Molecular imaging in a (pre-) clinical context

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Abstract

Molecular imaging can be defined as imaging of biological processes in a living organism at the molecular and/or cellular level. To achieve this goal, genetic information and new chemistries have to be combined into new imaging probes detectable by sophisticated imaging techniques. In contradistinction to conventional imaging, which mostly detects architectural, or morphological distortions (a late event), molecular imaging should be able to detect molecular changes that are at the basis of diseases. The detection of subtle pathologic changes in early, asymptomatic disease should have a tremendous impact on healthcare as a whole. (Acta gastroenterol. belg., **2008**, 71, **308-317**).

Introduction

Although a few years ago, the majority of the chemotherapies applied to oncologic patients were non-specific (i.e. preferential uptake by highly divided cells), new specific treatments directed against specific molecules are now emerging. The presence and/or absence of a specific receptor is now of paradigm importance for the determination of the best treatment. For example, cancers are known to be heterogeneous, certain cancers over-express epidermal growth factor receptor (EGFR), while others over-express Her2/neu receptors. The cancers over-expressing EGFR could be treated with an anti-EGFR anti body (as cetuximab), while Her2/neu over-expressing cancer could be treated with an anti Her2/neu antibody (as trastuzumab) (1,2). Nowadays, the presence of specific molecular targets on tumors is mainly determined by immunohistochemistry on biopsies. This approach has two major drawbacks. The first one is its relative invasiveness and the risk of associated complications, like hemorrhage or infection. The second one is due to tumor heterogeneity, since a small sample of the tumor being not necessarily representative of the entire tumor. Hence, the possibility of imaging the presence or absence of a specific molecular marker on a tumor *in toto* becomes now of prime importance and molecular imaging should be able to play this role. The present review is a comprehensive overview of existing and emerging methodologies for the molecular imaging in a clinical context, mostly based on magnetic resonance imaging (MRI) and fluorescence imaging.

From "conventional" imaging to "molecular" imaging

The term "conventional " imaging techniques may be used to describe the large body of modalities that are mainly used to display macromorphologic features of disease, namely Ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI).

These imaging methodologies still play a key role in the diagnosis and staging of solid tumors and metastatic sites ; detection and guidance of minimally invasive treatment. Given the high impact of oncologic imaging on cancer diagnosis, it is imperative that physicians involved with cancer care have an understanding of the tremendous potential and the impressive technological progress that is currently underway in the area of "conventional" imaging, such as whole-body MRI or hybrid imaging technologies combining Positron-emission tomography (PET) or single-photon-emission computed tomography (SPECT) with CT or MRI.

Nonetheless, despite the impressive advances in these "conventional" imaging techniques, our ability of differentiating between normal and transformed tissue by conventional imaging techniques, i.e. anatomical, structural, and endoscopical approaches is still limited, and "conventional" imaging techniques often fail to provide the essential information which is vital for the proper management of cancer patients, namely, about the presence or absence of microscopic tumor deposits and the viability of tumor tissue after treatment. Many aggressive, internal tumors, containing as few as 500'000 cells (~2 mm in diameter) are likely to pass undetected through most body-region scans, including CT, MRI, ultrasound, radionucleotide, and metabolic PET imaging because these imaging techniques are mostly based on bulk tissular features. Therefore, the resulting signal is epiphenomenal, being merely an expression of the sum of non-specific interactions of the imaging device with the tissue's structure, physiology and/or pathology. The degree to which a specific tumor can be visualized by these conventional methods is merely a function of its ability to differentially scatter, absorb, or emit radiation as compared to the healthy neighboring tissue. In addition, such modern imaging techniques are often cost intensive and time consuming. Furthermore, the interpretation of the resulting images is non-unequivocal and sometimes needs extensive experience of the physician.

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A variety of contrast materials are now commercially available for US, CT and MRI. Each of them has a specific pharmacodynamic behavior and distribution pattern in the different body compartments.

The most common imaging agents are non-specific small compounds, which have vascular distribution with leakage into the extracellular space.

These small molecules increase contrast of pathologies by differential rates of tissue/tumor perfusion, vascular leakage and clearance. Specifically, these molecules can help distinguish normal tissues (non-leaky) from tumoral ones (more leaky). Such small molecules are used everyday in clinics and comprise iodine for enhancement of computed tomography (CT) and gadolinium for magnetic resonance imaging (MRI) (3). The same approach could also be applied for fluorescence imaging (Fig. 1). In this example, fluorescent tracers of different molecular weight have been intravenously injected into tumor-bearing mice. Injection of a high molecular weight compound (50 kDa) does not increase the contrast between normal and abnormal tissues, since the relatively big hydrodynamic diameter of the compound precludes its extravasation, even from leaky vessels of the tumor. On the other hand, intravenous injection of a 30 kDa compound allows increasing the contrast between normal and abnormal tissues. This is based on the fact that 30 kDa is still too big to extravasate from a normal vessel, but small enough to extravasate form a more leaky vessel. Non-specific accumulation of molecules inside a tumor is known as the enhance permeability and retention (EPR) effect (4,5). The non-specific accumulation of small molecules is due to 1) an increase permeability of neo-vessels and 2) a poor lymphatic drainage inside the tumor, limiting the clearance of the accumulated compound. Hence, the size of small molecules could be optimized to enhance their accumulation inside a tumor (Fig. 1). The problems associated with this approach include the quite limited tumor to background ratio (making visualization sometimes difficult) and the lack of any specific cellular and molecular information.

In the past few years, new contrast media for MR cellular imaging have been approved for clinical used. These new contrast media consist of 1) iron oxide nanoparticles (ferumoxide, Endorem®, Advanced Magnetics and Guerbet or Ferucarbotran, Resovist®, Schering), which are taken up by the reticulo-endothelial system (RES), 2) gadolinium chelates a) gadobenate dimeglubine (Gd-BOPTA, Multihance®, Bracco Diagnostics, Inc) and b) Gadoxetate (Gd-EOB-DTPA, Primovist®, Schering), which are taken up by hepatocytes and 3) manganese chelate (Mangafodipir trisodium, Teslascan®, GE Healthcare) which is also taken up by hepatocytes.

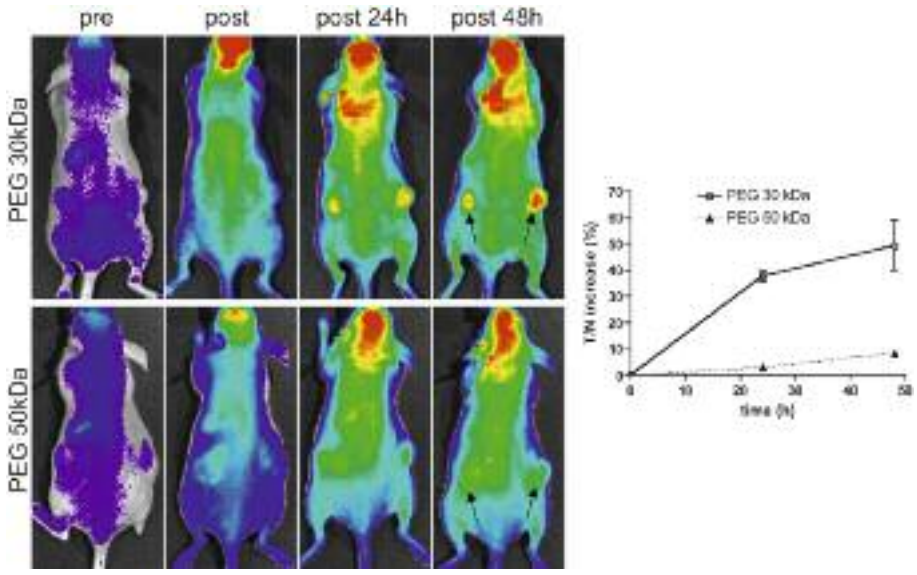

Fig. 1. — Enhance permeability and retention (EPR) effect of different sized PEG

Orthotopic PC3-M luc C6 tumors were implanted into the paralumbar region of nude mice and imaged after intravenous injection of a 30 kDa fluorescent poly-ethylene glycol (PEG) and of a 50 kDa fluorescent PEG, line A and B, respectively. A size-optimized small molecules will accumulate in the tumor (line A), whereas a bigger compound (too big to freely extravasate) will not (line B). The tumor to noise (T/N) increase over time is also presented. Please note that even if the tumor (arrows) imaged with the size optimized small molecule is easily visible, that fluorescent background is still very high (compare with the background of figure 7 : smart probes).

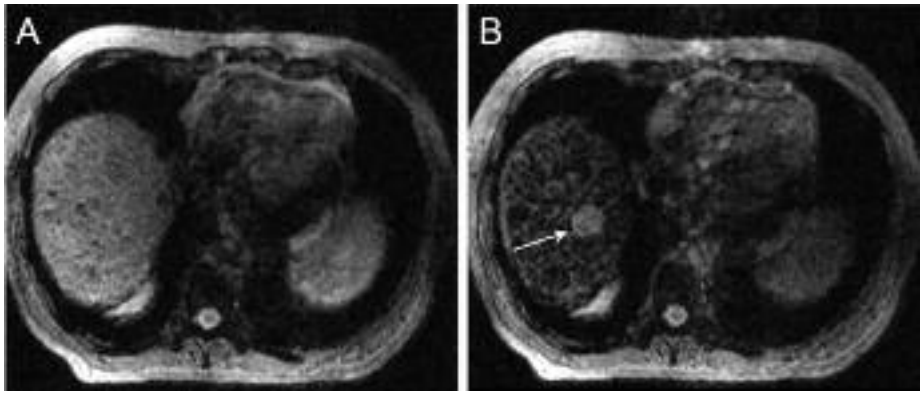

Fig. 2. — Iron oxide nanoparticle as RES contrast media for MRI

MR images of the hepatic dome before (A) and after injection of iron oxide nanoparticle (Ferumoxide : 1.4 ml) (B). The lesion (arrow) is clearly visible after injection of the iron oxide nanoparticle, but not before. The increase of contrast between the lesion and the liver is due to a darkening of the normal liver. Moreover, please note the heterogeneous aspect of the liver, corresponding to cirrhosis. In this context, the hepatic nodule is highly suspect of a hepatocellular carcinoma.

Iron oxide nanoparticles as cellular imaging agents for MRI

The first approved iron oxide nanoparticle for liver imaging was ferumoxide in 1984. As other particulate formulation, iron oxide nanoparticles are cleared from the blood by the RES (6). Hence, iron oxide nanoparticles are taken up by Kupffer cells in the liver, as well as by the spleen. This type of contrast media has a strong T2 and T2* effects on T2 or T2*-weighted MR images. On this type of MR sequences, iron oxide nanoparticles appeared as hypointense signal (black). Hence, the contrast between liver pathologies devoted of kupffer cells and the normal surrounding liver will be enhanced (Fig. 2). Intravenous injection of iron oxide nanoparticles allows clear visualization of a tumor located on the dome of the liver, whereas the lesion was not visible before injection. Hence, this hepatic lesion is devoted of Kupffer cells, opening a differential diagnosis of hepatocellular carcinoma or secondary lesions. This type of imaging has been proven useful in clinical practice in 1) increasing the sensitivity and specificity of diagnosis of malignant liver tumors (7) and 2) in characterizing liver hemangiomas (8).

Gadolinium chelates as hepatospecific imaging agents for MRI

Nowadays, two gadolinium chelates are approved for liver imaging, i.e. gadobenate dimeglubine (Gd-BOPTA, Multihance®, Bracco Diagnostics, Inc) and Gadoxetate (Gd-EOB-DTPA, Primovist®, Schering) both contrast media being taken up by hepatocytes. These types of contrast media have a strong T1 effect on T1-weighted sequences. On this type of sequences, Gd-chelates will appear hyperintense (white). The contrast between normal liver (white) and liver pathologies devoted of normal hepatocytes will again be increased (9,10).

Manganese chelates as hepatospecific imaging agents for MRI

Mangafodipir trisodium (Mn-DPDP, Teslascan®, GE Healthcare)-mediated liver imaging is another example of contrast agent that is also approved for clinical use. As hepatospecific Gd-chelates, this type of contrast media is taken up by hepatocytes. Once again, the differential uptake between normal and abnormal liver (devoted of normal hepatocytes, primarily hepatocellular carcinoma and metastases) is at the basis of the increased contrast (Fig. 3). This example illustrates the uptake of mangafodipir trisodium by normal hepatocytes (the liver becomes hyperintense), whereas the lesion, which is devoted of normal hepatocytes, does not take up the contrast media and hence stays black. Manganese-based contrast media have also been proven to ameliorate the sensitivity of detection of liver secondary lesion in a clinical context (11).

The advantage of cellular contrast media is that information about cellular content is amenable through imaging. Although this may enhance the impact of "conventional imaging" considerably and may be considered as the first step toward specific imaging, these approaches still lack molecular information.

For the above reasons, future imaging techniques should aim at earlier detection of disease in preclinical stages and thus need to be much more sensitive and disease-specific as compared to the "conventional" modalities. "Molecular" imaging is one possible answer to these specific needs. Molecular imaging can be defined as imaging of biological processes in a living organism at the molecular and/or cellular level. To achieve this goal, genetic information, proteomics, and new synthetic strategies have to be combined into new imaging probes detectable by sophisticated imaging techniques. In this context, magnetic resonance imaging

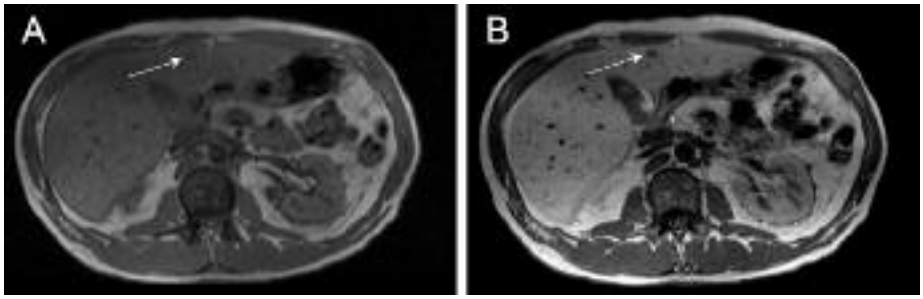

Fig. 3. — Mangafodipir trisodium as hepatospecific contrast media for MRI

MR images of the liver pre (A) and post injection of 25 ml of mangafodipir trisodium. The normal liver becomes hyperintense after injection of the contrast media. The lesion (arrows), which is devoted of normal hepatocytes does not. Hence, the differential uptake of the contrast media is at the basis of the increased contrast between the normal liver and the lesion. In this patient, where a primary tumor was known, the lesion is highly suspect of metastases.

and optical methods hold great promise for such imaging purposes (12,13). While the lower detection limit for tumors in human subjects is at best around 500'000 cells, optical methods have already been shown to reveal as little as approximately 1000 cells in animal models (14). Fluorescence imaging is one of such optical methods, which is characterized by its extremely high resolution and sensitivity. Furthermore, combined with modern endoscopic instrumentation, fluorescence imaging can be rendered minimally invasive and can give nearly unlimited access to hollow organs.

Molecular imaging techniques

Molecular imaging techniques are characterized by so-called imaging probes, i.e. the tagging mechanism used to study a specific biologic process or phenomenon. Overall, one might define three general classes of imaging probes that have been used to aid in visualization of pathologies : 1) small molecules, 2) actively targeted contrast-media and 3) activatable probes also termed as smart probes.

Small molecules

This group corresponds mainly to the above-described contrast materials, many of which are commercially available for their use with CT or MRI or both. However, true molecular information cannot be achieved with these agents, but requires the use of targeted contrast media and/or "smart" probes.

Targeted approaches

To overcome the limitations of non-specific imaging, targeted approaches have been developed to increase the localization of contrast-enhancing molecules in pathologies and to reduce their uptake in normal tissues. Such approaches consist of coupling specific targeting moieties to a contrast media. The targeting moiety has to be specific for a receptor over-expressed in a pathology, and could be an antibody, a sugar, or a peptide. The contrast media could be iodine (for CT imaging), gadolinium or iron oxide nanoparticles (for MRI) and fluorophores (for optical imaging). The benefits from such an approach for highly specific *in vivo* imaging were already evident in the mid 80s of the last century. Mach and coworkers have prepared antibodies of carcinoembryonic antigen with fluorescein (15) or a radioactive tracer (16) and tested their selectivity in humans. Although highly specific, not all tumors showed up in their studies. Furthermore, fluorescence distribution within the samples following resection was highly heterogeneous. These observations were preliminarily attributed to the large size of the full chimeric antibody, that impeded its penetration into the tumor mass.

More recently, multimodal contrast media have been synthesized, allowing combining the advantages of different imaging modalities. For example, using iron oxides nanoparticles coupled to a fluorophore allows realizing MRI and optical imaging on the same animal/specimen. One potential drawback of using targeted conjugates is the fact that target-to-background ratios can be limited by receptor density and/or availability, limited clearance kinetics from the interstitial space, and/or nonspecific cellular uptake or adhesion of certain fluorescent probes. In particular, it may be difficult to differentiate specifically bound from unbound ligands. Therefore, imaging is usually performed after non-specifically distributed excess probe has been cleared from the body.

Targeting activated endothelial cells of neo-vessels

Endothelial cells, the inner lining of the blood vessels, are probably one of the most straightforward systems to target by imaging techniques in the human body, since they are in direct contact with any compound injected in the circulating blood, without the need for the compound to pass the endothelial barrier to reach its target. Secondly, endothelial cells are activated in cases of inflammation or neo-vessels formation (angiogenesis).

These activated endothelial cells are known to express new membrane molecules in abundance, such as integrins and/or selectin, whereas quiescent endothelial cells do not. This differential expression between quiescent and activated cells is one of the major prerequisite for molecular imaging.

Angiogenesis, which is the development of new blood vessels from pre-existing vessels, is a tightly regulated process that plays a critical role in a variety of normal physiological events, including trophoblast implantation, wound healing and embryonic development (17-20). However, uncontrolled neovascularization can contribute to a number of pathological conditions like proliferative retinopathy, tumor growth and metastasis, and age-related macular degeneration (18,21-25).

The endothelium lining neovessels is an activated-type of endothelium and is known to express several surface proteins in abundance. One of these surface proteins is the integrin alpha(v)beta3, which plays important role in cell-cell and cell-matrix contacts (26,27). Integrin alpha(v)beta3 is strongly expressed on activated endothelial cells but also on tumoral cells (28-32). Another surface protein that can be easily targeted on neo-vessels is E-Selectin. E-selectin (also known as CD62E) is an inducible cells adhesion molecules belonging to the family of C-type lectin family (33). E-selectin is mainly implicated in the initiation of adhesion of neutrophils to vascular endothelium in an early step of inflammatory process (34,35), hence playing an important role in the control of leukocytes diapedeses (36). E-selectin play also an important role in angiogenesis in endothelial proliferation (37), migration (38), and tube formation (39).

Imaging of integrin alpha(v)beta3

The alpha(v)beta3 integrin can be specifically targeted with a small peptide containing the Arg-Gly-Asp (RGD) sequence or with a peptidomimetic.

Numerous imaging modalities have been tested to image the presence of integrin alpha(v)beta3. Among them, nuclear medicine (40,41), MRI (42) and optical imaging (43-47).

All these reports demonstrated the possibilities to specifically target the expression of integrin alpha(v)beta3 in vivo by imaging. Imaging of integrin alpha(v)beta3 has now been applied on patients with good results for tumor imaging (48). Moreover, response to a anti alpha(v)beta3 treatment could be predicted early after the beginning of the treatment by molecular imaging (48), which allows attributing the right subpopulation to these new types of expensive treatments and to stop the treatment in case of non-response.

Although the first reports on alpha(v)beta3 imaging were thought to be specific for neo-vessels imaging, it is now known, that integrin alpha(v)beta3 is overexpressed on activated endothelial cells, as well as on the surface of many tumor cells. Hence, integrin alpha(v)beta3 imaging has to be considered as tumor specific, and not angiogenesis specific. As said previously, multimodal contrast media have also been synthesized, combining the advantages of MRI and optical imaging (49). In this report, MRI was used to image the tumor *in vivo* in its entirety. Optical imaging was used to image the tumor in vivo by optical tomography (50), as well as to co-localize the fluorescence at a cellular level within specific cells (Fig. 4, reprinted with the permission of Neoplasia Press, Inc from (49)). Hence, this combination of contrast media integrates the advantages of high-resolution fluorescence microscopy and *in vivo* MRI of intact and opaque tissues.

Imaging of E-selectin

Numerous reports on E-selectin imaging have been published. The first reports were based on radiolabeled antibody (51) with then an evolution toward MRI (52,53) and finally to fluorescence imaging (54). As it was the case for the integrin alpha(v)beta3, it became clear with time that E-selectin is not only expressed on activated endothelial cells but also directly on tumor cells. Nevertheless, E-selectin targeted probes are tumor specific and could be used to sense the presence or absence of E-selectin receptor (Fig. 5, reprinted with the permission of Neoplasia Press, Inc from (54)). In this paper, we showed that E-selectin expression on tumor could be specifically targeted and imaged by epifluorescence imaging. The specificity of binding was demonstrated by synthesizing two optical probes. The first one contained a fluorophore and an E-selectin binding peptide, which is known to bind to E-selectin with an affinity in the low nanomolar range. The second one contained another fluorophore and a scrambled E-selectin peptide, which do not bind to E-selectin. The specificity of binding is then achieved by co-injection of both probes. The ratio of fluorescence between the targeted and non-targeted probe gave an estimation of the specificity.

Reaching the extra-vascular compartment

Using small enough targeted nanoparticles allow to target extra-vascular cells. The general principle of extravasation and uptake of nanoparticles into cells through endocytic routes is now well accepted. Discussion in many review articles suggest that, for this process to occur efficiently, particle sizes need to be less than 100 nm (4).

Targeting normal pancreatic exocrine cells

Hence, by using 30nm targeted nanoparticles it should be possible to reach a molecular target outside the vasculature. Proof-of-principle of extra-vascular targeting has already been published (55). In this report, specific molecular information on cells outside the vasculature

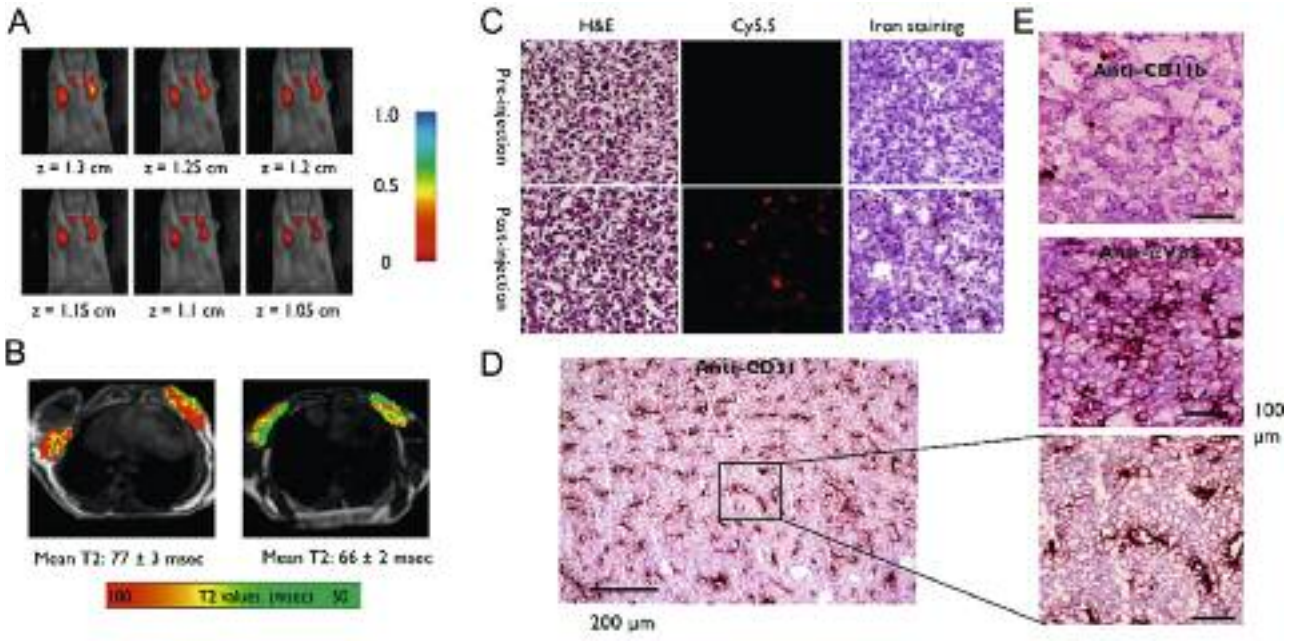

Fig. 4. — Multimodal (magneto-optical) nanoparticles targeted against integrin alpha(v)beta3, reprinted and modified with permission from (49).

Nanoparticle uptake by tumor cells of the BT-20 tumor : Imaging the accumulation of the targeted nanoparticle by fluorescence tomography (A) and magnetic resonance (B). Fluorescence tomography allows to reconstruct fluorescence images in 3D and to indicate the depths from where the signal comes from. The color scale indicates relative nanoparticle concentration in each plane. MRI of nanoparticle accumulation in the tumor (B). Tumors are presented as colorized T2 maps superimposed over a T2-weighted MR image (TR = 2000 ; TE = 50) at 24 hours postinjection. Values are average tumor T2 values ± 1 SD. In presence of iron oxide, the T2 values of the tumor decreased. Hence, a shortening of T2 values corresponds to iron oxide presence.

The distribution of the targeted nanoparticles within the tumor was then examined by iron staining or fluorescence (C). After nanoparticles injection, iron and fluorescence are broadly distributed throughout the tumor. The distribution of CD31 (endothelial cells) by immunohistochemistry is presented at low magnification (D). The tumor is highly vascularized. Distribution throughout the tumor of CD31 (endothelial cells), CD11b (macrophages), and alpha(v)beta3 is also presented (E). Alpha(v)beta3 integrin expressed in tumor cells is broadly distributed throughout the tumor, such as iron or Cy5.5 fluorescence from the nanoparticle. The figure demonstrated the unique advantages of combining the *in vivo* imaging by MRI and/or fluorescence, with the very high resolution of microscopy.

was achieved (Fig. 6, reprinted with permission from (55), copyright 2006, American Chemical Society). In this paper, we choose to target bombesin receptors, which are expressed on normal exocrine cells of the pancreas, but not on pancreatic ductal adenocarcinoma (56). The differential expression of surface molecules between normal and abnormal compartment is, as previously said, a major prerequisite. In this approach, we choose to target the normal compartment of the pancreas with targeted iron oxide and hence to increase the contrast by making the normal pancreas turning hypointense (black). Pancreatic tumors were clearly visible as hyperintense spot (white spot) after injection of the targeted nanoparticles, whereas the lesion was more difficult to see before injection.

These novel imaging capabilities open new roads in our assessment of pathology. It should now be possible not only to tell physicians that a tumor is present, but also to characterize them from a molecular point of view.

Activatable probes or smart probes

The third general class of imaging contrast agents are so-called smart probes. These agents change their physical properties after specific molecular interaction and are sometimes referred to as molecular beacons. The approach of using *in vivo* optical (near-infrared) smart probes has been pioneered by the group of Ralph Weissleder at Mass General Hospital to detect proteolytic activity (57). It is based on a quenching/dequenching paradigm. The probes are optically silent in their native (quenched) state and become highly fluorescent after enzyme-mediated release of fluorochromes, resulting in signal amplification of up to several 100-fold, depending upon the specific design (58). When several fluorophores are placed in close proximity to a polymeric backbone, efficient fluorescence resonance energy transfer (FRET) can occur between fluorophores thus rendering the entire compound non-fluorescent in this configuration. When

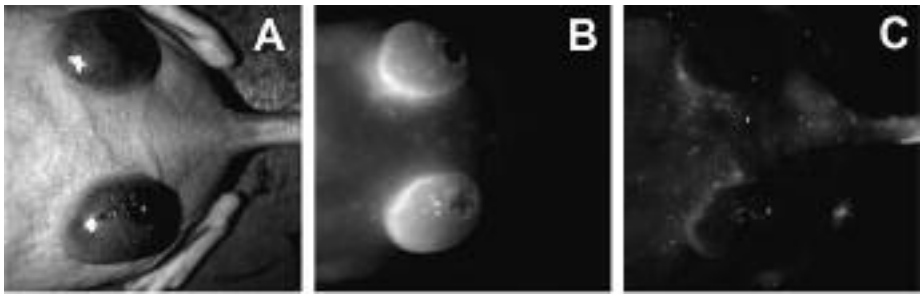

Fig. 5. — E-Selectin imaging by optical imaging, reprinted with permission from (54)

Fluorescence imaging of E-selectin with an E-selectin binding peptide (ESBP) coupled to a fluorescent iron oxide nanoparticle (CLIO = cross-linked iron oxide) in a subcutaneous tumor model. Lewis Lung Carcinomas (LLC) were implanted in nude mice followed by injection with a mixture of ESBP–CLIO(Cy5.5) and the control scrambled-nanoparticles, Scram–CLIO(Cy3.5). White light image (A), Cy5.5 fluorescence (B), and Cy3.5 fluorescence (C) are shown 24 hours after injection. The Cy5.5 channel shows a high fluorescence, whereas the Cy3.5 channel shows none, hence demonstrating a preferential uptake of the targeted nanoparticles.

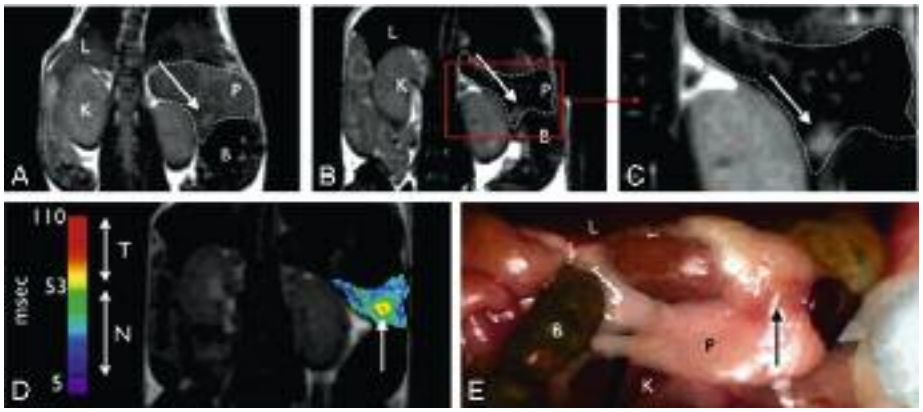

Fig. 6. — Pancreas imaging, reprinted with permission from (55)

Magnetic resonance images of the pancreas on T2-weighted sequences before and after intra-venous injection of a nanoparticle targeted to normal pancreas are presented.
The presence of the tumor (arrow) is faintly visible on the pre-injection images (A), whereas the tumor is easily seen after injection of the nanoparticle targeted to normal pancreas (B and C). C Image from B at higher magnification. (D) Postcontrast agent, colorized T2 map of a pancreas with implanted tumor. Tumor has higher T2s than normal pancreas (T2 of the normal tissues was decreased due to the presence on iron oxide nanoparticle). Abbreviations : L, liver ; P, pancreas ; K, kidney ; B, bowel. (E) Photograph of partially dissected mouse with pancreas and tumor visible.

an enzyme specifically cuts the probes and allows for the fluorophores to increase their mutual distance, fluorescent signal becomes restored (signal amplification after specific molecular information). The same approach has also been applied to MR contrast agent, where the magnetic property of the probe changes after enzyme activation. One of the key paper on this mechanism was published by Louie *et al.* (59). In this paper the authors demonstrated that a gadolinium-containing contrast media can be activated by a beta-galactosidase, if the access of water to gadolinium is restricted by a sugar, which can be cleaved. The contrast effect of the compound is only visible when water can interact with the gadolinium.

Of note, nonspecific and targeted agents have no amplification, and newly developed experimental MRI probes have < 3-fold amplification.

The probes typically consist of three biocompatible building blocks : (a) a delivery vehicle ; (b) near-infrared (NIR) fluorochromes ; and (c) enzyme-specific peptide substrates as linker between the former two entities. Since then, several different NIR probes based on this principle have been assembled with respect to the proteolytic activity in different kinds of pathologies (57,60-64).

Enzyme specificity is imparted through the use of enzyme cleavage-specific peptide sequences, which can

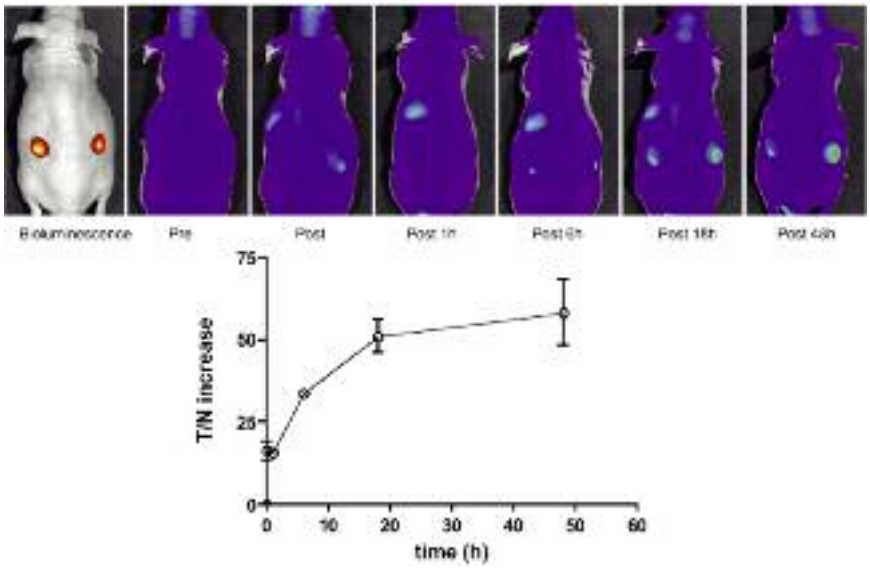

Fig. 7. — Smart probes

PC3M-luc-C6 cells were implanted in the paralumbar region of athymic nude mice. Bioluminescent signal (first images) confirm the presence of 2 small subcutaneous tumors. Before injection of the uPA probe (sequence specific : Gly-(L)Ser-Gly-(L)Arg-(L)Ser-(L)Ala-Gly), the tumors do not show fluorescent signal. After specific cleavage took place, the tumor start to fluoresce. Fluorescent signal is visible from 6h to 48 h. The tumor to noise (T/N) increase over time is also presented.
Please note that the fluorescent background is very low (compare with the fluorescent background of fig. 1 : effect of different sized PEG).

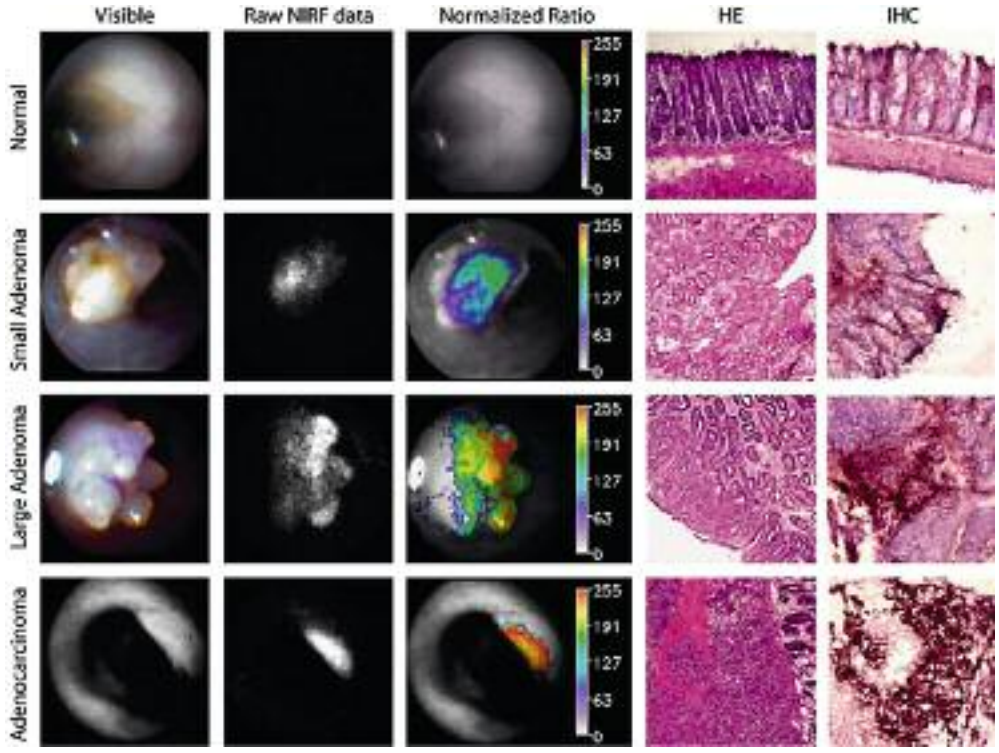

Fig. 8. — Colonoscopy after injection of a cathepsin-B specific probe, reprinted with permission from (66)

In vivo mouse colonoscopy. First row, Healthy colon is shown. Neither in the high near infra-red (NIR) (protease) channel nor by ratio imaging is a signal detected. Second row, Small APCMin+/– adenoma. Third row, Large APCMin+/– adenoma. Fourth row, Orthotopically implanted adenocarcinoma. The NIR protease channel shows areas of consistently high signal intensity and high signal-to-background ratio corresponding to the areas of neoplasia in rows 2 to 4. Ratio imaging shows moderately elevated values for the adenomas and high values for the adenocarcinoma. The corresponding H&E stained sections are presented in the fourth column (orig. mag. ×100). Immunohistochemistry (IHC) demonstrates progressive levels of cathepsin B from healthy colon to adenoma to adenocarcinoma.

be varied depending upon the desired protease to be visualized. For a more extensive list of examples of enzyme substrates used in such probes, the reader is referred to table 1 of reference 65. Moreover, other proteolytic processes are amenable to this activation scheme. This approach has several major advantages over simple targeting : (a) a single enzyme molecule can cleave multiple fluorochromes, resulting in one form of signal amplification ; (b) reduction of background signal of several orders of magnitude is possible because the quenched probe is optically silent when injected and remains so until it is activated by its target ; and (c) very specific enzyme activities can potentially be interrogated. All of these lead to better visualization of tumors based on their profile of enzyme overexpression.

Probes activation after intravenous injection is demonstrated on a heterotopic tumor model of PC3M-luc-C6 cells (Fig. 7). The tumors are almost non-fluorescent before injection of the uroplasminogen activator (uPA) activatable probe. After 6 h, the tumor becomes faintly visible, whereas after 18 and 48 hours, the tumor was easily demonstrated. Instead of using epifluorescence, the same approach could be rendered minimally invasive and applied to endoscopic techniques (66). In this paper, we take advantages of over expression of Cathepsin-B in colon tumor (67). After Cathepsin-B activatable probe injection, we were able to visualize and characterize small adenoma, large adenoma and adenocarcinoma based on fluorescent ratio (Fig. 8, reprinted with permission from (66)).

Summary

Despite the impressive progress of US, CT, MRI and PET and the introduction of new contrast materials, these current “conventional” imaging modalities are not sufficiently sensitive or specific to detect neoplastic disease in a suffiently early stage. Due to the advance of proteomic and genomic, new specific treatments are now emerging for clinical use. These treatments are specific for a given pathology and most of the time for the expression of a molecule at the surface of pathological cells. The presence (or absence) of such a molecule in a given patient is a primary importance. Therefore, new imaging techniques are required in order to detect neoplastic diseases at a much earlier stage. Molecular imaging techniques carry the potential to fulfill this need. In addition they may enable the radiologist to visualize the presence of a given molecule inside a pathologic process and hence to direct the treatment for a given patient, a principle also referred to as personalised treatment.

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