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Review article

Of what use is molecular biology to the practicing radiation oncologist?[1]

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Abstract

Background and purpose: We are in a period of rapid advance in understanding the basic mechanisms behind the induction and progression of cancer. The relevance of this new knowledge to the daily clinical practice of radiation oncology may not necessarily be readily apparent. Familiarity with a few of the concepts of molecular biology and biochemistry are necessary to fully appreciate the clinical relevance of the new biology.

Methods and results: To illustrate how the new knowledge affects the practice of radiation oncology, examples of the use of molecular biology are presented for different clinical aspects of clinical oncology, i.e. screening and prevention, prognostic factors, predictive factors, treatment decision, novel therapy and follow-up. A number of the molecular biology techniques are illustrated.

Conclusions: The advances from molecular biology directly impact the role of radiation oncologists in the clinic. While major new therapies are still in development in the laboratory, these will likely have a very significant role in patient care and cancer prevention in the not-too-distant future. Given the central role of radiation oncologists in cancer management, a basic knowledge of molecular biology techniques and their application is essential so that we can be current with our colleagues and patients and as a specialty, participate actively in improving the outcome of patients with or at risk of developing cancer. © 1998 Elsevier Science Ireland Ltd.

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1. Introduction

The new knowledge of cancer biology that has become available from molecular and cellular biology research is profoundly affecting our understanding of the diseases treated by radiation oncologists. The information in this paper was presented at the 1996 ESTRO meeting and is an expansion of a recent overview in Seminars in Radiation Oncology [15] in an issue dedicated to 'Molecular Biology and its Clinical Implications' [37].

When responding to the question 'what does all of the new biology mean to the practicing radiation oncologist?', one could answer 'very little', as there is a very minor impact on the day-to-day delivery of radiation therapy, 'moderately', since patients are aware of the new 'breakthroughs' in the news media and will often query their physician, or 'quite a bit', as we are now able to understand cancer in an entirely different way.

While an increasing number of radiation oncologists have become familiar with the terms and concepts of the new biology the rapid explosion in knowledge is daunting and for those trained only a few years ago there are terms and concepts that are not familiar. The purpose of this paper is to present an overview as to how the new biology is relevant to the day-to-day practice of radiation oncology and to demonstrate that with a few general concepts in hand, the information is readily understandable.

2. Scope of radiation biology research

Fig. 1 is an updated schematic representation of what is now within the scope of radiation biology research [18]. The phenotypic changes in the cell following radiation exposure, such as cell cycle arrest, restoration of damaged DNA and cell death are being understood mechanistically. The importance of the cellular microenvironment is far more complicated than the classic effect of hypoxia on cell survival

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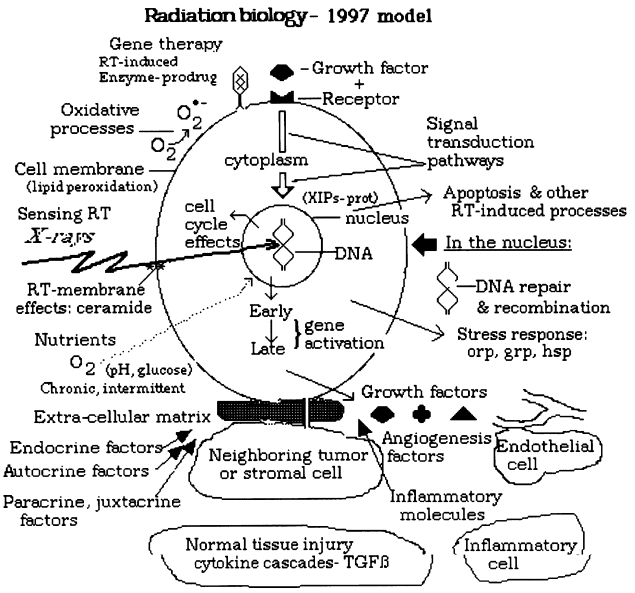

Fig. 1. Radiation biology research. The current areas of basic science investigation in radiation oncology and biology.

curves. The environmental effects on cellular phenotype include cell–cell interaction, cytokine and growth factor effects and oxidative stress, to name a few. For each of the processes in Fig. 1, there is extensive new knowledge as to the molecules and biochemical pathways involved. Radiation is but one of a number of stresses to which cells have had to adapt, therefore, there is some similarity between the cellular response to radiation and other stresses such as genotoxic agents, UV irradiation, hyperthermia and oxidative stress.

This paper will not review in detail the basic biology which has been discussed elsewhere [18,19,37]. It will indicate how some of the molecular biology techniques and concepts are now being directly applied to clinical radiation oncology. To make this clinically relevant, the clinical applicability will be presented using the steps along the way from carcinogenesis, through diagnosis to treatment and patient management. Table 1 [15] includes some of the steps and a few examples of each.

2.1. Screening and prevention

The linkage between a specific abnormal gene and the

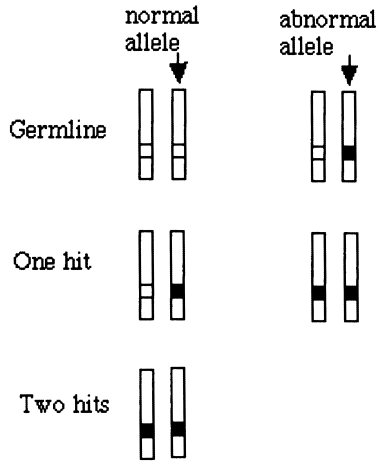

Fig. 2. The two-hit hypothesis. There are two types of mutations, dominant and recessive. A dominant mutation will produce its effect on the cell when there is a single mutation while a recessive mutation will be expressed only when both genes (alleles) are inactivated. The latter are referred to as tumor suppressor genes as the presence of one normal allele is sufficient to suppress the expression of the malignant phenotype. For a person born with two normal genes, both genes must be lost by mutation or deletion. For a person born with one abnormal gene, only one hit (mutation or deletion) is required. Such a person may have a cancer-family syndrome. An example of this is the Li-Fraumini syndrome where the p53 gene is mutated in one allele and only one additional mutation is necessary [54].

risk of developing cancer was first elucidated for retinoblastoma. It was through careful clinical observation that Knudsen [46] postulated the requirement for inactivation of both retinoblastoma genes well before molecular diagnostic techniques were available. The two-hit hypothesis is illustrated in Fig. 2.

Genes associated with common illnesses are now being described, excellent examples being BRCA-1 [20,49] and colon cancer associated genes [45,64]. Molecular biology techniques are used for determining who is a gene carrier and in defining the specific lesion. There are a number of components to a gene including the regulatory region, exons and introns. What is important for the cell is the gene product as this determines the actual function of the gene. To recall, the steps from DNA to function include DNA → transcription of mRNA → RNA processing within the nucleus → RNA transport to the ribosomes in the cytoplasm → translation of RNA into protein → post-translational modification of the protein. Post-translational modi-

Table 1

Examples of clinical applications of molecular and cellular biology

Screening and prevention	Prognostic factors	Predictive factors	Treatment decision	New therapy	Follow-up
Breast cancer, BRCA-1 Chemo-prevention retinoids	Proliferation rate, Ki-67 Metastatic potential Presence of metastases; PSA-PCR, or detecting translocation in lymphoma	Apoptosis genes, p53, bcl-2 Drug resistance phenotype MDR Hypoxia Proliferation rate	Use of chemo plus RT, ?topoisomerase inhibitors Pattern of bioreductive enzymes	New cytokines Novel sensitizers and protectors Gene therapy	Persistence of tumor, abnormal gene Complication, detecting MDS High risk, breast cancer

fication includes the addition by kinases or removal by phosphatases of a phosphate group. Kinases and phosphatases are critical in determining the function and structure of enzymes and proteins as the conformation of the protein is altered by the addition and subtraction of the phosphate group.

A gene is turned through its regulatory or promoter region when the proper proteins are attached and activated. This region is said to be upstream of the transcribed portion of the gene. RNA polymerase starts transcribing the DNA into mRNA. The part of the DNA that is transcribed is called the exon(s). Most genes have DNA sequences between the exons called introns. These are not transcribed but are important in regulation. Furthermore, there are parts of the DNA called tandem repeats [43] in which a two or three base sequence may be repeated many times. These are usually in silent portions of the DNA and do not effect the function of the gene product. Thus, the gene function is the same despite different sized genes, the size difference being due to the number of tandem repeats which will vary throughout the population.

Among the techniques to study DNA is gel electrophoresis. In a technique called Southern blotting, DNA is cut at specific sites by enzymes called restriction endonucleases and run on a gel under an electric current. DNA is separated based on the size of the fragment. Using DNA probes, a specific piece of DNA can be located on the gel, appearing as a band. This is illustrated in Fig. 3. Gel electrophoresis is also used to study RNA (Northern blot) and protein (Western blot). The lighter pieces of DNA will migrate further down the gel. A piece of DNA will migrate less far if it has something attached to it, like a transcription factor.

If there is a longer tandem repeat between the two cut sites at either end of the DNA fragment the piece of DNA

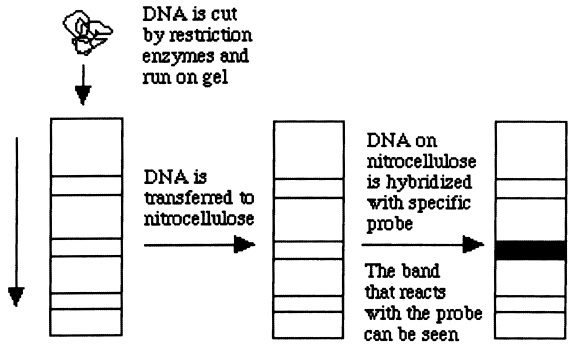

Fig. 3. Gel electrophoresis. Gel electrophoresis can be used to study DNA (Southern blot), RNA (Northern blot) or protein (Western blot). Illustrated is a Southern blot in which DNA is cut by restriction enzymes, run on a gel and then the DNA is transferred to nitrocellulose which is probed with DNA. The DNA probe will hybridize only with its match allowing one to identify the band which contains the piece of DNA of interest. This technique can also be used to examine whether or not a specific piece of DNA has been modified by having a protein attached to it as the added protein will slow down the migration of the piece of DNA. This is called an electrophoretic mobility shift assay (EMSA) or a gel shift assay (not illustrated).

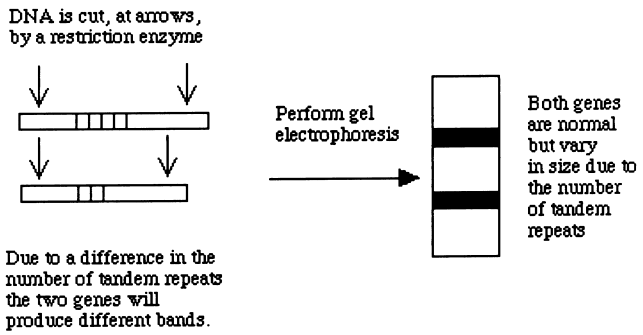

Fig. 4. Polymorphisms due to tandem repeats. There is variation in the size of normal genes due to the presence of tandem repeats. These alter the size of the DNA but not the function. When the number of tandem repeats differs, the two alleles of an individual will appear as two bands on a gel.

with the longer tandem repeat will migrate a shorter distance than the one with a smaller repeat, as shown in Fig. 4. A person may have two completely normal genes that have different lengths of a non-transcribed tandem repeat so that on gel electrophoresis, two bands will appear. This is called a polymorphism as the genes are both normal but migrate differently [42].

Fig. 5 illustrates how this information can be utilized. By looking at family members' DNA patterns, it can be seen which gene each member has. If one of the bands is known to contain an abnormal gene, then it can be ascertained which parent and which children have the abnormal gene. In this family, the mother has an abnormal gene that is passed on only to the second child.

Another use of gel electrophoresis is illustrated in Fig. 6. In this figure, the normal cells have two bands (heterozygous) but the tumor has only one. This indicates that one of the alleles has been lost; this is called loss of heterozygosity (LOH). A common means of tumor progression is the loss of heterozygosity of a tumor suppressor gene in a patient who was born with one mutated and one normal allele. Loss of the normal gene would allow the cell to lose a normal control mechanism.

By studying the molecular pattern of a tumor, one might

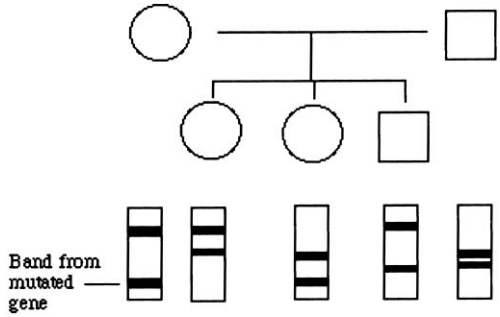

Fig. 5. Determining the presence of a certain gene in a family. Using the technique in Fig. 4, the pattern of each of the family members can be determined. If it is known which of the bands contains the abnormal allele, one can determine which family members are at risk. As indicated in this family tree, the second daughter carries the gene while the other children do not.

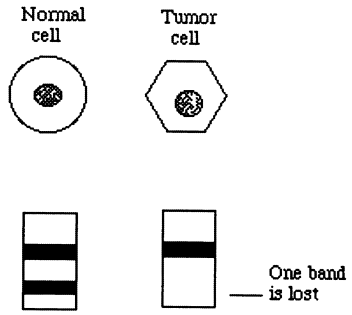

Fig. 6. Loss of heterozygosity. Using the two hit theory in Fig. 2, a normal cell may have two alleles, one of which is abnormal and the other normal. In this figure the lower band has a normal tumor suppressor gene while the upper band has a mutated suppressor gene that no longer functions. By mutation or deletion, the normal gene (lower band) is lost so that the tumor is no longer under control.

be able to tell if a tumor arises from a single clone or multiple clones. This is important for understanding pathogenesis of cancer and also in determining therapy as treatment might be much different for multicentric and unicentric cancers. Examples are the single clone of unilateral breast cancer [79] and bladder cancer [71] and bi-clonality of bilateral breast cancer [69].

Sophisticated techniques can be used to study the specific DNA lesion. There are probes that will only hybridize with a mutated gene, thus, a band will only be seen on a gel that has a specific mutation while no band will appear if there is not a mutation since the probe does not hybridize with the normal base sequence. It is possible to directly sequence the DNA either in the entire gene or in a certain portion. Automated techniques are making this easier to do but nonetheless this is an expensive undertaking. For some genes, certain mutations are more common (called 'hot spots' in the gene). At times, just a portion of the gene will be sequenced. As new cancer genes are discovered, a wide variety of mutations are found. Some mutations may be unimportant, therefore, simply finding a mutated gene does not mean that the person will have an increased risk of developing cancer.

Identifying disease-related genes raises a host of issues including societal dilemmas of confidentiality and insurability, personal dilemmas as to how one should live one's life with the knowledge of a susceptibility gene and therapeutic dilemmas, i.e. would the presence of such a gene make it dangerous to administer treatments that may be mutagenic, such as radiation and chemotherapy? Should a person undergo prophylactic removal of an organ at risk and does such a procedure eliminate or reduce the risk of developing cancer?

Cancer prevention is preferable to treatment. With the definition of specific genetic defects, it might be possible to target a corrective gene or to use a drug that diminishes the impact of the defective gene product. Current cancer prevention studies are less specific, often using agents such as retinoids or antioxidants [35,52]. As appealing as it seems to be able to utilize a simple vitamin (retinoids), studies of the basic mechanisms of chemoprevention at the molecular and cellular level will be necessary to understand the salutary effect and to develop relatively non-toxic chemoprevention agents. Chemoprevention will likely require long-term administration, therefore, the safety profile of the agents must be very strict. Of relevance to radiation oncologists is chemoprevention of metachronous cancers in the head and neck region [7,21] and secondary malignancies for long-term survivors of childhood cancer [56] and Hodgkin's disease [80].

2.2. Prognostic factors

Prognostic factors are useful in estimating the expected behavior or natural history of a tumor. Examples of commonly used prognostic factors include anatomic stage (TNM) for solid tumors, number or involved sites and bulk of disease for Hodgkin's disease and the non-Hodgkin's lymphomas [6] and abnormal blood chemistry, such as prostate specific antigen in prostate cancer [61] or LDH in lymphatic and hematologic malignancies [77]. These factors are usually determined by a retrospective review of a patient treatment series and might not necessarily be valid when tested prospectively.

There are efforts to develop molecular prognostic factors that will help determine a tumor's malignant potential. In an ideal world, each tumor could be individually characterized with the appropriate treatment then given. Examples of molecular prognostic factors include n-myc amplification or TRK gene expression in the primary tumor of patients with neuroblastoma [12,59], the use of a marker chromosome to detect circulating tumor cells in follicular lymphoma [62] and markers of tumor proliferation, such as Ki-67 [13]. The overall treatment plan may depend to some extent on the prognostic factors. For example, if a patient is determined to be at high risk for metastatic disease, systemic disease would be part of the initial treatment. Determining the risk of metastases, which is now based on clinical staging, may ultimately be based on the presence of specific invasion and metastases genes within the tumor.

An example of a molecular prognostic factor for metastases might be the presence of a circulating cell with a chromosome translocation. Follicular lymphomas have a characteristic 14:18 translocation [81]. Circulating cells can be collected and using a process called polymerase chain reaction (PCR), a specific piece of DNA can be amplified many-fold [26]. Logically, the presence of a translocation would lead to the conclusion that there are still some malignant cells present, even though a patient may be in a clinical complete remission. A few studies have been reported indicating that circulating cells can be detected in some patients with follicular lymphoma in clinical remission [28,62]. However, not all cells with a translocation may be malignant. For example, one recent study has shown that this 14:18 translocation is seen in the peripheral blood of patients with Whipple's disease which disappears when the disease is treated with antibiotics [27]. A second paper using

very sensitive techniques found this translocation present in normal blood donors [22]. It is possible that as they differentiate normal lymphocytes may produce a gene rearrangement that includes the 14:18 translocation and that this by itself is not necessarily an indication of malignancy. This is an example of how molecular biologic approaches must be interpreted with some caution.

2.3. Predictive factors

Unlike prognostic factors which are useful for estimating the overall course of the patient's illness, predictive factors are useful in determining a mode of therapy for an individual patient based on their likelihood of benefiting from the therapy. Prognostic factors may also be useful as predictive factors. For example, proliferation rate may be a good estimate of overall prognosis but may also dictate an appropriate radiation fractionation scheme or the need to use antiproliferative drugs in conjunction with radiation [4,5]. Tumors can become resistant to chemotherapeutic agents through a number of mechanisms, the most studied being the multi-drug resistance phenotypes (mdrs) [8]. Tumors with a high level of expression of P-glycoprotein [83] might be treated with drugs that are not affected by this cellular phenotype. While there is some potential overlap between prognostic and predictive factors, one must be careful in how they are used when applying therapies to individual patients [48].

Among the more interesting molecules that may be important in selecting therapy is p53. Cells with aberrant p53 have a number of abnormal phenotypes including the loss of the G_1 cell cycle checkpoint and a diminished ability to undergo apoptosis [70,84] after ionizing irradiation. Does the ability to undergo apoptosis determine treatment outcome? Maybe [58]. Is the measure of p53 by itself sufficient to predict the presence or absence of apoptosis? Probably not. For example, there are two recent papers investigating the role of mutant p53 on treatment outcome for patients with head and neck cancer which demonstrate opposite results, i.e. one shows the mutant p53 to be an adverse prognostic factor [47] and the other shows the mutant p53 to be a favorable prognostic factor [32]. Can a cell with abnormal p53 be made to undergo apoptosis by a p53 independent mechanism? Yes and that would be a great therapeutic strategy [78]. Can a normal p53 protein function abnormally in a disturbed microenvironment? Possibly if the biochemical state of the microenvironment (redox state) alters the conformation of a normal protein [36]. Clearly, the role of any one specific gene in predicting treatment outcome or selecting therapy is complex so that studying the effect of one gene at a time may give conflicting information. Therefore, the genotype of the cell, for example, mutant or wildtype p53, may not be predictive of the phenotype, for example, the response to radiation [11].

Apoptosis is the process by which a cell that is no longer needed undergoes a suicide process [24,44,75]. The DNA is degraded and the cell is broken into fragments that are ingested by the neighboring cells or inflammatory cells. This is a physiological process and not the same as necrosis. In apoptosis, enzymes called nucleases digest the DNA and proteases digest the protein. Logically, a cell will carefully regulate this suicide process. Among the molecules involved is bcl2 [33], which is an anti-apoptosis gene seen in certain lymphomas. Studying the presence of absence of bcl2 by itself is not sufficient to predict whether or not a cell will undergo apoptosis. There are other molecules that interact with bcl2 and that enhance or inhibit apoptosis (e.g. bax, bcl-X_L, bcl-X_S, Bad, BAG-1 and Bak) [14,25,30,33,66,76] and there is careful control of the activation of proteases, the enzymes which degrade cellular protein [53,60].

The role of hypoxia in tumor progression and treatment outcome still remains to be defined. In addition to causing radiation resistance [16], hypoxia can dramatically alter cellular phenotype and may be important in tumor progression [34]. Oxygen electrode techniques have demonstrated the presence of hypoxia in human tumors, but in only about half of the tumors studied [16,41]. As easier methods become available, the detection of hypoxia either before or during a course of radiation therapy may be useful in determining which patients should receive anti-hypoxic therapy [16,63].

2.4. Treatment decision

That molecular and cellular studies may be useful in treatment selection is exemplified by hypoxia. The presence of hypoxia itself, while indicating a general therapeutic strategy, does not indicate which of the anti-hypoxic therapies would be best. There is currently great interest in the use of bioreductive drugs, such as tirapazamine and EO9 [82], which are enzymatically activated within the tumor. Preferential activation in the tumor compared to normal tissue will depend on the presence of hypoxia and on the particular enzyme profile that would activate the drug [1,82]. Thus, knowing the cellular biochemistry in addition to its microenvironment may ultimately allow for the decision as to whether or not a patient should receive a certain treatment or for the selection of a drug that is specific for a patient's tumor.

Another example of a new area of interest is the use of inhibitors of topoisomerase-I in conjunction with radiation therapy [10]. Logically, such an approach would be useful with tumors that express adequate levels of the enzyme. Although topoisomerase-I appears to be constitutively expressed and not vary with cell cycle distribution [72], this might not be the case for other target enzymes which might only be transiently expressed. A drug that selectively acts in S-phase would not be of great use in a tumor that has few cells in S-phase or it might be necessary to administer such a drug more frequently to catch cells as they enter S-phase. Furthermore, radiation itself might reduce the level of the target enzyme, as has been described with topoisomerase-I [9]. Fig. 7 includes some of the potential means of

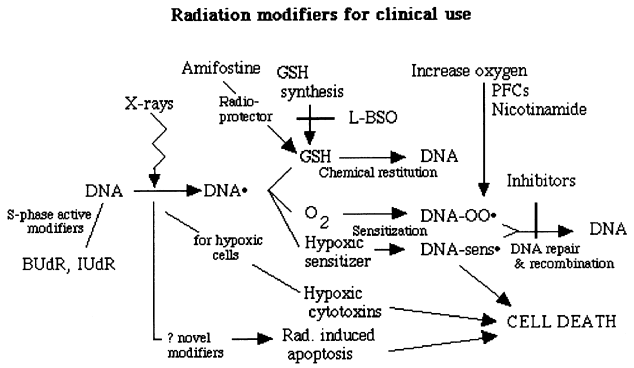

Fig. 7. Current approaches to radiation modification. The current clinical approaches to radiation modification are illustrated. These include the halopyrimidines, oxygen mimetic drugs, bioreductive drugs, some chemotherapeutic agents, agents to modify glutathione and radioprotectors (from Ref. [18]). Knowledge of the tumor's biochemistry and physiologic state would be useful in selecting the treatment for the specific tumor. DNA·, DNA radical created by ionizing radiation; DNA-OO·, peroxide DNA radical; DNA-sen·, DNA sensitizer radical (where sensitizer is an oxygen mimetic sensitizer); GSH, glutathione; L-BSO, buthionine sulfoximine, an inhibitor of de novo synthesis of GSH; BUdR, IUdR, halopyrimidines bromo- and iodo-deoxyuridine; PFC, perfluorochemicals.

modifying radiation therapy now in the clinic. Being able to measure particular molecular and biochemical processes may allow the clinician to select the drug to use and the proper timing of drug administration.

When determining the radiation dose to use, normal tissue tolerance is the limiting factor for the treatment. Usually, radiation oncologists select a dose that will produce a $<5\%$ incidence of serious late toxicity. The question has been raised as to whether there is a subpopulation of patients who have an increased radiosensitivity, perhaps by virtue of a defect in response to radiation as seen in ataxia-telangiectasia [23,57], severe-combined immunodeficiency (SCID) [73], or some other repair gene. It might be that the AT heterozygotes have a slight increase in sensitivity so that knowing this, it might be possible to design radiation therapy based on knowledge of normal tissue radiobiology. The sensitive patients would receive a reduced dose while the 'normal' patients might receive more treatment. Furthermore, the pathogenesis of late effects may be due to a cytokine cascade [2,65] and it might be possible to interrupt the development of late normal tissue injury at some point in time following the radiation. This may make it possible to intervene with the late effect process following a course of radiation therapy so that one might selectively effect normal tissue injury and not the tumor kill. While both of these normal tissue injury concepts are somewhat hypothetical, they may present unique opportunities for therapeutic intervention.

2.5. New therapy

The radiation modifiers currently in clinical use [18] have been developed based on the more classical radiation biology models such as hypoxia (radiation sensitizers and enhancers, altered oxygen delivery), the competition model (thiol depletion and radioprotectors) and increasing susceptibility of DNA to radiation damage (halopyrimidines). Recently, entirely novel classes of compounds have been shown to alter the radiation response, including cytokines, such as IL-1 [68] and growth factors, such as bFGF and TNF [29,38]. More speculative but very interesting is the use of radiation-induced gene therapy [39] which has some of the generic limitations of gene therapy and gene delivery [31,40,45].

Fig. 8 illustrates the complexity involved in bringing a concept to a beneficial clinical result. New treatments such as gene therapy have exciting promise yet expectations must be realistic and patience is necessary as the complex steps are resolved [55].

Another example of a new form of treatment is the use of anti-sense oligonucleotides [3]. These are generally small pieces of DNA (oligonucleotides) that are made to interact with mRNA and prevent the mRNA from being translated into protein. In essence, the gene product of a gene of interest is not produced due to the presence of the antisense molecule. Due to the inherent instability of the oligonucleotides, molecules called phosphorothioates may be used that substitute sulfur for oxygen in the backbone of the molecule

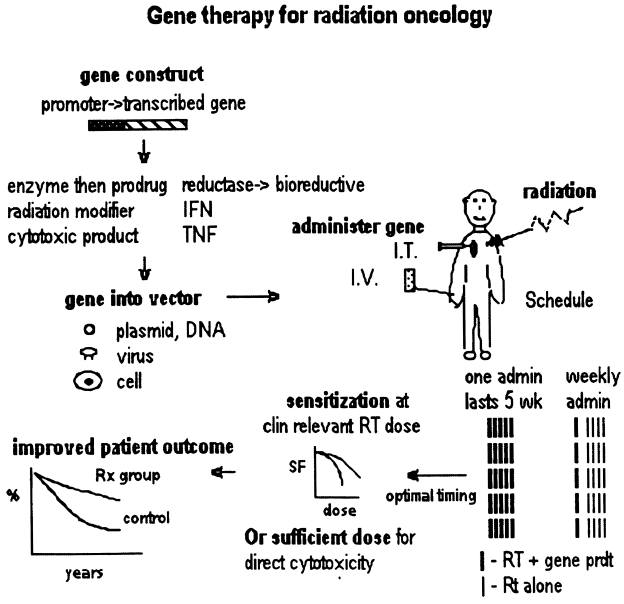

Fig. 8. Steps in radiation-induced gene therapy. A radiation-inducible promoter is attached to a gene the product of which is of therapeutic use. This might be to produce a protein that is toxic to the cell, an enzyme that can activate a prodrug, a protein that replaces a normal pathway such as apoptosis or a protein that stimulates the immune response. This gene must be placed in a vector by which it can get into the target cell. The vector must then reach the tumor, possibly through intratumoral injection or by systemic administration. The gene product must reach all or enough of the cells and must be present for an appropriate length of time. This may mean it must be there every day or just on some of the treatment days as might be true for a very effective cytotoxic agent. The enhancement ratio produced by the gene product must be large enough to demonstrate a therapeutic effect in the tumor and finally this effect must have a beneficial outcome to the patient in a clinical trial.

[51]. While such molecules may show a therapeutic effect in animals, the mechanism may not necessarily be that of blocking the mRNA. An example is a recent study using phosphorothioate antisense to c-myc in which the effect may have been due to direct interaction of the antisense molecules outside of the cell with extracellular growth factors and not by acting as an intracellular antisense molecule [51,74].

2.6. Follow-up

Molecular biologic approaches may be useful in evaluating the efficacy of treatment and in long-term follow-up. The persistence of a cell population with an abnormal gene may be a useful surrogate for the ability of the treatment to eradicate the cancer [50], with the precautions as noted above in the discussion of the 14:18 translocation. Some treatment regimens may cause a myelodysplastic syndrome [74] and early detection of a malignant clone may allow for the use of a chemoprevention agent or an aggressive treatment approach using bone marrow transplantation to replace the damaged stem cells.

The intensity of follow-up may depend on the patient's genetic composition. Patients with a cancer susceptibility gene might require more intensive long-term follow-up than those with a sporadic cancer.

3. Conclusion

For radiation oncology to remain a vigorous component in cancer care, as a specialty we must remain current. This requires that all of our residents are adequately trained in the basic science terminology and concepts and that we in both university and private practice not only support basic research but make an effort to remain conversant with the newer concepts and terms [17]. It is an aim of this paper to show that it is not necessary to know all the details of cell and molecular biology, but it is important to appreciate the general role that it may have in furthering our knowledge of cancer induction, progression and treatment. Review articles, refresher courses and national and international 'schools' of molecular biology are among the mechanisms available to help the busy practitioner remain reasonably up-to-date.

The author's answer to the question 'what does all of this mean to the practicing radiation oncologist?' is as follows. If the basic molecular and cellular mechanisms are relevant to the cancer cell, how can they not be relevant to any practicing oncologist? Our ability to prevent, diagnose and treat cancer will likely change dramatically over the next few decades. To date, much of our research has focused on going from cellular phenotype to genotype, that is, trying to understand what genes are causing what effect. As the Human Genome Project moves toward completion [67], based on both the knowledge of the DNA sequence and the techniques developed to study the DNA, we will soon be able to determine the entire genotype of a cancer cell. Rather than just studying a single aspect of each cell and hoping that it is the important one, we will be able to assess many processes at the same time. The challenge will then be to understand what functions are abnormal and how they interact. While there are many complexities to be worked out, it is imperative that radiation oncologists follow the big picture so that, as a specialty, we are able to appropriately blend our technological expertise with emerging biological knowledge.

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