we hope it will be possible to find the dose delivery that can maximize the complication-free cure.

Up to the present, models have been used mainly as research tools, to better understand dose-response relations and to describe, in numerical terms, the potential gains of, e.g. new fractionation schemes, new treatment techniques or

new methods and equipment. There is now an increasing demand for the use of models also as clinical routine tools. With appropriate modelling, it is possible to identify potentially dangerous treatment schedules before they are introduced, to improve the design of clinical trials and to avoid medico-legal issues.

The radiobiological models of different tumours and normal tissues are frequently described by the dose needed to deliver a specified effect in 50% of the patients (D_{50}) and the normalized slope of the dose-response relation (γ). When the dose-fractionation schedule is changed from the regular 2 Gy per day, a larger number of parameters are needed, such as the a, b, c and the α/β values characterizing the shoulder of the dose-response relation (50). To account for the response in greater detail, the tumour repopulation rate (δ), the volume dependence of the response of normal tissues as described by the relative seriality (s) and the repair and reoxygenation rate (μ, ρ) should also be considered (51). Recent radiobiological results also indicate a need to consider the hypersensitivity at low doses when large volumes of normal tissues receive multiple low doses (a, b, c or α_{sen}, D_{c}.) (50, 52). For the analyses of dose-response data, it may also be desirable to utilize some of the new dose-effect measures such as the effective uniform doses (D_{eff}) (53), the equivalent uniform dose (EUD) (54) and D (55).

In the first approximation, the optimal treatment outcome is obtained when the probability of curing the patient without inducing severe morbidity to normal tissues is as high as possible. This probability is well described by the equation: $P_+ = P_B - P_I + \delta(1 - P_B)P_I$. Here P_B is the probability of beneficial treatment—(tumour cure, i.e. TCP) whereas P_I (approx. NTCP) is the probability of inducing severe injury, and δ is the fraction of patients with a truly statistically independent response in the tumour and surrounding normal tissues. This fraction is often in the order of 0–20% and thus quite small. The complication-free cure is consequently often approximately equal to the probability of tumour cure minus the probability of severe injury. Based on the above equation, it is possible to intensity modulate the dose delivery, select angles of incidence of the beams, the radiation types and the fractionation scheduled such that P_+ is maximized.

It is also possible to perform the maximization with a number of constraining conditions whereby the dose or biological effect in certain tissues could be limited to acceptable values. The most advanced optimization strategy is probably first to maximize P_+ and then introduce a constraint so that the final P_+ should not be allowed to decrease more than about 0.3% from this maximum value, while the probability of inducing severe injury is minimized as far as possible. This so-called P_{++} strategy often allows a 3–5% reduction of the probability of severe injury while P_+ is only marginally reduced (0.3–0.5%).

An interesting mechanism presently being tested by modern radiobiologically based optimization algorithms is that the dose can be reduced in a small part of the tumour in order to minimize local complications in neighbouring sensitive normal structures, provided the dose can instead be increased in other parts of the tumour. The combined therapeutic effect is thus maintained and possibly even increased, at least in terms of the probability of achieving a complication-free cure (56). This is possible since a curative dose that causes 90% tumour control ($P_B \approx 90\%$) also causes, on average, a clonogenic tumour cell survival $\bar{N}$ of only 0.1 cell since, according to Poisson statistics, $P_B = e^{-\bar{N}} = e^{-0.1} \approx 0.9$. Hence, if the dose is allowed to increase by about 1 D_0 in, say, half the tumour, so that only 0.02 ($\approx$ 0.5·0.37) cells survive there, the dose in the other half may instead be allowed to decrease by almost 1 D_0, so that no more than 0.08 cells would survive there, and still fewer than 0.1 cells will survive, on average, over the whole tumour.

When the dose modifications are applied in even smaller volumes, obviously higher dose modifications are possible and significantly improved complication-free cure is possible by improving the distribution of the highest therapeutic dose level. In the first approximation, or more exactly if the dose variations are small, it is the mean dose to the target tissues that counts and determines the tumour cure (56). Since the mean tumour dose is an important quantity for the response, irrespective of the exact radiation sensitivity of the tumour, intensity-modulated beams can be applied quite generally to improve the treatment outcome for complex tumours (28, 29). It is even more important to take the properties of the normal tissues into account, since most tissues have a fairly parallel organization of their functional subunits and therefore might tolerate local hot spots without significant loss of tissue function (46, 57, 58).

Critical issues

- The accuracy of biologically based treatment optimization can never be better than the clinical data the models are based upon. Thus, the most critical issue is the relevance of the models and the parameter values in the models. To ensure this, it is important to carry out adequate long-term follow-ups in order to collect tumour-response data and acute and late normal tissue-response data, for the benefit of future patients.
- Besides this overriding critical issue, it is important to be able to locate and quantify the most radiation-resistant tumour cell compartment.
- It is also important to improve our understanding of the functional organization and relevance of functional subunits of normal tissues.
- To better quantify the clinical difference in normal tissue response between a low dose in a large volume and a high dose in a small volume, considering low-dose

hypersensitivity and normal tissue organization (serial/parallel).

Future aspects and potentials

Clinical data on the radiation effect on tumours and normal tissues have been collected at an increasing rate during the past few decades (51, 56, 57, 59–65). Still, there is currently insufficient clinical data to establish the model parameters or to enable us to choose between the models themselves and thus to quantify with confidence the clinical gains from one technique over another. This is true whether the comparisons are performed in an individual patient in clinical routine practice or in comparative exploratory studies. Since many parameter values are known with reasonable accuracy, it is usually safe to favour one technique or one dose distribution over another. Better possibilities to quantify the gains are required, however.

Identification of the most resistant tumour cell compartment may be fundamental for the responsiveness of the tumour even if that compartment is small (57, 66). The tumour may be dominated by well-oxygenated cells, but a small hypoxic compartment may dominate its radiation response at high doses. For modelling purposes, the simplest assumption is that oxygen is purely dose modifying, but several more advanced compartment models have been described (67, 68).

For advanced tumours with complex infiltrative growth, the improvement in complication-free cure by the biologically based P_+ and P_{++} optimization strategies can be calculated to be as high as 25–40%, largely irrespective of the dose-response model used (46). The main reason for using biological optimization, besides the fact that it may direct research to such increases in therapy outcomes, is that it allows a more efficient accumulation of accurate dose-response data, since the optimization ensures maximum use of the tolerance of the patient.

For normal tissues, the arrangement of functional subunits of a tissue is fundamental for its response to heterogeneous or partial irradiation (30, 31, 51, 57, 69). Continued development in this area is important. An important aspect when reporting the result of clinical trials of radiation therapy for various tumour sites is that the correlation and covariance of the responses of the tumour and of the surrounding normal tissues are carefully studied. The customary practice of reporting tumour response and complications separately is insufficient since knowledge about the correlations between these two endpoints is important for estimation of the probability of a truly positive outcome (66).

Opinions about the clinical use of models vary from the standpoint that they should not yet be applied, to those advocating rapid incorporation and extensive use. The former and more nihilistic opinion is that the main task at present is to use or create large prospectively collected clinical materials so that the models can become highly valid. Those advocating the models state that they are also tools with the potential to minimize the need to use randomized trials to suboptimal treatment schedules. It has been suggested that accurately collected response data can be used as a historical control arm against which all the therapy outcomes can be compared (56). Although the biological reality is complex, use of the biological knowledge we possess and recognizing the limitations will likely advance our knowledge much more rapidly than a nihilistic approach.

REFERENCES

1. Rich T, Allen RL, AH W. Defying death after DNA damage. Nature 2000; 407: 777–783.
2. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature 2000; 408: 307–10.
3. Tanya TT, Rogakou EP, Yamazaki V, et al. A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. Curr Biol 2000; 10: 886–95.
4. van Gent DC, Hoeimakers JHJ, Kanaar R. Chromosomal stability and the DNA double-stranded break connection. Nat Rev Genet 2001; 2: 196–206.
5. Brown JM, Wouters BG. Apoptosis, p53, and tumor cell sensitivity to anticancer agents. Cancer Res 1999; 59: 1391–9.
6. Steel GG. The case against apoptosis. Acta Oncol 2001; 40: 968–75.
7. Taccioli GE, Gottlieb TM, Blunt T, et al. Ku80: product of the XRCC5 gene and its role in DNA repair and V(D)J recombination. Science 1994; 265: 1442–5.
8. Stone HB, Dewey WC, Wallace SS, et al. Molecular biology to radiation oncology: a model for translational research? Opportunities in basic and translational research. Radiat Res 1998; 150: 134–47.
9. Smilenov LB, Brenner DJ, Hall EJ. Modest increased sensitivity to radiation oncogenesis in ATM heterozygous versus wild-type mammalian cells. Cancer Res 2001; 61: 5710–3.
10. Andreassen C, Alsner J, Overgaard J. Does variability in normal tissue reactions after radiotherapy have a genetic basis—where and how to look for it? Radiother Oncol 2002; 64: 131.
11. Reya T, Morrison SJ, Clarke MF, et al. Stem cells, cancer, and cancer stem cells. Nature 2001; 414: 105–11.
12. Kummermehr JC. Tumour stem cells—the evidence and the ambiguity. Acta Oncol 2001; 40: 981–8.
13. Bach SP, Renehan AG, Potten CS. Stem cells: the intestinal stem cell as a paradigm. Carcinogenesis 2000; 21: 469–76.
14. Shackney SE, Shankey TV. Cell cycle models for molecular biology and molecular oncology: exploring new dimensions. Cytometry 1999; 35: 97–116.
15. Wilson GD. A new look at proliferation. Acta Oncol 2001; 40: 989–94.
16. Turesson I, Bernefors R, Book M, et al. Normal tissue response to low doses of radiotherapy assessed by molecular markers. A study of skin in patients treated for prostate cancer. Acta Oncol 2001; 40: 941–51.
17. Begg AC, Haustermans K, Hart AA, et al. The value of pretreatment cell kinetic parameters as predictors for radiotherapy outcome in head and neck cancer: a multicenter analysis. Radiother Oncol 1999; 50: 13–23.
18. Corvo R, Paoli G, Giaretti W, et al. Evidence of cell kinetics as a predictive factor of response to radiotherapy alone or

chemoradiotherapy in patients with advanced head and neck cancer. Int J Radiat Oncol Biol Phys 2000; 47: 57–63.
19. Haustermans K, Fowler JF. Is there a future for cell kinetic measurements using IdUrd or BdUrd? Int J Radiat Oncol Biol Phys 2001; 49: 505–11.
20. Wilson GD. Tpot and head and neck cancer: where are we now? Anticancer Res 1998; 18: 4801–5.
21. Fowler J. Biological factors influencing optimum fractionation in radiation therapy. Acta Oncol 2001; 40: 712–7.
22. Brenner DJ, Martinez AA, Edmundson GK, et al. Direct evidence that prostate tumors show high sensitivity to fractionation (Low α/β ratio), similar to late-responding normal tissue. Int J Radiat Oncol Biol Phys 2002; 52: 6–13.
23. Joiner MC. Models of radiation cell killing. In: Steel GG, ed. Basic clinical radiobiology, 2nd ed. London: Arnold; 1997. p. 52–7.
24. Joiner MC, van der Kogel AJ. The linear-quadratic approach to fractionation and calculation of isoeffect relationships. In: Steel GG, ed. Basic clinical radiobiology, 2nd ed. London: Arnold; 1997. p. 106–21.
25. Brenner DJ, Hlatky LR, Hahnfeldt PJ, et al. The linear-quadratic model and most other common radiobiological models result in similar predictions of time-dose relationships. Radiat Res 1998; 150: 83–91.
26. Thames Jr HD, Withers HR, Peters LJ, et al. Changes in early and late radiation responses with altered dose fractionation: implications for dose survival relationships. Int J Radiat Oncol Biol Phys 1982; 8: 219–26.
27. Williams MV, Denekamp J, Fowler JF. A review of α/β ratio for experimental tumors: implications for clinical studies of altered fractionation. Int J Radiat Oncol Biol Phys 1985; 11: 87–96.
28. Intensity Modulated Radiation Therapy Collaborative Working Group. Intensity-modulated radiotherapy: current status and issues of interest. Int J Radiat Oncol Biol Phys 2001; 51: 880–914.
29. Tubiana M, Eschwege F. Conformal radiotherapy and intensity-modulated radiotherapy—clinical data. Acta Oncol 2000; 39: 555–67.
30. Schultheiss TE. The controversies and pitfalls in modeling normal tissue radiation injury/damage. Semin Radiat Oncol 2001; 11: 210–4.
31. Yorke ED. Modeling the effects of inhomogeneous dose distributions in normal tissues. Semin Radiat Oncol 2001; 11: 197–209.
32. Marples B, Joiner MC. The response of Chinese hamster V79 cells to low radiation doses: evidence of enhanced sensitivity of the whole cell population. Radiat Res 1993; 133: 41–51.
33. Marples B, Lambin P, Skov KA, et al. Low dose hyper-radiosensitivity and increased radioresistance in mammalian cells. Int J Radiat Biol 1997; 71: 721–35.
34. Joiner MC, Marples B, Lambin P, et al. Low-dose hypersensitivity: current status and possible mechanisms. Int J Radiat Oncol Biol Phys 2001; 49: 379–89.
35. Wouters BG, Sy AM, Skarsgard LD. Low-dose hypersensitivity and increased radioresistance in a panel of human tumor cell lines with different radiosensitivity. Radiat Res 1996; 146: 399–413.
36. Short SC, Kelly J, Mayes CR, et al. Low-dose hypersensitivity after fractionated low-dose irradiation in vitro. Int J Radiat Biol 2001; 77: 655–64.
37. Joiner MC, Denekamp J, Maughan RL. The use of 'top-up' experiments to investigate the effect of very small doses per fraction in mouse skin. Int J Radiat Biol 1986; 49: 565–80.
38. Joiner MC, Johns H. Renal damage in the mouse: the response to very small doses per fraction. Radiat Res 1988; 114: 385–98.
39. Withers HR. Biological aspects of conformal therapy. Acta Oncol 2000; 39: 569–77.
40. Brenner DJ, Curtis RE, Hall EJ, et al. Second malignancies in prostate carcinoma patients after radiotherapy compared with surgery. Cancer 2000; 88: 398–406.
41. Travis L. Therapy-associated secondary solid cancer. Acta Oncol 2002; 41: 323–33.
42. Mothersill C, Seymour C. Radiation-induced bystander effects: past history and future directions. Radiat Res 2001; 155: 759–67.
43. Wu LJ, Randers-Pehrson G, Xu A, et al. Targeted cytoplasmic irradiation with alpha particles induces mutations in mammalian cells. Proc Natl Acad Sci USA 1999; 96: 4959–64.
44. Azzam EI, de Toledo SM, Little JB. Direct evidence for the participation of gap junction-mediated intercellular communication in the transmission of damage signals from alpha-particle irradiated to nonirradiated cells. Proc Natl Acad Sci USA 2001; 98: 473–8.
45. Mothersill C, Seymour C. Medium from irradiated human epithelial cells but not human fibroblasts reduces the clonogenic survival of unirradiated cells. Int J Radiat Biol 1997; 71: 421–7.
46. Brahme A. Development of radiation therapy optimization. Acta Oncol 2000; 39: 579–95.
47. Brahme A, Nilsson J, Belkic D. Biologically optimized radiation therapy. Acta Oncol 2001; 40: 725–34.
48. Coleman CN. Radiation oncology—linking technology and biology in the treatment of cancer. Acta Oncol 2002; 41: 6–13.
49. Hall EJ. Do no harm—normal tissue effects. Acta Oncol 2001; 40: 913–6.
50. Lind BK, Persson LM, Edgren MR, et al. Repairable conditionally repairable damage model based on dual Poisson processes. Radiat Res 2003 (in press).
51. Ågren-Cronquist AK. Qualification of the response of heterogeneous tumours and organized tissues to fractionated radiotherapy. Stockholm University. Thesis; 1995.
52. Turesson I, Joiner MC. Clinical evidence of hypersensitivity to low doses in radiotherapy. Radiother Oncol 1996; 40: 1–3.
53. Brahme A, ed. Dosimetric precision requirements and quantities for characterizing the response of tumours and normal tissues. In: Radiation dose in radiotherapy from prescription to delivery. Rio de Janeiro: IAEA; 1996. p. 49–65.
54. Niemierko A. Reporting and analyzing dose distributions: a concept of equivalent uniform dose. Med Phys 1997; 24: 103–10.
55. Nordic Association of Clinical Physics (NACP). Specification of dose delivery in radiation therapy. Acta Oncol 1997; 36 (Suppl 10): 1–32.
56. Brahme A. Dosimetric precision requirements in radiation therapy. Acta Radiol Oncol 1984; 23: 379–91.
57. Källman P, Ågren A, Brahme A. Tumour and normal tissue responses to fractionated non-uniform dose delivery. Int J Rad Biol 1992; 62: 249–62.
58. Fowler JF. Biological factors influencing optimum fractionation in radiation therapy. Acta Oncol 2001; 40: 712–7.
59. Ågren-Cronquist A-K, Källman P, Turesson I, et al. Volume and heterogeneity dependence of dose-response relationship for head and neck tumours. Acta Oncol 1995; 34: 851–60.
60. Emami B, Lyman J, Brown A, et al. Tolerance of normal tissue to therapeutic irradiation. Int J Radiat Oncol Biol Phys 1991; 21: 109–22.
61. Rubin P. A direction for clinical radiation pathology. The tolerance dose. In: Vaeth JM, ed. Front Rad Ther Oncol. Basel: S Karger; 1972. p. 1–16.

62. Thames HD, Hendry JH, eds. Fractionation in radiotherapy. London: Taylor & Francis; 1987.
63. Wigg DR. Applied radiobiology and bioeffect planning. Madison Wis: Med Phys Publish; 2001.
64. Gagliardi G. Modeling heart and lung complication data in radiation therapy of the breast: Stockholm University: Thesis; 1998.
65. Gagliardi G, Bjohle J, Lax I, et al. Radiation pneumonitis after breast cancer irradiation: analysis of the complication probability using the relative seriality model. Int J Radiat Oncol Biol Phys 2000; 46: 373–81.
66. Brahme A, Ågren A. On the optimal dose distribution for eradication of heterogeneous tumours. Acta Oncol 1988; 26: 377–85.
67. Jones B, Dale RG. Mathematical models of tumour and normal tissue response. Acta Oncol 1999; 38: 883–93.
68. Olive PL, Banáth JP, Aquino-Parsons C. Measuring hyopoxia in solid tumours. Is there a gold standard? Acta Oncol 2001; 40: 917–23.
69. Withers HR, Taylor JMG, Maciejewski B. Treatment volume and tissue tolerance. Int J Radiat Oncol Biol Phys 1988; 104: 751–9.