Seeing into cells

The promise of *in vivo* molecular imaging in oncology

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Medicine is undergoing a revolution. Disease is increasingly being redefined in terms of underlying molecular abnormalities, as opposed to the signs and symptoms of the patient. This new definition is referred to as the 'molecular signature' of a disease and the current developments in biomedical research are accordingly termed 'molecular medicine'. In the case of cancer, we know that tumours result from genetic alterations of cells, which may involve overexpression or underexpression of normal genes, or mutations that generate abnormal gene products. This may affect any of the molecules within the cell, the cell membrane or the cancer-cell milieu. In addition, the microenvironment of a tumour, including stromal and vascular endothelial cells, is important for the growth and persistence of the tumour. This entire constellation of the abnormal molecular biology of tumour cells and their microenvironment is the molecular signature of cancer (Hanahan & Weinberg, 2000).

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One consequence of this new understanding of disease is the increased effort to develop targeted drugs that interact specifically with the altered genes or their products and either limit the progression of or kill tumour cells. The hope is that such therapeutics will selectively target tumour cells and leave normal cells untouched, thereby reducing the common side effects of current anticancer therapies such as radio- and chemotherapy. There is an increasing number

Table 1 | Targeted drugs to treat cancer

Cancer	Drug	Action
Colorectal cancer	Bevacizumab (Avastin®)	Angiogenesis inhibitor
	Cetuximab (Erbix®)	EGFR inhibitor
	Irinotecan (Campto®)	Topoisomerase I inhibitor
	Capecitabine (Xeloda®)	Inhibits DNA/RNA synthesis
	Oxaliplatin (Eloxatin™)	Inhibits DNA/RNA synthesis
Multiple myeloma	Bortezomib (Velcade®)	Proteasome inhibitor
Lung cancer	Gefitinib (Iressa®)	EGFR tyrosine kinase inhibitor
	Erlotinib (Tarceva®)	EGFR tyrosine kinase inhibitor
Chronic myeloid leukaemia	Imatinib mesylate (Gleevec®, Glivec®)	Tyrosine kinase inhibitor
Breast cancer	Exemestane (Aromasin®)	Aromatase inhibitor
	Letrozole (Femara®)	Aromatase inhibitor
	Anastrozole (Arimidex®)	Aromatase inhibitor
	Tamoxifen	Oestrogen receptor antagonist
	Fulvestrant (Faslodex®)	Oestrogen receptor antagonist

EGFR, epidermal growth factor receptor

of such targeted therapeutics being developed and made available for clinical use. For example, until about five years ago, there were few options available for treating colon cancer beyond surgically removing the affected part of the colon and using standard chemotherapy. However, since 1999, the US Food and Drug Administration (FDA) has approved at least five targeted drugs for the treatment of colorectal cancer. This is not an exception—the past few years have seen the advent of more targeted therapeutics to treat other cancers as well (Table 1).

To help in testing these new drugs for safety and efficacy, and to aid clinicians in selecting from the various alternatives, diagnostic tools to detect the molecular signature of cancer have become more important. In this context, molecular imaging is a valuable tool for basic researchers and clinicians for several reasons. First, molecular imaging is, in essence, an

in vivo assay. The greatest potential value of molecular imaging is its ability to report on the molecular state of a tumour in its normal milieu. Cancer cells do not behave *in vitro* the same as they do *in vivo*—as soon as cells are removed from the body, by biopsy for example, their pattern of gene expression changes, reflecting the dependence of the tumour cell on its microenvironment. *In vivo* imaging, therefore, has the potential to display the molecular expression of tumours as they function *in situ*. This information may be a better indicator of the effects of targeted therapeutics than the molecular expression observed in the same tumour cells when cultured.

Second, cancer-cell phenotypes change over time and if such changes occur during the course of therapy, they often result in resistance to the administered drug. Serial information from molecular imaging can therefore assist physicians in determining whether the current therapeutic choices are

still relevant in treating the specific phenotype of the tumour. Third, cancer is often distributed at various locations within an organ or throughout the body. *In vitro* information from a single biopsy may not reflect the full heterogeneity of molecular changes that have taken place within the tumour cells of a patient. Fourth, molecular imaging—when used during therapeutic trials—can provide kinetic and dynamic data on a drug that cannot be obtained from a static biopsy.

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In general, any form of medical imaging involves administering a known amount of energy to the body and measuring, with spatial localization, the energy that is transmitted through, emitted from or reflected back from various organs and tissues. The difference between the administered and the recorded energy provides information about some properties of the tissue with which the energy interacted. The energy most commonly used is some form of electromagnetic energy, such as X-rays or light, but occasionally other forms are used as well, such as mechanical energy for ultrasound scans. For most of the past century, the property of matter with which clinicians have been most concerned in medical imaging is structural or anatomical information. However, the developments in medicine that are described above are based on targeting individual genes and molecules, which means that information is needed about the chemical properties of matter—hence the term ‘molecular imaging’.

In cancer therapy, *in vivo* imaging has become an increasingly important tool in helping clinicians select patients with the appropriate molecular phenotype for a given therapeutic, provide quantitative information about the optimum biological dose and timing of the therapy (as opposed to the present paradigm of administering the maximum tolerated dose) and assess appropriate biological end points, which may not necessarily be the reduction of tumour size.

In some cases, it is possible to obtain such molecular information directly by measuring the difference between energy administered to and recorded from the body—for instance, using magnetic resonance spectroscopy to measure the choline peak in breast carcinomas during therapy gives clinicians an earlier indication of histological improvement than does measuring overall tumour size (Meisamy *et al*, 2004). Similar results have been recorded in prostate cancer (Kurhanewicz *et al*, 2002).

However, the amount of chemical information that can be obtained directly from analysing energy changes alone is limited. Thus, most molecular imaging must be performed with the addition of an imaging agent or probe that targets selected molecules. Imaging agents are molecules that interact with the altered products of gene expression, or the altered genes themselves, to affect the recorded energy in such a way that more chemistry-specific information is obtained. There is a wide variety of molecular mechanisms that can be used for developing imaging agents. Some of these involve binding to cell-membrane structures whereas others exploit transport mechanisms into the cell, and subsequent enzymatic or other biochemical reactions within the cytoplasm. Others may be localized to intracellular structures such as mitochondria or within the nucleus itself. No single molecular mechanism in the cell precludes all others for clinical utility.

Similarly, just as there is no single molecular mechanism, so there is no single technology that is superior to all others. Molecular imaging agents and methods have been developed for a variety of systems using different forms of energy. These include nuclear medicine methods—such as positron emission tomography (PET) and single photon emission computed tomography—magnetic resonance imaging (MRI), ultrasound methods, computed tomography (CT), and optical technologies. Although the term ‘optical’ implies the use of visible light, it is often more broadly applied to include near-infrared (NIR) methods as well; the term ‘photonics’ is sometimes used to describe both visible and non-visible radiation. These technologies have different advantages and drawbacks. For example, CT and MRI are able to portray anatomical detail exceptionally well, whereas nuclear medicine and optical methods have very high sensitivity for detecting specific molecules but cannot portray anatomical

detail with high spatial resolution. Increasingly, methods with complementary strengths are combined in clinical practice, such as the CT–PET systems that are now commercially available.

As the fundamental basis of cancer is at the gene level, molecular imaging methods that report directly on gene function would be particularly useful

The most common example of molecular imaging in oncology is the use of fluorine (F)-18-labelled fluorodeoxyglucose (FDG) PET scanning (FDG-PET). Most cancer cells have increased glycolysis and an increased uptake of FDG. FDG-PET scans show the distribution of tumour deposits; the relative intensity of FDG uptake correlates with the activity of cancer cells. Decreases in FDG uptake therefore indicate that the patient is responding to the selected therapy. For example, FDG-PET has been used to measure the response of gastrointestinal stromal tumours to imatinib (Fig 1; Stroobants *et al*, 2003). A more targeted example is uptake of F-18-labelled fluoroestradiol (FES), which correlates with the response of breast-cancer cells to hormonal therapy (Fig 2; Mankoff *et al*, 2001).

As the fundamental basis of cancer is at the gene level, molecular imaging methods that report directly on gene function would be particularly useful. Reporter genes could be used in one of two ways. One method is to insert a gene that codes for an enzyme. A labelled substrate for that enzyme could later be administered and the trapped probe would identify those cells that express the reporter gene. The second method is to insert a gene that codes for a cell-surface receptor and later administer a labelled ligand specific to that receptor. Unfortunately, in most diagnostic and therapeutic situations it is not practical to insert reporter genes into a patient's native cells. However, genes could be inserted into genetically engineered cells or viruses that were administered to a patient, such as in stem-cell or vaccine therapies.

The concept of nanoparticles as generic platforms for imaging agents is also under intense investigation. A single particle, such as a dendrimer, liposome or other construct, acts as a general platform to which a variety of signalling moieties is attached,

Fig 1 | Example of functional imaging used to measure the response of gastrointestinal stromal tumours (GIST) to imatinib. First column (left) shows a pre-therapy fluorine-18-labelled fluorodeoxyglucose-positron emission tomography (FDG-PET) scan (top) and a contemporaneous computed tomography (CT) scan (bottom). GIST lesions are present in the liver (single arrowhead) and stomach (double arrowheads). There is intense FDG uptake in both lesions. The radioactivity seen in the pelvis is due to normal urinary excretion of the compound. The middle column shows similar scans one month after the start of therapy. The tumour masses are still present, although smaller, on the CT scan but there is no FDG uptake in either lesion. The lack of FDG uptake indicates a biological response to therapy and predicts a long-term beneficial response, despite the tumours being present on the CT scan. Normal collections of radioactivity are present in the urinary bladder and the heart. The third column (right) shows scans one year later. Continued absence of FDG uptake in the liver or stomach indicates an absence of tumour activity. The residual lesions seen on the CT scan are due to scar tissue. Images courtesy of Annick D. Van den Abbeele, Dana Farber Cancer Institute, Boston, MA, USA.

along with one or more targeting molecules that can be substituted as required. Such imaging agents have already been developed for nuclear medicine, ultrasound, magnetic resonance and optical applications (Sullivan & Ferrari, 2004).

Photonic methodologies have also attracted much attention in recent years as they have the advantages of not involving ionizing radiation and of being cheaper than traditional clinical imaging techniques. Photonic technologies that do not require the administration of any exogenous contrast materials, and which measure photon reflection, transmission, refraction or fluorescence from endogenous fluorophores, have already been developed. Other photonic methods use similar physical properties, but require the administration of agents that either fluoresce or bioluminesce.

Organic dyes that fluoresce have been used clinically for some time, but their value in the molecular imaging of humans is limited because of tissue absorption. There is also considerable interest in using quantum dots or other nano-constructed particles that fluoresce much more intensely than organic dyes (Gao *et al*, 2004). Such particles can be engineered to fluoresce at a variety of wavelengths, while still being stimulated by light of a single wavelength. A major drawback of bioluminescence is that it requires the combination of an enzyme (such as luciferase) and its substrate (luciferin), and most methods insert the enzyme's gene into target cells using genetic engineering (Contag & Bachmann, 2002). As a result, this method is unlikely to be clinically useful, but it has become an enormously valuable technique for basic research.

Another potentially important characteristic of optical probes is the development of so-called 'activatable' probes, which exploit the phenomenon of fluorescence resonance energy transfer (FRET). Two fluorescent molecules can be held in a steric configuration such that they will not fluoresce when stimulated by the appropriate wavelength of light. If something disturbs that configuration, they will fluoresce. In biological situations this is exploited by attaching the fluorophores to a substrate—such as a polymer—by peptide linkers, which are themselves the substrate for a particular enzyme, such as a protease. When the protease is present, for example in cancer cells that overexpress it, the peptide linkers will be cleaved and the fluorophores will move away from the substrate and fluoresce, thereby signalling that the protease of interest is present and active (Ntziachristos *et al*, 2003). Similar types of 'activatable' agents that use magnetic resonance contrast materials have been reported (Louie *et al*, 2000). Activatable agents that use radioisotopes are not feasible, because the radioactive decay phenomenon cannot be controlled to respond to a particular molecular event as in photonic or magnetic resonance situations.

The main drawback of photonic imaging techniques is that visible light and NIR are highly absorbed by water and tissue. Therefore, the future role of photonic methods in patients, in which light may have to traverse many centimetres of tissue, is not yet clear. Nevertheless, photonic techniques could be valuable for the assessment of mucosal surfaces where the majority of human cancers originate. Engineering simulations have also suggested that it may be feasible to use photonic methods in deep tissue, although such applications are still far from clinical use (Ntziachristos *et al*, 2003).

It is a huge challenge to first identify the molecular signature of all cancers and pre-malignant lesions, to subsequently develop an array of targeted therapies to address this enormously heterogeneous disease and to concomitantly develop targeted imaging agents to work in conjunction with these therapies. It will probably take decades for a range of such applications to reach patients. An alternative approach, that can and should be developed in parallel with the molecular approach, is the use of image-guided, minimally or non-invasive,

focally ablative therapies. Abnormal tissue can be ablated, or removed, by a variety of means such as heating or freezing to induce protein coagulation, using ionizing radiation to initiate apoptosis or directly applying toxins or poisons. In the past, these methods were directed primarily towards large tumours, and the intent was usually to palliate rather than to cure. However, technology has improved to the point at which it is now possible to precisely administer ablative therapies to very small deposits of abnormal tissue virtually anywhere in the body. As methods improve for detecting small, early cancers or pre-malignant lesions—for example, by whole-body imaging screening or serum proteomic tests—there is an increasing need to develop such focally ablative techniques.

At present, the main limitation of these methods is the lack of imaging technologies that define the equivalent of the surgical tumour-free margin. In other words, when the surgeon removes a malignancy, he or she relies on the histological diagnosis of a tumour-free margin to decide whether the surgical procedure was adequate. If the margin was not tumour-free, a re-excision is necessary. For image-guided ablations, there is no opportunity for histological evaluation and an alternative means for assessing adequacy of the procedure is therefore required. This is another task for molecular imaging. It does not necessarily require a broad array of targeted imaging agents; rather a limited, hopefully small, number of agents that could be used to identify with microscopic precision the edge, extent or presence of tumour cells. This is necessary to give some indication after an ablative procedure is performed that the procedure is complete in terms of eliminating all cancer cells.

Another problem is the interpretation of imaging data, which usually involves some subjective evaluation by a radiologist or other specialist. The variability of these interpretations is an issue of concern. More than 50 years ago, Birkelo *et al* (1947) reported that a study of the relative effectiveness of four methods of chest imaging for tuberculosis was inconclusive, because the variation of opinion among observers was greater than the variety of techniques. More recently, wide variability has also been found in the interpretation of the same set of mammograms by 108 randomly chosen radiologists (Beam *et al*, 1996). The reasons for such interpretive variability

Fig 2 | Images illustrating the potential for targeted imaging agents to stratify patients for therapy. Coronal images from two patients (top and bottom rows) of fluorine-18-labelled fluoroestradiol (FES; left column) and F-18-fluorodeoxyglucose (FDG; middle column) uptake before therapy, along with FDG uptake six weeks after hormonal therapy (right column). The patient in the top row is a 44-year-old woman who was previously treated with adjuvant tamoxifen and had a sternal recurrence of breast cancer 4 years later. Her sternal lesion had high pre-therapy FES uptake, which indicated a high level of oestrogen receptors in the tumour (arrowhead; image also shows normal liver and bowel uptake). Such tumours are more likely to respond to hormonal agents such as letrozole. FDG images taken before and after six weeks of letrozole treatment show a significant decline in FDG uptake, with subsequent excellent clinical response. The patient in the bottom row is a 69-year-old woman with newly diagnosed metastatic breast cancer that was not previously treated. Bone metastases were documented by multiple imaging modalities, including FDG-positron emission tomography (PET). Her pre-therapy FES scan showed no uptake in the bone metastases. The patient received multiple letrozole treatments with no response shown by the bone metastases, which is indicated by the post-therapy FDG-PET. (Images courtesy of D. Mankoff. Reprinted from *Sem Nucl Med* 34: 224–240 with permission from Elsevier.)

are not well understood, and the resulting clinical inconsistency is therefore difficult to minimize. Thus, an important goal for the future of molecular imaging is to develop objective quantitative technologies that are independent of observer variability. It will remain a major challenge for several reasons, including the presence of statistical noise in medical images and the fact that spatial resolution is not yet sufficient at the molecular level, thus making determination of the exact boundaries of tumours and normal tissue problematic. The development of automatic, or semi-automatic, computer assisted detection or diagnosis algorithms for clinical images is an important priority for research in the imaging sciences. At the same time, it may be unrealistic to expect that interpretation or evaluation of clinical

images can ever be made completely autonomous by computer algorithms. Therefore, there is a need to improve the understanding of the perceptual and cognitive processes involved in image interpretation, so that variability and errors can be minimized.

Many other methodological and developmental issues must be addressed for the full potential of *in vivo* molecular imaging to be realized. Development costs for imaging agents are substantial and may be similar in magnitude to drug-development costs. Industry may view these costs as prohibitive because the clinical value of the agent under investigation is not likely to be known and the expected market value for any imaging probe is far less than that of a successful therapeutic. To

overcome this bottleneck, the US National Cancer Institute and the FDA have set up an Interagency Oncology Task Force. One of its goals is to define a developmental pathway to 'first in man' studies that would allow a much more efficient evaluation of the clinical merit of targeted imaging probes in order to decrease developmental time and costs. Once new imaging probes pass preclinical and safety testing, they must be evaluated in phase II and III studies for their clinical applications, including early detection, diagnosis, staging, prognosis and measuring tumour response to therapeutic intervention. Many of the imaging probes being developed will probably be sufficiently robust for the FDA to accept them as biomarkers for cohort selection, for measuring pharmacodynamic endpoints in dose setting, and as potential biomarkers for clinical use.

Medical imaging has been an important part of medical care for more than a century. During the past 25 years, it has expanded beyond the use of X-rays to include a variety of technologies and to achieve anatomical resolution that is now in the sub-millimetre range. Anatomical and structural imaging will always be essential in health care, but the research focus of the next few decades will be on the development of methods that give quantitative, functional or molecular information with very high spatial fidelity. These improvements in spatial resolution and signal sensitivity in imaging systems are needed to meet the future demands of molecular medicine.

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