MOLECULAR IMAGING AND TARGETED THERAPIES

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Abstract

Molecular medicine represents a new approach to therapeutics and has significant implications for the practice of oncology in the future. Leveraging the rapidly increasing pace of technological and scientific innovation in molecular biology, there has been an explosion in the understanding of the key drivers of malignant transformation. New target discovery and development of therapeutic agents against these targets creates new challenges for the oncology community. Traditional staging and therapeutic response paradigms have limited capacity for detecting these targets and whether they are modulated by therapeutic intervention. Molecular imaging, which is reviewed below, offers unique promise as the means to select and monitor molecular targeted therapy. Use of radioactive chemicals and radiopharmaceuticals, directed at specific cellular targets, is an example of molecular targeted therapy and will logically be enhanced by improved understanding of tumour biology.

Elsewhere in this edition of *Cancer Forum* the progressive move from conventional chemotherapy toward molecular targeted therapies is described. In parallel with this trend towards molecular medicine, development of new approaches to tumour characterisation and therapeutic response assessment is required. This is vital because the recognised limitations of conventional structural imaging are likely to be even more compromised with respect to novel therapies, the success of which is likely to be based on target expression and its modulation.

To facilitate rational use of molecular targeted therapies, new laboratory diagnostic tests have developed to profile molecular markers known to be associated with particular clinical patterns of tumour behaviour. The concept of 'biomarkers' that have either prognostic significance, or are predictive of response to a particular therapy, has become entrenched in the development and validation of new cancer treatments. Hormone receptors, peptide receptors and tumour-associated antigens are routinely assayed in tissue samples to improve disease characterisation and to guide therapy selection for individual patients. With increasing frequency, analysis of the genetic characteristics of particular tumours using DNA microarrays and advanced proteomic analysis are being used to predict the natural history of the tumour and the likelihood of therapeutic response. In this context, it is salient to ask: What role may molecular imaging play?

Nuclear medicine as a quintessential molecular imaging tool

A number of imaging modalities have the capacity to move beyond structural characterisation of malignancy. These include magnetic resonance spectroscopy and targeted contrast agents for MRI or ultrasound. However, this discussion will be confined to nuclear medicine, particularly Positron Emission Tomography (PET), because radiotracer techniques are ideally suited to play a leading role in the coming era of targeted cancer therapy, and are generally at a more advanced stage of clinical development.

Nuclear medicine techniques depend upon molecular mechanisms operative in vivo. Minute (trace) quantities of radioactive materials, chosen because of their ability to participate in biological processes of interest, can provide highly sensitive indications of body function in health and disease. Largely independent of structural disturbances, nuclear medicine scanners increasingly offer high spatial resolution, but more particularly, high contrast. There is also a growing trend to incorporate CT scanners into hybrid imaging devices. Disordered metabolism or physiology can be detected with high sensitivity and the anatomical distribution of abnormality can be determined with greater precision than ever before. Nuclear medicine technology has an additional attraction in cancer medicine because tracers may become therapeutic agents if administered in high doses, or with substitution of the radioactive moiety to a radionuclide with appropriate particulate emissions.

Radioactive iodine as a prototypical molecular targeted therapy

Radioactive iodine-131 (^{131}I) has been used in clinical medicine since the early 1940s, yet this 'old' molecular imaging agent provides a good illustration of the important contribution to cancer care that can be made by radioactive compounds. Disturbed iodine metabolism of thyroid cancer cells has been successfully exploited to stage and delivers curative treatment to patients with this malignancy. As a substrate for the sodium/iodide symporter, tracer quantities of radioactive iodine can demonstrate even minute metastatic lesions, particularly in the absence of normal thyroid tissue. Iodine-avid metastatic lesions may be completely ablated using high doses of ^{131}I. Indeed, this tightly targeted radioactive agent was one of the first curative therapies for disseminated metastases from solid malignancy.

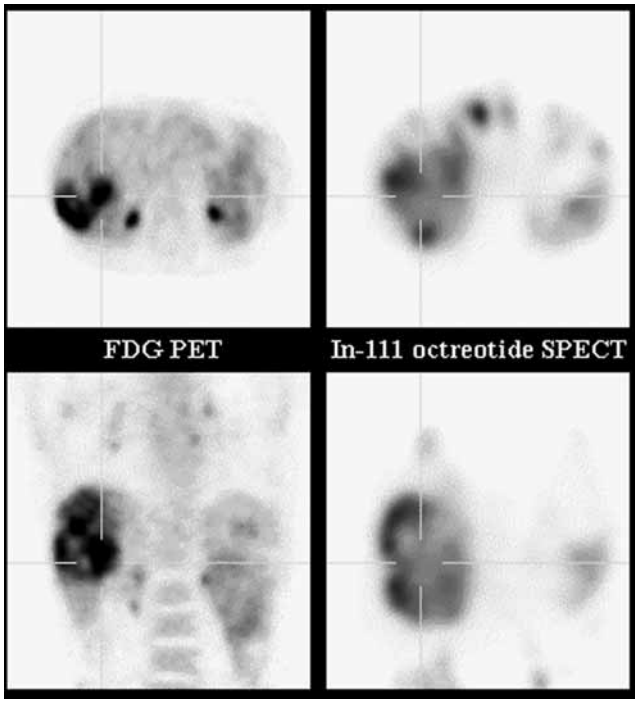
However, not all thyroid cancers are iodine-avid, reflecting heterogeneity of tumour biology between patients, and further molecular characterisation, for example using PET, has been shown to have prognostic and therapeutic implications.[1]

Tumour heterogeneity and targeted therapy

Heterogeneity of malignant cell clones in different sites within a single tumour and between different tumour sites in the body is a manifestation of the genomic instability that characterises cancer cells.[2] Heterogeneity may manifest itself as a differential therapeutic response to conventional chemotherapy agents and poses a greater threat to successful therapeutic outcomes when using highly selective molecular targeted therapies. Detection of different populations of cancer cells is impossible for structural imaging and limited by restricted tissue sampling for pathological techniques. However, as differing clones of malignant cells have different metabolic characteristics, there is a potential for *in vivo* profiling of these differences with molecular imaging (figure 1).

It is important to realise that there is a wide range of existing radioactive tracers that can be used with traditional single photon techniques and also for PET.

Figure 1: *Clonal heterogeneity appears to be a particular feature of neuroendocrine malignancy. In this patient with metastatic neuroendocrine carcinoma, co-registered images in transaxial (above) and coronal (below) planes demonstrate sites (cross-hairs) of high FDG uptake on PET (left panels) lacking in somatostatin receptor expression based on In-111 octreotide SPECT (right panels) scanning. This was despite high-uptake of In-111 octreotide at multiple other sites within the liver. These findings indicate that peptide-receptor radionuclide therapy would be unlikely to control all sites of disease if used as a single agent. Despite combined use of chemotherapy and peptide receptor radionuclide therapy, this patient demonstrated rapid disease progression of the FDG-avid disease.*



The rapidly expanding knowledge of molecular mechanisms of cancer provides an expanding array of relevant molecular targets that may be investigated radiolabelled agents. The following sections will provide some examples of existing radiolabelled molecular imaging probes that are being used in clinical or trial circumstances.

Altered substrate metabolism for assessment of response to targeted therapy

Several key genes involved in malignant transformation lead to uncontrolled cellular proliferation. This necessitates activation of cellular machinery controlling basic substrate fluxes and differentiates cancer cells from many normal tissues. PET tracers have been developed that reflect altered cancer cell glucose metabolism (F18- fluorodeoxyglucose or FDG), altered amino acid and protein metabolism (F18-fluoroehtyl-tyrosine or FET), altered sterol metabolism associated with increased cell membrane turnover (F18-fluorocholine of FCH) and increased nucleic acid formation (F18-fluoro-thymidine or FLT).

Although all of these PET tracers have been used as molecular imaging probes in basic and clinical cancer research settings, FDG has a pre-eminent role at present by virtue of a better predictive value for detecting most cancer types than conventional structural imaging standards.[3] Although it has been demonstrated repeatedly that the degree of disturbance of glucose metabolism in individual tumours carries independent prognostic information, FDG PET is mainly used for identifying the extent of cancer.[4-6] FET and FLT have found clinical utility in the brain for detecting active tumours, primarily because of the low uptake of these tracers in the normal brain compared to tumours (contrasting with FDG where normal brain uptake is very high and tumours may be difficult to distinguish).[7,8] However, the generally low uptake of these tracers into the malignant cells has rendered them less useful as predictors of cancer extent outside the brain. FCH has proved useful as a predictor of cancer extent in patients with breast and prostate cancer, particularly in tumour types of a more indolent nature where FDG uptake may be minimal or absent, and also for detecting active malignancy in the brain.[9]

In therapeutic monitoring applications, change in the degree of metabolic abnormality appears to be more important than the morphological extent. Reduction in FDG uptake has been shown to correlate with reduction in viable cell numbers, to precede lesion shrinkage in the setting of conventional cytotoxic therapies and to provide useful prognostic information.[10,11] It is likely that the advantages of molecular imaging over structural methods will become even more pertinent in the context of molecular targeted therapies that may arrest the growth of cancer cells rather than killing them. In patients where a chosen therapy proves ineffective, the delay inherent in relying upon measuring tumour response using standard anatomical paradigms may greatly disadvantage patients. The current response assessment paradigm relies on a percentage increase in tumour dimensions. For larger lesions to meet this criterion, the total volume of disease may need to increase very markedly. This increase in

tumour burden, accompanied by cumulative toxicity and cost, limits the opportunity of instituting alternative therapies. This will become an increasingly important consideration as the range of available cancer therapeutics inevitably increases.

In the era of targeted molecular therapy, tumour response assessment with FDG PET has already proven invaluable in monitoring the therapeutic effect of imatinib on gastrointestinal stromal tumours (GIST). A key molecular driver of these tumours is mutation of the C-KIT oncogene leading to constitutive activation of signaling pathways involved in cell growth, survival and proliferation. Imatinib normalises glucose transport into these tumours, and therefore FDG uptake, within days of commencing treatment. Despite significant improvement in patient survival, tumour regression is often undetectable or delayed using structural response criteria. In contrast, normalisation of FDG uptake demonstrated with PET scanning provides a reliable guide to the effectiveness of imatinib long before measurable tumour response criteria are satisfied and metabolic response is predictive of outcome.[12,13] Furthermore, some GIST patients have primary resistance to imatinib and the majority develop resistance to imatinib during treatment.[14] This drug resistance associated with absence of the relevant molecular target can be demonstrated using FDG PET imaging as a surrogate marker long before therapeutic failure is apparent from structural imaging. This is clinically important because second-line drugs such as sunitinib can also block the aberrant tyrosine kinase and improve clinical outcome in a percentage of imatinib resistant patients.[15]

FLT also has potential as a therapeutic monitoring tool for new molecular targeted therapies.[16] However, FLT PET is predominantly being undertaken in trial settings thus far.

Targeted therapy directed at neovascularisation and hypoxia

Lethal malignancy only develops when growing tumours are able to establish effective blood supplies. Molecular targeted therapies devised to interrupt tumour angiogenesis are now in regular clinical use. One such agent, bevacizumab, is a monoclonal antibody that blocks the interaction of vascular endothelial growth factor (VEGF) with vascular receptors. Trials have demonstrated effectiveness only in combination with standard chemotherapy agents and only in a percentage of patients.[17] Radiotracers that target VEGF receptors have been developed and explored in animal imaging.[18] While molecular imaging using such agents has the potential to enable identification of patients who will benefit from agents such as bevacizumab, or individual patient dosing, clinical demonstration is lacking.

Despite angiogenesis, it is often insufficient to supply adequate perfusion and the consequent tumour hypoxia has profound consequences for cancer therapy, because hypoxic cells are both radio and chemo-resistant. Special radiotherapy techniques such as dose painting and chemotherapeutic agents, that are active in a hypoxic environment, offer the potential for improving treatment outcomes for patients with hypoxic cell components within their tumours. The potential for improving treatment of hypoxic tumours with molecular imaging has been explored using several available PET radiotracers that are retained by hypoxic cells. F18 fluoro-misonidazole (FMISO) and F18-fluoro-azomycinarabinoside (FAZA) are two such PET agents that have been extensively investigated at the Peter MacCallum Cancer Centre in Melbourne. FAZA demonstrates higher quality images than FMISO due to more rapid washout from normal tissues, but both enable imaging of hypoxia *in vivo* that is not possible using invasive probe based measurements.

Using FMISO PET, it has been demonstrated in a Phase 2 trial that addition of hypoxia activated chemotherapy agent tirapazamine, to standard chemoradiotherapy in patients with advanced head and neck carcinoma, decreased loco-regional failure rates, predominantly in patients who demonstrated tumour hypoxia on PET imaging.[19]

Targeted therapy directed at cell receptors

Malignant cells frequently express large numbers of cell surface antigens that are present in a minority of normal cells, or that are expressed at much lower concentrations. The role of these over-expressed surface proteins is often unknown. However, it is clear that these cell surface components may fulfil an important role in cancer cell growth and development. For example, the abnormal tyrosine kinase of GIST tumours was originally recognised as a tumour-associated antigen CD 117. This antigen served to refine the pathological identification of these tumours before the functional role of the associated protein in tumorigenesis was recognised.

Over-expression of peptide hormone receptors has been recognised as a defining characteristic of neuroendocrine tumours for several decades. Symptoms relating to excessive hormone secretion may be clinically debilitating for patients with disseminated neuroendocrine tumours. Despite this, these tumours often display very indolent growth patterns with long survival, even without therapy. Chemotherapy and radiotherapy have generally low efficacy. However, molecularly targeted agents, such as octreotide, bind to over-expressed somatostatin receptors and can bring marked amelioration of symptoms caused by hormone secretion and diminish tumour growth rates.

Metabolic imaging with radiolabelled somatostatin analogues (indium 111-pentetreotide (Octreoscan) for single photon imaging and gallium 68-DOTA-octreotate for PET) can detect somatostatin receptor expressing tumours with very high sensitivity and thereby improve the staging and therapeutic planning. Radiolabelled peptides are an exciting option for therapy.[20] Agents such as high dose indium 111-pentetreotide and lutetium 177-DOTA-octreotate (LuTate) have been shown to provide patients who express a high density of somatostatin receptors on molecular imaging studies with significant relief of symptoms and low toxicity. LuTate therapy has the additional advantage of producing measurable tumour responses in a significant percentage of patients.[21]

Of note, metabolic heterogeneity is common in neuroendocrine tumours, and lesions in the same patient that appear identical on CT often demonstrate quite different molecular imaging characteristics. This information is crucial for treatment planning because tumour deposits that demonstrate low uptake of somatostatin radiotracers do not respond to radionuclide therapy. Interestingly, these tumour deposits are probably less differentiated as they usually display enhanced glucose metabolic activity on FDG scanning. In contrast, somatostatin receptors expressing tumours generally do not accumulate FDG to a significant degree. At the Peter MacCallum Cancer Centre patients with widespread neuroendocrine tumours are commonly assessed by molecular imaging to characterise both glucose metabolic status and somatostatin receptor expression prior to treatment selection. If a significant component of the tumour burden has high FDG avidity without somatostatin receptor expression, platinum and etoposide based chemotherapy is usually used as first line therapy, as this more aggressive component of the tumour burden generally determines the patient's outcome.

Monoclonal antibodies developed against cell surface antigens have been investigated for many years as therapeutic agents. For example, rituximab, a monoclonal antibody that targets the CD 20 surface antigen expressed on the surface of normal and malignant B cells, is both effective and has a low toxicity profile for the treatment of non Hodgkin-lymphoma. Several radiolabelled forms of anti-CD 20 monoclonal antibodies, including yttrium-90 -tiuxetan-ibritumomab (Zevalin) and iodine-131-tositumomab (Bexxar), have been approved for therapeutic use. Iodine-131-rituximab is also produced at Fremantle Hospital in Perth and Peter MacCallum Cancer Centre for use in therapeutic doses in patients with relapsed B cell NHL that is refractory to other therapies. Significant effectiveness and very low toxicity have been reported, and in clinical use the therapeutic dose is calculated on the basis of preceding tracer dose molecular imaging.[22]

Cancer research and drug discovery

The potential for molecular imaging in small animals to increase knowledge of drug effects in models of human cancer has been recognised around the world and has been embraced as a means of decreasing the time taken to identify agents that merit clinical trial and to decrease the cost of drug development.[23] The role of molecular imaging with radiolabelled tracers for research into molecular targeted therapies is important to recognise, but further discussion is outside of the scope of this article.

Conclusion

The revolution in molecular biology has led to an evolution of existing molecular imaging techniques to align themselves to become a vital component of a new paradigm in cancer management. The ability to assay non-invasively and in vivo the presence of a molecular target and its modulation during therapeutic intervention provides a unique and invaluable tool for molecular medicine. The future clinical application of PET will likely have greater impact in characterising the biology of disease than its current impressive role of counting lesions.

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