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Overview

Molecular Biology for the Radiation Oncologist: the 5Rs of Radiobiology meet the Hallmarks of Cancer

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ABSTRACT:
Recent advances in our understanding of the biology of cancer have provided enormous opportunities for the development of novel therapies against specific molecular targets. It is likely that most of these targeted therapies will have only modest single agent activities but may have the potential to accentuate the therapeutic effects of ionising radiation. In this introductory review, the 5Rs of classical radiobiology are interpreted in terms of their relationship to the hallmarks of cancer. Future articles will focus on the specific hallmarks of cancer and will highlight the opportunities that exist for designing new combination treatment regimens. Harrington, K. *et al*. (2007). *Clinical Oncology* 19, 561–571

Key words: Hallmarks of cancer, molecular biology, radiobiology, targeted therapy

Introduction

It is an extremely exciting time to be a clinical oncologist. We work in a specialty that has undergone seismic shifts in routine practice in the last decade. These include: (i) the application of technological advances in radiation delivery; (ii) the demonstration of the superiority of chemoradiotherapy over radiotherapy alone for a range of tumour types; and (iii) the development of novel targeted therapies for integration within standard combination strategies. Indeed, it is not an exaggeration to claim that clinical oncology is currently in the middle of a renaissance and at its heart is the realisation that combinations of start-of-the-art radiotherapy, chemotherapy and new targeted drugs will probably yield significant therapeutic advantages to a large number of patients in the foreseeable future.

After decades of stagnation, technological developments have brought three-dimensional conformal radiotherapy and intensity-modulated radiotherapy within the reach of most departments [1]. In fact, with a few exceptions, the pace of introduction of the new technologies has outstripped our ability (or willingness) to conduct carefully controlled randomised clinical trials comparing them with conventional radiotherapy. In addition, the use of particulate radiation (protons, carbon ions) is receiving renewed attention and large collaborative projects have been established in Europe and the USA [2,3]. The next 20 years will probably require significant research effort by clinical oncologists as they focus on implementing the new technologies for radiation delivery.

More recently, after much previous debate and controversy, meta-analyses have clearly shown the clinical benefit of adding concomitant cytotoxic chemotherapy to radiotherapy in a number of tumour types in both radical and adjuvant postoperative settings [4–9]. As a consequence, concomitant chemoradiotherapy has become the standard of care for many tumour types. This change in practice has brought with it new problems, including the selection of appropriate patients for chemoradiotherapy and the management of the increased acute (and possibly late) toxicity of chemoradiotherapy [10,11].

While these changes in clinical practice have been taking place, we have witnessed fundamental changes in our understanding of the biology of cancer and, as a consequence, we are just beginning to reap the rewards of this research in the form of novel targeted agents. For example, a recent phase III randomised study of radiation with or without a targeted monoclonal antibody (cetuximab) in patients with head and neck cancer showed a very significant advantage for the combined regimen [12] and this agent is now undergoing evaluation in randomised studies with chemoradiotherapy [13]. Undoubtedly, the next decade will see a wide range of new targeted drugs coming to the clinic for use alongside standard chemoradiotherapy regimens. Indeed, it is not inconceivable that in due course some of these agents may replace cytotoxic chemotherapy in combination strategies.

Therefore, in addition to possessing expertise with the new technologies, clinical oncologists will be expected to conduct and assess trials of novel targeted agents in

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combination with (chemo)radiotherapy. It is of paramount importance that the specialty embraces this challenge in order to ensure that the direction of clinical studies is informed by sound radiobiological principles, such that the focus is on maximising the effect of the most important component of the treatment (i.e. radiation). Failure to rise to this challenge means that clinical oncologists will take a passive role in the development of new strategies and will run the risk of being relegated to the role of radiation technicians.

In this series of review articles, we will discuss the key advances in the molecular biology of cancer as they relate specifically to the practice of clinical oncology. In this introduction we will briefly describe the key features or 'hallmarks' of cancer [14] as a prelude to subsequent articles that will explore the potential effect of each of these processes on the field of clinical oncology. We will also attempt to show how information derived from studies of the molecular biology of cancer can be used to breathe new life into the 5Rs of radiobiology and make them relevant to the new generation of radiation oncologists.

Radiobiological Determinants of Treatment Outcome

Radiotherapy is an extremely effective treatment for cancer, especially when the disease presents at an early stage. However, despite its undoubted activity, localised radiotherapy (with or without cytotoxic chemotherapy) frequently fails to eradicate all of the clonogenic cells within a cancer and the tangible reality of this failure is a local or regional recurrence of disease. Alternatively, radiotherapy (with or without cytotoxic chemotherapy) may be used as an intensive local therapy for a disease that has already slipped the leash and spread to distant sites, with the inevitable consequence of disease recurrence outside the radiation portals.

The 4Rs of radiobiology were initially described in an attempt to provide a means of understanding the success or failure of localised radiotherapy [15]. The differential repair of tumour and normal cells between treatment fractions, the redistribution of cells into more or less radiosensitive phases of the cell cycle, the repopulation of tumour cells between fractions and the re-oxygenation of tumour cells during treatment were all invoked to explain the net outcome of radiotherapy. Later, the system was revised to include intrinsic radiosensitivity in the 5Rs of radiobiology [16]. With a few exceptions, this final addition to the quintet was an admission of our inability to explain at the mechanistic level the different radioresponsiveness of diseases like seminoma, lymphoma, glioma and melanoma.

Nonetheless, the 5Rs have served an extremely important function in providing a framework within which to examine new therapeutic strategies from the point of view of both tumour and normal cells. Each of the Rs can be viewed as a double-edged sword such that changes can occur in either direction to increase or decrease the net therapeutic effect. For example, if a tumour cell has acquired a defect in its DNA repair pathway, it is more likely than an adjacent normal cell to be killed by a dose of radiation [17—19]. However, the abnormal DNA repair pathway may already have allowed the tumour cell to accumulate non-lethal mutations in other important genes that allow it to tolerate unrepaired DNA damage (or to repair it in an inaccurate manner that only serves to enhance genetic instability). Similarly, the enhanced tumour cell division that occurs during a course of radiotherapy is generally viewed negatively as the driving force behind accelerated repopulation, but it may also make a tumour cell more susceptible to radiation-induced death by causing it to enter mitosis with unrepaired DNA damage (so-called mitotic catastrophe).

Our new insights into the molecular biology of cancer have now put us in a position to reinterpret the classical 5Rs of radiobiology in terms of their underlying mechanisms. As we shall see below, a direct one-to-one translation of each of the Rs of radiobiology into a single biological mechanism is not possible. For example, DNA damage repair can be influenced by growth factor receptor autonomy and evasion of apoptosis. However, the particular strength of describing cancer in terms of its molecular biological hallmarks is that it leads naturally into a discussion of potential new targeted therapies that may favourably modulate the tumour response and increase the therapeutic index [20].

The Molecular Biological Hallmarks of Cancer

There is ample evidence to support the hypothesis that human tumours arise as part of a sequential multi-step process, with each step reflecting the accumulation of genetic alterations that confer a survival advantage on the evolving malignant cell population [21—24]. Hanahan and

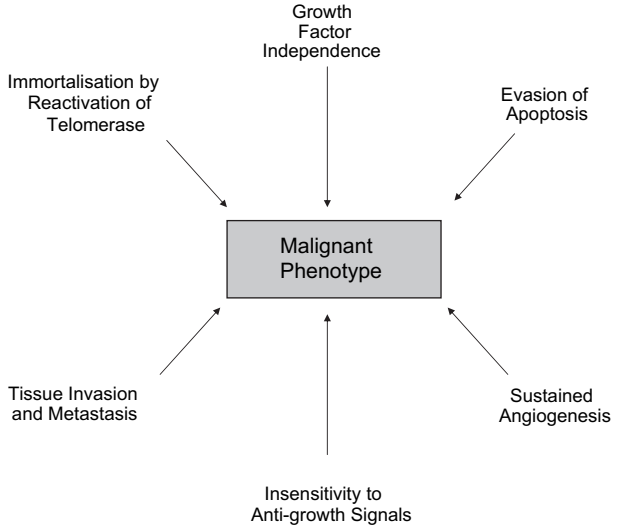

Fig. 1 — The six hallmarks of cancer.

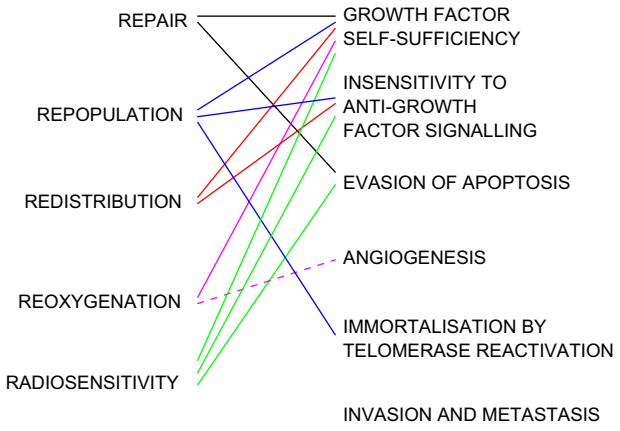

Fig. 2 — Potential relationships between the 5Rs of radiobiology and the hallmarks of cancer.

Weinberg [14] provided a useful framework for thinking about the steps that play a key part in the development, progression, spread and response to treatment of most cancers (Fig. 1). From analyses of the evolution of tumours through their pre-malignant precursors to their frankly malignant metastatic manifestations, two things have become clear: (i) single genetic abnormalities are rarely sufficient to cause cancer; and (ii) the sequence in which multiple abnormalities accumulate is not necessarily important. Nonetheless, each of the steps in the process of malignant transformation represents an opportunity for therapeutic intervention and many of them have specific relevance to the practice of radiation oncology. As we shall see, many of the hallmarks of cancer can be invoked (singly or in combination) to explain the fundamental observations enshrined in the 5Rs of classical radiobiology (Fig. 2).

Self-sufficiency in Growth Factors

A general scheme for the role of growth factor receptors and their ligands in promoting cell growth (and other functions) is shown in Fig. 3. Binding of a growth factor (the cognate ligand) to its specific ligand-binding domain on the extracellular component of the receptor leads to a signal being passed from the membrane to the nucleus via a cascade of intermediary messengers such that the binding of a protein on the cell surface is able to influence the behaviour of the cell [25,26].

Under normal circumstances, the activation of growth factor receptors is very tightly controlled — as is the synthesis and release of the ligands that stimulate them. Cancer cells frequently usurp normal signalling through growth factor receptors and use this to promote unrestrained cell division. Cancer cells exploit three main strategies for achieving autonomy in growth factors: (i) they manufacture and release their own growth factors that are able to stimulate their own receptors (autocrine signalling) and those of their immediate neighbours (paracrine signalling) [27,28]; (ii) they alter the number, structure or function of the growth factor receptors on their surface such that they are more likely to send a growth signal to the nucleus (even in the absence of the cognate ligand) [29,30]; (iii) they deregulate the growth signalling pathway downstream of the growth factor receptor such

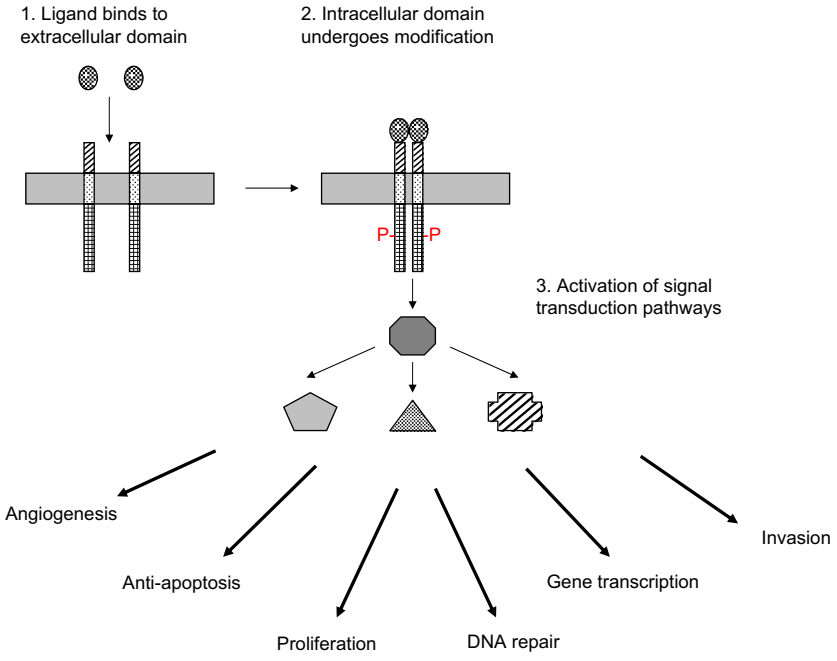

Fig. 3 — Simplified diagram illustrating activation of a tyrosine kinase growth factor receptor. Binding of ligand to the extracellular domain of the receptor leads to dimerisation, phosphorylation of the intracellular domain and signal transduction through second messengers that lead to phenotypic changes.

that the pathway is permanently turned on (constitutively active) [31].

An increased appreciation of the molecular mechanisms underlying autonomy in growth factor signalling significantly affects our understanding of a number of the 5Rs. Growth factor receptor signalling is up-regulated in irradiated cells and plays a key role in repopulation, DNA repair and intrinsic radiosensitivity (through interactions with the apoptotic machinery; see below) [32]. In addition, growth factor receptor signalling is involved in promoting angiogenesis, which may affect tumour re-oxygenation [33]. Therefore, there is a sound radiobiological basis for developing agents that target growth factor receptor pathways and combining these with radiation.

Evasion of Apoptosis

Normal cells are permanently held in a state in which their continued existence depends on a very tight balance between survival and death signals. In a normal cell, the accumulation of DNA damage leads to the arrest of cell growth (cell cycle arrest) while the potential for repair is assessed. If the extent of the damage exceeds the capacity to repair it without leaving residual genetic abnormality, the balance of survival and death signals tips and the cell activates its apoptotic signalling pathway and commits suicide [34—37]. This prevents the maintenance of DNA damage and avoids the risk that mutations could be passed to future progeny. This mechanism represents a very powerful barrier to the development of cancer. Therefore, it should come as no surprise that the loss of normal apoptotic pathway signalling is an extremely common event in cancers. Indeed, two of the best known cancer-associated genes (p53 and bcl-2) are intimately involved in the apoptotic process [38,39]. The two main mechanisms of apoptotic signalling (intrinsic and extrinsic pathways) are illustrated in a highly simplified form in Fig. 4. Cancer cells are able to evade apoptosis through an ability to ignore signals sent through the extrinsic pathway or by re-setting the balance of intracellular pro- and anti-apoptotic molecules in favour of the inhibition of apoptosis.

By circumventing apoptosis, cancer cells are able to sustain DNA damage without it causing cell death (unless the damage is to a gene that is absolutely necessary for cell survival) [40]. Therefore, cancer cells that have switched off their apoptotic pathway are more likely to be intrinsically resistant to radiation and/or chemotherapy. In fact, the use of these treatments may promote the accumulation of other mutations that may have a negative influence on the biology of the disease. In addition, the failure to trigger apoptosis in response to DNA damage may also allow cancer cells a greater period of time to attempt DNA repair. Dissection of the changes that occur in the apoptotic signalling pathways in cancers has resulted in the production of drugs that are able to either reactivate death signals or inactivate survival signals [41]. A number of agents are in clinical trials and these will probably have direct relevance in combination with radiation and/or chemotherapy [42—52].

Sustained Angiogenesis

In normal tissues, the growth of new blood vessels (angiogenesis) is held very tightly in check by a balance between positive (pro-angiogenic) and negative (anti-angiogenic) signals [28,29,53]. Aberrations in new blood

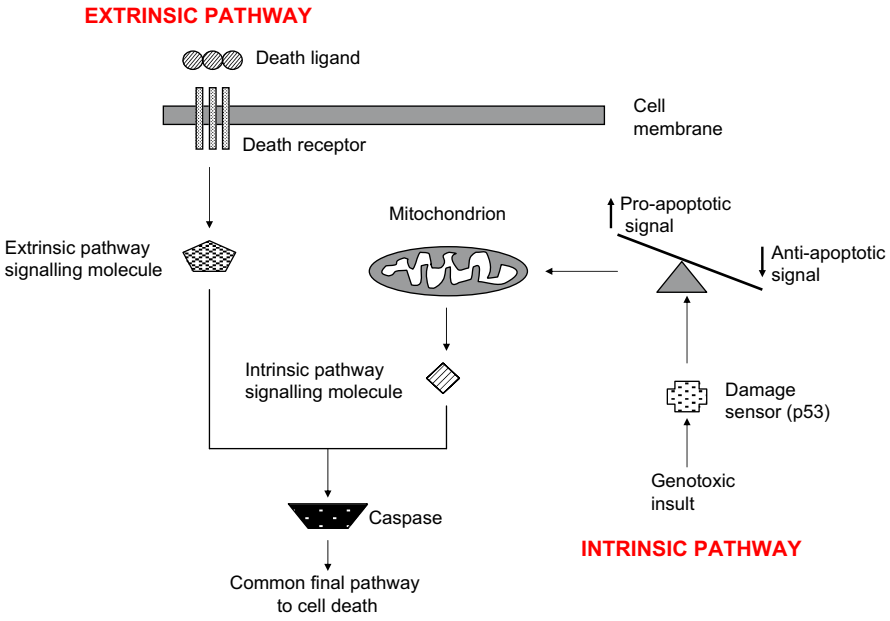

Fig. 4 — Extrinsic and intrinsic apoptotic pathways. Activation of the intrinsic pathway occurs if there is an alteration in the balance between pro- and anti-apoptotic signals and is mediated at the level of the mitochondrion. Activation of the extrinsic pathway occurs after binding of specific death ligands to death receptors.

vessel growth are associated with non-malignant conditions, including proliferative diabetic retinopathy, but they have been increasingly recognised as being of fundamental importance to the formation, progression and spread of cancers [54,55]. The growth of cancer deposits is intimately related to their ability to secure a blood supply. A small cluster of cancer cells can grow to 60–100 μm by deriving a supply of oxygen and nutrients by direct diffusion, but beyond this size the fledgling tumour must have a dedicated blood supply of its own. Cancers acquire the ability to grow a new blood supply by subverting the balance between pro- and anti-angiogenic factors. Essentially, cancers switch to an 'angiogenic phenotype' by up-regulating the production of pro-angiogenic proteins such as vascular endothelial growth factor and/or by down-regulating the production of anti-angiogenic proteins such as thrombospondin-1 [56,57].

The description of the biology of angiogenesis does not translate conveniently into an understanding of re-oxygenation as one of the Rs of radiobiology. The former seeks to provide a means of characterising interactions of tumour and normal cells that lead to the formation of functional vessels, whereas the latter is a term that describes a number of processes that may result in improved oxygen delivery into tumours during a course of radiotherapy. Nonetheless, the wealth of data that have been derived from studies of tumour oxygen levels using Eppendorff electrodes, immunohistochemical staining for pimonidazole and CA-9, and non-invasive imaging modalities such as dynamic contrast-enhanced magnetic resonance imaging and positron emission tomography with tracers such as Cu-ATSM all point towards the clinical importance of the partial pressure of oxygen in the tumour at the time of irradiation [58]. Furthermore, a meta-analysis of data derived from studies of interventions that aim to improve tumour oxygenation (blood transfusion, hyperbaric oxygen, hypoxic cell sensitisers) during radiotherapy showed an odds ratio of 1.35 (95% confidence intervals 1.20–1.53) in favour of hypoxia modification [59]. Therefore, any future attempts to modify the radiation response by targeting tumour-associated blood vessels must be based on a sound understanding of the historical data generated from studies of tumour oxygen levels.

The new blood vessels associated with tumours present a diverse array of potential therapeutic targets to be exploited by the radiation oncologists. Novel agents may have the ability to switch off signalling through the pro-angiogenic pathway or switch on signalling through the anti-angiogenic pathway [60–62]. Both of these effects can be mediated either at the level of the ligand or its receptor. Alternatively, treatment may seek to destroy the new tumour-associated blood vessels (anti-vascular drugs) by exploiting differences between them and their normal tissue counterparts. The relevance of the angiogenic phenotype of cancers to their susceptibility to radiation is complex. Clearly, for a microscopic tumour deposit, activation of angiogenesis represents a quantum leap in its ability to grow and spread and, as such, can only be viewed as a negative development. However, in the setting of an established large tumour, it is generally believed that the presence of a good blood supply is a pre-requisite for radiosensitivity. Therefore, rather perversely, angiogenesis may be viewed as beneficial by radiation oncologists. By direct extension of this logic, on first consideration it would seem that drugs that target angiogenesis may be detrimental to the radiation response by reducing tumour oxygenation. In fact, experimental models have shown that careful titrated use of anti-angiogenic agents may lead to enhanced sensitivity to radiation by 'normalisation' of the vasculature [63]. Future clinical trials will need to address these issues by studying very carefully the effects of anti-angiogenic and anti-vascular agents on tumour blood and oxygen supply and the resulting effects on treatment outcome.

Anti-growth Signal Evasion

In keeping with the theme of normal cells being maintained in a stable state by the maintenance of a balance between competing biological factors, there are a number of normal anti-growth signals that counteract positively acting growth signals described briefly above. Anti-growth signals function either by forcing cells into quiescence (G0 stage of the cell cycle) or by inducing their terminal differentiation, such that they are permanently unable to re-enter the cell cycle. Anti-growth signalling is mediated by ligands (e.g. transforming growth factor beta) that act on cellular receptors (e.g. transforming growth factor beta receptor) and send signals to the nucleus via second messengers. These pathways are mainly involved in controlling the cell cycle clock and mediate their effects through proteins, which include retinoblastoma protein (Rb), cyclins, cyclin-dependent kinases (CDK) and their inhibitors (CDKi) (Fig. 5) [64–66]. Abnormalities in anti-growth signalling pathways are extremely common in cancer and play a role in helping cancer cells to progress through the cell cycle. Therefore, the loss of Rb and members of the CDKi family and the overexpression of certain cyclins and CDK have been shown to occur in a large number of tumour types [67–69]. Indeed, a detailed analysis of the role of Rb in the causation of both hereditary and sporadic forms of childhood retinoblastomas was a key part of our understanding of tumour suppressor genes [66].

Failure to heed anti-growth signals (or ignorance of their presence) plays a role in a number of the 5Rs of radiobiology, including cell cycle redistribution, repopulation and inherent radiosensitivity. By taking the brakes off cancer cell division, abnormalities in anti-growth signalling facilitate continued (and accelerated) proliferation of tumour cells during a course of treatment. Until now, attempts to exploit anti-growth pathways for therapeutic gain in cancer have lagged considerably behind those that target the growth factor receptors. Nonetheless, therapies that target anti-growth factor signalling are being developed and have enormous potential for combination with radiation therapy.

Immortalisation by Reactivation of Telomerase

Normal somatic cells are only able to undergo a finite number of cell divisions before they enter a period of

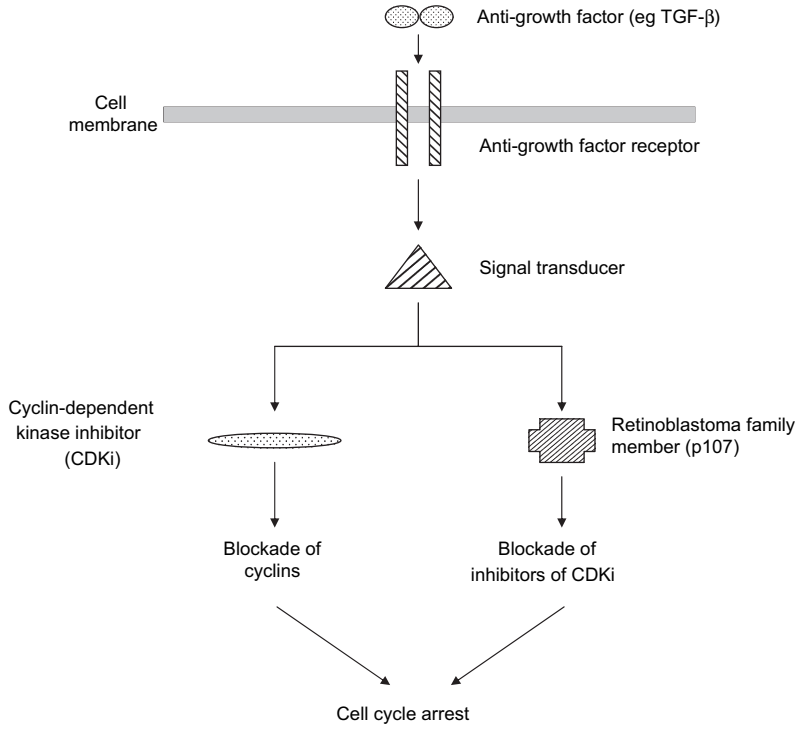

Fig. 5 — Anti-growth signalling pathways. Ligand binding to cellular receptors activates signal transduction pathways that cause cell cycle arrest.

permanent growth arrest known as replicative senescence (the Hayflick limit) [70]. This process occurs as a result of the cells' inability to replicate the ends of their chromosomes (the telomeres) fully at each division — the so-called end-replication problem. Therefore, over time the telomeres get progressively shorter, effectively acting as molecular clocks that count down the cells' life span. Stem cells and malignant cells, by contrast, have acquired immortality through the ability to maintain the length of their telomeres [71]. In most tumours, this occurs through up-regulation of the enzyme telomerase [72], but in 10—15% of cases a different mechanism called alternative lengthening of the telomeres is responsible [73]. The enzyme telomerase is an extremely complicated structure that comprises a large number of proteins. Its two main components are an RNA template (hTR) and a reverse transcriptase enzyme (hTERT): the reverse transcriptase uses the hTR RNA template as a guide in the resynthesis of the DNA sequence of the telomere [74]. Therefore, tumours that have reactivated the expression of telomerase are able to re-build the parts of their telomeres that they lose with each round of cell division and, so, are able to avoid being sidelined into replicative senescence. It is this phenomenon that underlies our understanding of cancer clonogens as the sub-population of tumour cells with the potential for limitless replication.

Tumour cell immortalisation by reactivation of telomerase does not neatly translate into any of the 5Rs of radiobiology. Instead, it should be viewed as an underlying property of cancer cells that gives them licence to exhibit properties such as repair, redistribution, repopulation and radiosensitivity. In addition to being rather difficult to pigeon-hole in radiobiological terms, telomerase reactivation represents a difficult target for drug development. Although drugs that inhibit the function of telomerase have been derived, it is difficult to design strategies that involve using them in combination with radiotherapy. The main reason for this problem is the lag time between telomerase inhibition and the critical shortening of the telomeres (a process that may require multiple rounds of cell division). Alternative approaches that are in clinical development include using viral vectors to deliver therapeutic genes that will only be expressed in cells that have activated telomerase expression [75,76].

Tissue Invasion and Metastasis

Distant metastases are the cause of 90% of cancer deaths [77]. Invasion and metastasis involve careful orchestration of a series of extremely complex biological processes: (i) the tumour cell detaches from its immediate neighbours and the stroma of the local site; (ii) it invades the extracellular matrix by enzymatic digestion followed by specific directional motility; (iii) it penetrates a blood or lymphatic vessel (intravasation) and forms a tumour embolus; (iv) it survives in the circulation until it reaches its destination (which may be chosen on the basis of the fact that it contains a favourable supply of appropriate growth factors); (v) it adheres to the endothelium of blood vessels at its destination and extravasates from the vessel; (vi) it

begins to proliferate and invade its new location and sets about recruiting a new blood supply [30,53,78—80]. Each of these steps may be seen as a potential target for therapeutic intervention.

However, at present, most of these processes only have limited direct relevance to radiation oncology because once a tumour has escaped from the local site and spread systemically it can no longer be regarded as curable by radiotherapy, although radiation may still play a role in securing local control at the primary site and first echelon lymph nodes. Nonetheless, therapeutics capable of preventing tumour invasion and dissemination might be valuable if used before, during or after a course of radiotherapy because recent research suggests that the pathways involved in tumour cell invasion and spread, such as the chemokine signalling through CXCR4 and CCR7, may also be important in sending survival signals to cancer cells [81]. There is also the possibility that specific knowledge of the metastatic process may give rise to more effective systemic therapies, with a resulting increase in the importance of securing control of the primary site with modalities such as radiotherapy [82]. Therefore, it is important that radiation oncologists are aware of this important area of cancer biology and are able to exploit the therapeutic opportunities that it offers.

Clinical Trial Design in the Era of Targeted Drugs

The success of molecular biology in defining specific molecular defects in cancers and the low single-agent response rates of almost all of the new selective inhibitors mean that the quest for combinations of targeted therapies with radiotherapy or chemoradiotherapy is a rapidly expanding area of research. In fact, radiotherapy is a form of targeted therapy in its own right, but it differs fundamentally from the two other major branches of anti-cancer treatment (surgery and chemotherapy) in the way in which it can be used in conjunction with targeted agents. Like surgery, radiotherapy is a local treatment modality — but it is one that is delivered over a protracted period of time. Therefore, in direct contrast to surgery, there exists the possibility of gaining incremental local benefits on a day-to-day basis by combining radiotherapy with targeted drugs. The local nature of radiotherapy also presents opportunities that are denied to cytotoxic chemotherapy because the lack of systemic toxicity with radiotherapy means that interactions (both positive and negative) are largely restricted to the volume of irradiated tissues.

Careful analysis of the different molecular aberrations in specific tumour types offers the prospect of designing smart targeted therapies that will exert preferential mechanistically favourable effects to yield additive, synergistic or even independent anti-tumour actions in combination with (chemo)radiotherapy. Pre-clinical studies will also allow us to optimise combinations of different classes of targeted drugs to select the most appropriate schedules to maximise the therapeutic gain. However, it must be appreciated that such data may not translate readily to the clinic. For most patients the use of chemoradiotherapy already represents a form of treatment that causes toxicities at, or close to, their limit of tolerability. It would be naïve to imagine that we are going to be able to add a cocktail of new targeted agents to standard chemoradiotherapy regimens without encountering severe dose-limiting toxicities. There also exists the possibility that targeted drugs may exacerbate normal tissue toxicity or antagonise anti-tumour efficacy of the standard therapy.

These considerations dictate that clinical trials will have to be designed very differently in the future. The current model of clinical trials in which phase I trials define the maximum tolerated dose, phase II trials the response rates in specific tumour types and phase III trials the comparison with the current gold standard will have limited utility. Instead, pre-clinical studies will identify tumour types in which a specific molecular abnormality drives the biology of the disease (e.g. epidermal growth factor receptor [EGFR] in head and neck cancer) and will assess the interaction between specific targeted therapies and chemotherapy and/or radiotherapy in *in vitro* and *in vivo* studies. These studies will allow phase I trials of the new agent to be conducted in patients with specific tumour types and the objectives will include identification of the optimal biological dose (OBD) rather than the maximum tolerated dose. Definition of the OBD will depend on the analysis of the molecular target in tumour (or normal) tissue samples and may avoid needless escalation of the drug dose beyond a level at which the maximum biological effect is seen. In turn, this approach will limit the patients' exposure to the toxicity of the new agent. It is also probable that this design of phase I studies will give more useful information on therapeutic efficacy than a conventional phase I study. In the future, it is possible that these studies will involve functional imaging modalities (dynamic contrast-enhanced magnetic resonance imaging, positron emission tomography/computed tomography and perfusion computed tomography) as a means of defining early changes in tumour biology as predictors of eventual outcome. Thereafter, it will be necessary to conduct preliminary phase I combination studies with (chemo)-radiotherapy — but prior definition of the OBD will limit the need for sequential dose-escalation cohorts and patient exposure to doses of the drug above those that cause maximum knock-down of the putative therapeutic target. This point is particularly important as these studies will need to be carried out in patients who are receiving full-dose (chemo)radiotherapy with curative intent. In this group of patients, excessive additional toxicities due to the introduction of a new agent would run the risk of causing unscheduled treatment breaks, which are known to have a negative effect on outcome in a number of tumour types [83—88]. As a result of this type of biologically driven design and analysis of phase I studies, it is probable that subsequent phase II studies will have a randomised design that will allow clinicians to obtain an

initial impression of the efficacy of the new combination in comparison with the standard therapy. In theory, if the pre-clinical data are able to define suitable groups for clinical trials on the basis of the underlying biology of the cancer, these phase II studies may have sufficient statistical power to yield answers that previously have required phase III trials. As a result, it is probable that phase III studies may evolve to require smaller numbers of patients with tumours that are biologically homogeneous in which testing of a new targeted drug represents a rational strategy. Such careful target definition in early phase I–II studies will hopefully avoid the sort of debacle that occurred with the testing of gefitinib in combination with gemcitabine and cisplatin (INTACT 1) or paclitaxel and carboplatin (INTACT 2) in two large phase III studies involving more than 2000 patients before the importance of EGFR mutation status to the likelihood of response was understood [89–91].

Development of Targeted Therapies Based on an Integration of the 5Rs of Radiobiology and the Hallmarks of Cancer

The real challenge that faces clinical oncologists at this time is the question of selecting the best candidate molecules for evaluation alongside radical chemoradiotherapy. The obvious corollary of this challenge (if we accept the premise that we will not be able to add cocktails of three, four or five new drugs concomitantly to standard chemoradiotherapy regimens) is that we will need to exploit the potential opportunities of other targeted agents in the neoadjuvant or adjuvant setting. Thus, we will need to use an integrated understanding of the biology of cancer derived from the 5Rs of radiobiology and the hallmarks of cancer to guide treatment design. Figure 6 provides a schema that illustrates the conceptual links between the 5Rs of radiobiology and the hallmarks of cancer. Consideration of these links allows us to build up a picture of a future trial design that aims to hit the various targets on offer at a biologically optimal time (these timings may differ for different tumour types). By way of illustration, we shall consider how we may build a strategy for introducing new drugs into a standard chemoradiotherapy approach in head and neck cancer (Fig. 6).

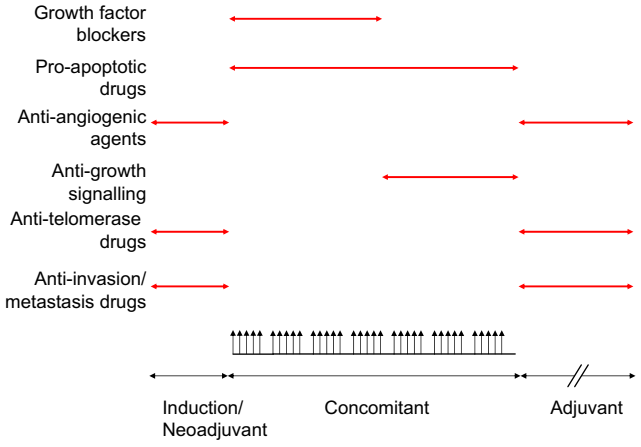

Fig. 6

The link between growth factor self-sufficiency and all 5Rs of radiobiology represents a compelling case for the concomitant use of drugs that interact with this process during chemoradiotherapy. Indeed, the benefit of using EGFR blockade with radiotherapy has been shown in a randomised setting in patients with head and neck cancer [12]. The importance of the restoration of sensitivity to anti-growth signals to tumour cell repopulation, redistribution and radiosensitivity means that drugs that are able to target these pathways should also be considered for concomitant use with chemoradiotherapy. Similarly, the link between the evasion of apoptosis and DNA damage repair and radiosensitivity argues strongly for the administration of drugs that re-set the apoptotic balance of cancer cells in a pro-apoptotic direction concurrently with radiation. Therefore, we immediately have three groups of drugs competing for a place in a combination regimen with chemoradiotherapy. In order to exploit each of these opportunities to the maximum extent, it might be reasonable to deliver a drug that modulates tumour apoptosis throughout the whole treatment course, but schedule an EGFR blocker for the first 4 weeks of treatment and a drug that restores anti-growth factor signalling for the last 3 weeks of treatment (or *vice versa*). Such a strategy may represent a rational approach to targeting accelerated repopulation of tumour clonogens in the latter half of treatment.

The role of angiogenesis in establishing and maintaining a tumour blood supply makes this an ideal candidate for targeting in a neoadjuvant or adjuvant setting. In addition to having anti-tumour effects, anti-angiogenic agents may be capable of the 'normalisation' of aberrant tumour-associated blood vessels with a resulting improvement in tumour oxygenation and drug delivery [63]. Therefore, short duration neoadjuvant administration of anti-angiogenic drugs may prime tumours for a better response to subsequent chemoradiation. The growth dependence of metastatic colonies on the activation of angiogenesis also means that anti-angiogenic drugs may be very good candidates for adjuvant use against micrometastatic disease. The role of telomerase reactivation as an overarching feature of cancer biology means that targeting this hallmark may yield therapeutic gains at any time during the treatment of cancer. As such, at present, there is no compelling reason for scheduling agents that target this process concomitantly with chemoradiation. Instead, it may be advantageous to use anti-telomerase strategies in the neoadjuvant or adjuvant settings.

Finally, it is difficult to define a clear role for agents that target tumour cell invasion and metastasis. Nonetheless, it is reasonable to hypothesise that any drugs capable of targeting this process might have a useful role in neoadjuvant treatment (before surgery or definitive chemoradiotherapy) as a means of limiting the local extent of disease (anti-invasion) or the likelihood of tumour cell spread (anti-metastasis). Similarly, as these signalling

pathways also seem to play a role in tumour cell survival, the use of targeted drugs in the adjuvant setting may be worthy of evaluation.

Conclusions

Interpretation of the 5Rs of radiobiology in the context of the hallmarks of cancer presents enormous therapeutic possibilities to clinical oncologists. We now have an opportunity to design combination therapies using drugs that can enhance many of the fundamental biological effects of radiation. At this time, it is extremely important that clinical oncologists are familiar with the new biology of cancer so that they can play a leading role in the rational development of clinical trial protocols. In the series of articles that will follow this introduction, we will attempt to describe how an integrated biological view based on the 5Rs and the hallmarks of cancer has the potential to guide the future direction of clinical oncology.

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