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Invited reviews

The future of radiation therapy in the post-genomic era

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The cloning of the human genome has generated a tremendous resource of information that will improve treatment of cancer, and other diseases. Allied to these discoveries are powerful new investigative tools that have been, and are being, developed. These are being used to give a comprehensive biological profile of individuals and their cancer that will allow better classification, as well as identification of pathways that might be targeted with therapeutic benefit. The hope is that these approaches will allow intervention that is tailored to the needs of the individual patient and that the targeted cancer therapies will be associated with less toxicity than those currently used. This raises questions as to how best to use the new biotechnologies to predict responses to conventional therapies and indeed will conventional therapies, like radiation therapy, have a role in cancer treatment as specific biologically targeted drugs become commonplace. Here, it is argued that even the molecular staging of cancer that is currently being performed, if exploited correctly, will greatly aid patient selection for radiation therapy and that this should be the starting point for further studies aimed at developing predictive profiles for improving treatment outcome. It is also argued that because the biological anti-cancer agents target molecular pathways that overlap with those responsible for radiosensitivity, and because on their own they have little cytotoxic power, radiation therapists should incorporate biological agents into combined modality regimens and that this is likely to be a standard form of treatment in the next decade.

Przyszłość radioterapii w erze genomu

Sklonowanie ludzkiego genomu dostarczyło ogromnej ilości informacji, które z pewnością przyczynią się do postępu w dziedzinie leczenia wielu schorzeń, nie tylko o charakterze nowotworowym. Rozwój ten wspomagany jest również przez potężne narzędzia badawcze, które znalazły się ostatnio w naszym zasięgu. Dzięki zdobytej wiedzy i możliwościom możemy w chwili obecnej analizować profile biologiczne nowotworów i ich „żywicieli", przyczyniając się do zwiększenia skuteczności leczenia oraz do zmniejszenia jego toksyczności. Niestety, jak przy każdej nowej metodzie, tak i w tym przypadku rodzą się pytania – jak zastosować najnowsze osiągnięcia biotechnologii dla przewidzenia odpowiedzi na leczenie konwencjonalne i na metody bardziej nowoczesne. Istotne jest również przewidzenie miejsca radioterapii w leczeniu chorych w chwili, gdy wysoce specyficzne preparaty celowane biologicznie staną się elementem rutynowego postępowania. Wydaje się być celowym zaznaczenie, że "staging" molekularny guza nowotworowego może znacznie wspomóc wyselekcjonowanie grupy chorych, którzy najbardziej skorzystają na leczeniu radioterapią, i że właśnie taki sposób rozumowania powinien leżeć u podstaw prac mających na celu dalszy rozwój terapii w onkologii. Co więcej, postuluje się, że drogi metaboliczne, określane dzięki wnikliwej analizie guzów, mogą, do pewnego stopnia, pokrywać się z mechanizmami promieniowrażliwości, a zatem lekarze radioterapeuci mogą sięgać po preparaty biologicznie celowane, aby poprawić skuteczność swego postępowania. Należy sądzić, że tak zmodyfikowane leczenie stanie się standardem w czasie nadchodzącego dziesięciolecia.

Słowa kluczowe: radioterapia, genom, promieniowrażliwość
Key words: radiotherapy, genome, radiosensitivity

Introduction

The genomic era was ushered in when the human genome project began cloning each of our >30,000 genes. Since this endeavor is essentially complete we are, in a sense, entering the post-genomic era. The explosion in biological information issuing from the human genome project promises major improvements in health care and a revolution in the treatment of numerous diseases, including cancer. At the same time, while this may be the crowning achievement of the biotechnological revolution, it represents only a part of the progress that has been made. In cancer diagnosis and therapy, novel biology-based paradigms are being generated that threaten to profoundly alter the practice of cancer therapy.

A keystone concept (Figure 1) is that we will be able to use genome-based biotechnology approaches to accu-

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rately define the molecular profile of the patient and of their cancer and identify pathways that can be specifically targeted by "smart" drugs. In this way therapeutic intervention will be tailored to the individual patient to maximize therapeutic benefit. It is always difficult to predict the future, and this presentation should be considered as containing personal and at times deliberately provocative views, but it seems that this is an appropriate time to consider the place of radiation therapy in the proposed new world of cancer therapy.

These new technologies should allow characterization of
- the molecular profile of the patient and define the
  - risk of getting cancer
  - risk of normal tissue injury
  - the outcome of a given therapy
- the molecular profile of the cancer with
  - improved molecular staging and diagnosis
  - identification of prognostic biomarkers
  - identification of candidate targets for biological intervention
  - development of molecular surrogates of therapeutic outcome

i.e. tailor therapy to individual patient needs

The current state of biotechnology

The human genome project may be rewriting the language of biology, but it is the high throughput biotechnologies that are generating the individual words and it is the field of bioinformatics that is charged with generating the syntax that allows this language to be placed into a readable, or at least semi-readable, form. They allow a holistic approach to scientific investigations that complements the more traditional reductionist mechanistic approach.

At the genetic level, variation between individuals is being assessed at the level of single nucleotide polymorphisms (SNP). Over a million SNPs have been detected in the normal population. The picture that is being painted is that they are responsible for about 90% of individual variation and that there is around 1-2 variants/gene. Such approaches augment classical genetic strategies that are themselves becoming more efficient and powerful. Advances in our ability to manipulate individual genes is best seen in the numerous targeted knock-in and knock-out strains of mice that have been developed and that have given many new animal models of human disease.

It is only natural that mRNA has emerged as a prominent macromolecule in this quest for knowledge. mRNA expression profiles are a reasonable reflection of cell activity and function and can be read with a high level of efficiency and reproducibility using cDNA or oligonucleotide microarrays, which are becoming common currency in the search for cancer-related cellular alterations. Quantitation of the "transcriptosome" is improved by techniques such as serial analysis of gene expression (SAGE). In addition, RNA is a dynamic versatile macromolecule, attributable in part to the fact that non-coding, as well as the coding, regions of the DNA are transcribed and confer properties such as molecular stability. RNA-based science is a promising approach in therapeutics. mRNA is an accessible target for specific inhibition of gene products, for example using anti-sense agents or ribozymes, the RNA versions of enzymes that digest specified sequences of mRNA, These approaches are being tested in phase I trials, for example, to target the transcript for the vascular endothelial growth factor (VEGF) receptor.

It should not be forgotten that the current genocentric view of cancer is just that, a current view. Bridging genotype with phenotype is the major feat that researchers and clinicians need to accomplish in this post-genomic era. The proteome is still difficult to comprehensively interrogate. Protein analyses are less reliable and more restrictive than those at the genome or mRNA level and the magnitude of the challenge is greater. The proteome may be 100 fold larger than the genome due to alternative splicing of mRNA and post-transcriptional and post-translational processing but progress is being made even at this level, in particular using mass spectrometry techniques. In addition, tissue arrays are now commonly used to examine expression of multiple proteins by immunohistochemistry in patient biopsy samples. These form a natural practical extension of gene arrays that can be focused on expression of defined prognostic molecular profiles and at the same time confirm gene expression at the protein level, testing up to 100 products on one slide. At the same time, transcriptional silencing and post-transcriptional modification of protein expression levels is becoming increasingly recognized as important in carcinogenesis, prognosis, and therapy.

A current impediment to progress is the lack of methods for analyzing how gene products network in an ontological sense to generate meaningful subcellular and cellular systems that are capable of social molecular interactions and intelligent responses to external signals. The basis for this field of bioinformatics is embedded in current literature on molecular circuitry of the cell and the need to bridge genotype with phenotype in this post-genomic era. It is difficult to use expression profiles to identify nodal molecules within networks that dictate and direct downstream events and that could serve as critical targets for biological therapeutic intervention.

The tools for intervention are however becoming increasingly available in the form of small molecule inhibitors, monoclonal antibodies, gene therapeutics, or elaborations of these approaches. An alternative approach to identifying potentially critical molecular targets for cancer treatment is systematic searching of the entire genome of a wide range of cancer types in the hope of identifying common mutations. This approach has already achieved some success with the identification of BRAF mutations associated with 59% of melanomas [1, 2]. The hope is that the definition of specific molecular targets for intervention will collude with

high throughput drug production and testing systems that are already well developed and are resulting in production of a wide range of new biological therapeutics.

The new paradigm therefore presents a compelling view of the future (Figure 1). The genetic profiles of the individual patient and their cancer are typed and therapies are chosen off-the-shelf that "fit" the profile for that specific patient. In other words, they provide a molecular readout that maximizes the therapeutic benefit. Following initiation of treatment, molecular analyses will determine the level of toxicity and response to therapy by employing surrogate markers to assess benefit at early time points rather than rely on tumor recurrence or tumor-free survival as endpoints. The importance of early assessment of response cannot be underestimated. It allows patient selection during treatment, the cost of clinical trials to be minimized, and an increase in the number of compounds that can be tested. Currently, the number of promising compounds being produced exceeds the number of patients that might readily be entered into a trial and the basis for a compound "making" it to Phase III clinical trials is frequently of dubious scientific validity.

Prioritizing questions to be asked with the new biotechnology

Given the potential impact of the emerging technologies, this is a good time to prioritize the questions that might be asked so that their implementation in cancer prognostics, diagnostics, and therapy might be optimized.

In recent years it has become clear that therapies induce molecular responses and that these may influence therapeutic outcome. Predicting outcome might therefore be possible by determining the therapy-induced molecular profile in normal tissues and cancers in individual patients. However, progress may be slow. For example, preliminary data suggest 150-200 genes may be activated rapidly following exposure to DNA damaging agents [3]. Evaluation of responses is further complicated because different genes can be switched on at different times and to differing extents in different tissues. Furthermore, late responses, such as those to radiation therapy that generally limit the dose that is given, are likely to reflect differences in genetic programs related to healing rather than a direct response to radiation.

Greater value might be obtained by assessing variation between individuals and cancers prior to therapy, using SNPs, microsatellite, gene methylation, and similar analyses. It seems highly possible that radiation or chemosensitive individuals will have polymorphisms in specific genes. For example, polymorphisms in the thymidylate synthase enhancer region can influence protein levels and survival of colorectal cancer patients receiving 5-FU based therapy [4], as can polymorphisms in glutathione S-transferase gene in patients receiving 5-FU/oxaliplatin chemotherapy [5]. Gene expression profiles prior to therapy might therefore identify individuals and cancers that are intrinsically sensitive to certain therapeutics. Such studies would also be important in establishing the baseline for studies on radiation-induced gene expression.

The concept of genetically determined variation in normal tissue response seems anathema to some radiation oncologists, but animal and clinical data suggest that sensitive individuals exist although their frequency and the magnitude of their sensitivity are uncertain [6, 7]. Currently, it seems that radiosensitivity might be impacted not so much by heterozygous expression of classical genes such as those mutated in ataxia telangiectasia (ATM) and other extreme radiosensitivity disease states, but more by genes involved in DNA, cell, and tissue repair. If this endeavor is successful, identification of "radiosensitive" individuals will do away with the concept of standard tolerance doses of radiation that are currently applied to all patients, irrespective of genotype.

It is germane to ask whether predictive assays for tumor radioresponsiveness will be of value and whether these will relate most to molecular phenotype of the cancer, proliferation markers, radiation-induced gene expression pattern, hypoxic pattern, or all of the above. Some clues can be obtained from the information that is beginning to emerge from the use of genetic profiling of cancers.

A major current focus of gene microarray analysis for cancer is molecular staging. The aim is to develop prognosis classifiers to distinguish tumor subsets. If these are of manageable size they can be validated for patient outcome at the protein level using archival tissue arrays. Most would agree that, at least for solid tumors, current staging criteria lack predictive power and that apparently homogenous cancers show marked variation in behavior and response to therapy. In breast, lung, and lymphoid malignancies in particular, sufficient evidence is already available to suggest a revolution in cancer staging is coming, even though the data are from studies involving tens of samples rather than the hundreds that would be desired [8].

The data that are generated by gene microarrays are prodigious in quantity. For analysis, unsupervised hierarchical clustering algorithms are commonly used to cluster genes in one dimension based on similarity in expression levels and samples are clustered in the other dimension based on their similarities. Filters are imposed to select genes that vary maximally between patients. While these filters make the data of a convenient size to be handled, information that may be valuable can be discarded. However, the power of the approach is already obvious.

In lung, Garber used microarrays to show that large cell (LCC), small cell (SCLC), squamous cell (SCC), and adenocarcinomas can be distinguished from each other based on molecular profile with reproducibility that is superior to conventional histological assessment [9]. As might be expected, LCC had markers associated with epithelial/mesenchymal cells, SCLC of neuroendothelial, and SCC of well-differentiated cells. In addition, three subgroups of adenocarcinomas were defined. Interestingly, patients in these subgroups showed markedly different disease-free survival. It was concluded that, in

general, molecular classification can be achieved with less than 100 genes and this meets the goal of identifying a small subset of prognosis classifier genes for each tumor type.

There is increasing suggestive evidence that in many cases intrapatient variation, for example between primary tumor and metastatic deposit, may be considerably less than variation between patients. This suggests that the metastatic phenotype is expressed within the primary. Indeed, van't Veer et al. [10] have used expression profiling of tumors from node negative women under 55 with sporadic breast tumors less than 5cm to predict from the profile of the primary tumor those patients who are likely to fail at distant sites and could benefit from adjuvant therapy. Sensitivity was 91% and specificity 73%. Currently, only 20% of patients actually benefit from adjuvant treatment, while 70-90% may be advised to have it. Using molecular forecasting, it was predicted that only 20% would receive unnecessary treatment without an increase in wrong assignments saving a large number of patients the toxic and emotional damage associated with administration of adjuvant chemotherapy.

There is a need to express caution concerning this and similar studies. Normally the sample size is small; typically less than 100 and the studies are retrospective in nature. Those that use clinical outcome to guide the selection of a prognosis classifier cluster of genes may have an intrinsic bias. Furthermore, most studies performed so far can be considered to be using gene profiling only to establish proof-of-concept. Treatment and clinical factors are not given much consideration. However, the potential is obvious and it is worth asking what impact molecular staging will have on radiation therapy and the design of further molecular investigations involving radiation therapy.

It is well recognized that plots of tumor control probability (TCP) with dose from clinical data show a flatter dose response curve than would be expected from theoretical considerations. There are many possible reasons for flat TCP curves [11]. Variation in tumor volume/number of clonogens between tumors contributes, but intertumoral variation in intrinsic radiation sensitivity, proliferation potential, and hypoxic status would have the same end-result. It has long been recognized that the proportion of cells surviving radiation in vitro varies roughly with histological tumor type and this pattern is generally consistent with clinical radiocurability. Variation in intrinsic cellular radiosensitivity has been considered to be evidence that the way tumors perceive and respond to radiation damage is important in determining outcome i.e. it is not only the energy that is deposited that matters but also the molecular wiring of the cell [12]. Furthermore, the pathways involved can be altered by mutations in oncogenes and tumor suppressor genes that often, but not invariably, increase resistance to the cytotoxic effects of radiation [13]. More accurate staging using molecular criteria may therefore identify tumor subtypes prior to therapy that vary in their intrinsic radiation response. One result may be steeper TCP curves and better definition of dose response relationships. The extent of the improvement in tumor control cannot be predicted, but it important that the radiation community becomes involved in correlating outcome from radiation therapy with molecular staging profiles.

The broader hope is that molecular profiling will be able to redefine at the molecular level the impact of the 4 R's of radiobiology that relate most closely to dose fractionation in radiation therapy. Accurate molecular staging would seem to be a prerequisite for these studies. For example, intrinsic molecular markers of hypoxia, such as CA-IX, HIF-1α, and VEGF, in some tumor sites may correlate with disease-free survival better than with local control following radiation therapy. In other words hypoxia, in particular chronic hypoxia, may reflect tumor progression and be of less radiobiological interest and related more to cancer biology than has been suggested in the past [14, 15]. If this is true, clearly, any analysis of hypoxia-induced gene profiles would be more powerful if the broader molecular subtype of the tumor was included in the analysis. Similarly, molecular staging would provide a more powerful experimental framework for study of the relationship between proliferation and cell cycle markers, apoptotic markers, expression of repair enzymes, and radiation response. There is optimism that this will be possible. For example, a method for analysis of data gleaned from microarrays that assigns a score to each of 6800 genes on the basis of change in expression relative to a standard deviation in repeated measurements has identified 34 radiation-responsive genes in human lymphoblastoid cells [16]. Of these 34, 19 were involved in cell cycle regulation, 13 in apoptosis, and 4 in nucleotide excision repair. Further clinical studies will be required to develop a full picture of radioresponsiveness and this will take considerable effort in collection of tumors, clinical data, and automated analyses of a large number of specimens, but are essential if radiation therapy is to be tailored to the individual patient.

Targeted therapies

There is optimism that advances in molecular forecasting will lead to identification of treatment options that are tailored to individual patients. This requires that the molecular aberrations associated with a tumor that distinguishes it from normal tissue are defined so as to allow specific biological targeting. In theory, such approaches should be highly tumor-specific and not associated with the toxicity that limits the use of radiation and chemotherapy. This begs the question as to whether biological targeted therapies will eventually replace conventional therapies.

Several potential problems with tumor-specific biological therapies have been noted. For example, while cancer is a clonal and genetic disease, it is associated with a constellation of changes that may occur in unique or mutation-specified temporal sequences. These multiple genetic changes give rise to heterogeneity, even within one tumor, as can epigenetic influences, such as gene

silencing through exon/promoter methylation or post-translational modifications in gene expression.

Targeting any one cancer-related alteration may therefore be of limited value, although some proof of principle that genetic alterations can be targeted has been obtained with the use of herceptin for the EGFR member Her2neu in breast cancer and Gleevac for Bcr-ABL in CML. In both cases, prescreening has been critical for establishing efficacy and in the case of Gleevec post-treatment monitoring of crk phosphorylation and loss of the Philadelphia chromosome has been useful in assessing response. The message is that molecular analyses will be important if other similar strategies are to show efficacy.

The argument can be made that individual tumors are "addicted" to a particular pathway or that specific mutated molecules act as "nodal" directors of multiple pathways and that, in spite of multiple genetic changes, targeting one molecule may be sufficient to achieve a therapeutic benefit. While this is possibly true, it is not easy to define the critical pathway or molecule and intratumoral heterogeneity in their expression suggests that escape variants are likely to occur that will be responsible for failure of even effectively targeted agents.

It would be useful if the impact of specific oncogene or tumor suppressor gene mutations could be readily identified and downstream events identified that might indicate alternative targets and the importance of the pathway for tumor survival. Sorlie et al [17] were able to identify ER-ve and +ve groups of breast carcinomas that differed by about 150 genes. Interestingly, only 15% could be called ER discriminators and only about half of these were directly estrogen responsive. In other words, most of the genes distinguishing these subsets are secondarily responsive or related to tumor origin or progression. Alternatively, the gene microarray approach may not be sufficiently discriminatory to reliably identify distinct pathways. Certainly, the analysis, which typically includes an increase or decrease in mRNA expression levels of 2 or 2.5 fold is arbitrary and may bear little relationship to protein expression levels. They are also heavily influenced by gene copy number involving large segments of the genome [18]. This highlights the potential problems of identifying meaningful targets associated with molecular survival pathways. On the other hand, Sorlie did identify an ErbB2 rich group of ER-ve breast carcinomas that had a distinct phenotype and outcome [19]. These could be distinguished from basal cell and 3 luminal mammary carcinoma subgroups. p53 mutation was associated with several subsets. Using patient survival in supervised learning programs to guide the clustering showed that 90% of those that correlated best with survival also self-organized into the main tumor molecular subtypes. The conclusions from this study were that gene expression profiling can define subtypes within the biological diversity of tumors even in the presence of oncogenes and tumor suppressor gene mutations, and that these mutations can be associated with a distinct molecular profile that relates to patient survival.

It is worth considering the lessons learnt so far with biologically targeted therapies. Perhaps the most popular current target for preclinical and clinical investigation is the EGFR-related system and its downstream pathways. EGFR is overexpressed in many cancers and targeting EGFR blocks tumor cell proliferation and encourages cell death [19]. Monoclonal antibodies, small molecule inhibitors, and gene therapy approaches have all been developed. Indeed, perhaps the great diversity of agents that are in preclinical and clinical development [19] serves as an indication of how inept we are at judging potential efficacy. In preclinical in vivo models, the monoclonal antibody C225 has been shown to slow tumor growth [20], as have many small molecular inhibitors, but tumor regression is rare. There are many small molecule inhibitors in preclinical and clinical development, but those in the most advanced stages of development, like Iressa, show limited efficacy and dose-limiting toxicity even when combined with chemotherapy [21].

In short, in spite of the enormous enthusiasm for biological targeting, the available evidence suggests that there are problems to be overcome. In addition to heterogeneity of expression and escape variants, specificity can be an issue. The small molecule inhibitors of EGFR have side effects related to normal tissue expression of EGFR, perhaps more than with monoclonal antibodies because of their more homogeneous distribution [21]. Also, many inhibitors are not truly tumor-specific. Even Gleevac was designed to inhibit a different target than Bcr-Abl, namely platelet-derived growth factor, and is not without normal tissue toxicity. However, arguably the most serious drawback is that biologically targeted agents tend to be cytostatic rather than cytotoxic, or at least are not toxic for all tumor cells. To cure cancer you need to kill all the cancer cells! Partial responses are not of great therapeutic value. It is therefore not surprising that, in most cases, biologically targeted agents work far better when combined with classic cytotoxic agents like radiation or chemotherapy.

Another problem is that, while small molecule inhibitors and monoclonal antibodies may be of use in targeting oncogene-related mutations and overexpressed or aberrantly expressed molecules, they are of little value for tumor suppressor gene (loss of function) mutations. Most strategies aimed at these mutations rely on gene therapy, which has considerable limitations. Gene therapy is a lovely concept, but inefficiency of the vectors is a major issue. The best transduction efficiencies we are able to achieve, even in preclinical tumor models were about 50% [22]. This dictates that a bystander effect is needed to affect the other cells in the tumor that could not be transduced. Current approaches, even using replicating vectors or tumor-specific vectors, are therefore limited. Further, tumor suppressor gene replacement is generally cytostatic not cytotoxic and, while for example p53 gene replacement in preclinical models slows tumor growth in vitro and in vivo, examples of cures are rare [22]. In general, tumor suppressor gene replacement

seems to work far better if combined with a powerful cytotoxic agent like radiation therapy.

Conclusions

The above discussion suggests that molecular forecasting of the response of individual patients and their cancer to therapy is going to be greatly improved by the new biotechnologies and that this should lead to the identification of novel targets and strategies with a high therapeutic index. The tools for specific biological targeting of cancer are also becoming readily available and biologically targeted therapies are going to become common currency in cancer therapy. However, they are unlikely to be effective as sole therapies. They will still need to be combined with powerful cytotoxic agents, like radiation. In order for radiation oncologists to derive benefit from the biological revolution on behalf of their patients, the concept that molecular signaling pathways may be activated in cancer that distinguish cancer from normal tissues and determine cellular radiation responses needs to be embraced by the radiation community and trials developed to investigate these new combined therapies. The baseline for these therapeutic investigations, as well as prognostic studies in radiation therapy, should be that cancers are molecularly staged since this may more precisely profile the disease. The influence of hypoxia, cell cycle, proliferation, and repair should be evaluated within this context. Radiation oncologists should embrace this formula and use the new biotechnologies, with associated critically important clinical assessment and sample collection, as a basis for evaluating combined therapy approaches using novel biologically targeted drugs and gene therapy approaches along with radiation therapy.

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