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Fundamentals of Molecular Imaging: Rationale and Applications With Relevance for Radiation Oncology

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Molecular imaging allows for the visualization and quantification biologic processes at cellular levels. This article focuses on positron emission tomography as one readily available tool for clinical molecular imaging. To prove its clinical utility in oncology, molecular imaging will ultimately have to provide valuable information in the following 4 pertinent areas: staging; assessment of extent of disease; target delineation for radiation therapy planning; response prediction and assessment and differentiation between treatment sequelae and recurrent disease. These issues are addressed in other contributions in this issue of *Seminars in Nuclear Medicine*. In contrast, this article will focus on the biochemical principles of cancer metabolism that provide the rationale for positron emission tomography imaging in radiation oncology.
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Molecular imaging can be defined as "the visual representation, characterization and quantification of biological processes at the cellular and subcellular level."[1] Imaging techniques available for this purpose include nuclear medicine techniques (in particular positron emission tomography [PET]), magnetic resonance imaging (MRI) with dedicated imaging sequences and molecular contrast agents, and optical imaging (including bioluminescence and immunofluorescence imaging). The goals of molecular imaging include

- To improve our understanding of tumor biology (cancer development, progression, and metastasis);
- To visualize and quantify noninvasively the presence and biologic status (active/inactive) of receptors and pathways involved in tumor development and progression;
- To study the pharmacokinetics and pharmacodynamics of novel anticancer "targeted therapies"; and
- To measure and predict the response to such novel anticancer drugs early during the therapy. (Here, one would particularly like to know how sensitive and specific the molecular imaging information is and whether molecular imaging as part of treatment monitoring will ultimately improve patient outcome, for instance, by avoiding side effects from continued drug exposure if that drug has no therapeutic efficacy or when secondary resistance develops.)

Molecular imaging thus differs greatly from anatomic imaging, which is used to visualize structural abnormalities that are usually already the endpoint of the underlying molecular process. The need for molecular imaging has also been recognized by radiation oncologists. Traditionally, radiation therapy design has been based on the concepts of the anatomically defined gross tumor volume (GTV), planning target volume (PTV), and clinical target volume (CTV). However, it has become obvious that target design based on structural abnormalities alone has many limitations, leading to overtreatment of healthy tissues or undertreatment of sites of disease. The new concept of a biologic target volume (BTV) therefore also considers functional parameters that may affect the response to irradiation, such as cancer metabolism, proliferation and hypoxia.[2]

The radiosensitivity of malignant tumors depends on many factors, including cell cycle phase, growth fraction, dose rate, radiation damage repair capacity, and the presence and severity of hypoxia. Some of these features can be studied by molecular imaging and may be considered when designing a biologic target volume, determining the radiation dose schedule, and determining the need for adjuvant chemotherapy or treatment with specific radiation sensitizers.

A number of recent review articles have discussed molecular imaging in cancer and other diseases.[3-10] For the purpose of this article, we will only address positron emission tomography (PET), the most widely used technique of molecular

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imaging. This article will focus on a few principles of biologic imaging with clear clinical applicability and expected relevance for radiation oncology in the near future.

In 2000, Douglas Hanahan and Robert Weinberg, a biophysicist and a biologist, published their now much-quoted paper "The hallmarks of cancer." These hallmarks were defined as limitless replicative potential, self-sufficiency in growth signals, insensitivity to subgrowth signals, sustained angiogenesis, tissue invasion and metastasis, and evading apoptosis.[11] One might add that a number of metabolic abnormalities are also characteristic for cancer.[12-14] Of those, the upregulation of aerobic glycolysis, as first described by Warburg and coworkers,[15,16] is probably the only discovery that, thus far, has had an obvious and long-lasting impact in the clinical arena in the form of ^{18}F-fluorodeoxyglucose (FDG)-PET imaging for the detection, staging and response assessment in cancer.

The rational application of molecular imaging in oncology requires a fundamental understanding of biochemistry and molecular biology. The aim is to characterize (image) certain features of the malignant phenotype (such as the presence and activation status of receptors, activation or inhibition of tumor pathways, response to external stress in the form of chemo- or radiation therapy, mechanisms of cell death) that may have implications for cancer diagnostic, assessment of prognosis, choice of therapy, and determining treatment response. Ultimately, however, molecular imaging will have to show a measurable clinical utility, for instance by providing valuable information in the following 4 pertinent areas:

- staging; assessment of extent of disease;
- target delineation for radiation therapy planning;
- response assessment and prediction; and
- differentiation between treatment sequelae and recurrent disease.

These clinical questions can likely be addressed by using currently already available PET tracers for imaging fundamental properties of cancer: glucose and fatty acid metabolism, proliferation, hypoxia, angiogenesis, and apoptosis. Conceptually and for the interpretation of imaging data, it is important to realize that any malignant tumor consists of cancer cells and surrounding stroma and can thus be considered a "community of cells." One should therefore distinguish between tumor-specific properties (such as the expression of oncogenes and antigens, tumor metabolism, expression and activity of receptors and transporter molecules) and more general features (such as blood flow, hypoxia, inflammation, composition of tumor matrix, and the enzymatic breakdown of tissue barriers required for the process of metastases).

Glucose Metabolism

The first steps of glucose metabolism (cellular uptake and phosphorylation by hexokinase) can be traced using the glucose analog FDG. Aerobic glycolysis and an overall increase in glucose metabolism are characteristics of cancer cells as compared with normal tissue.[12,15,16] The increased glucose metabolism in cancer is mediated through increased expression and activity of glucose transporters (GluT) in the cell surface membrane[17] and through characteristic changes in glycolytic enzyme expression and activity.[18,19] Despite the presence of oxygen, glucose is largely metabolized to lactate (aerobic glycolysis). These alterations in glucose metabolism are an early event in cancer development.[20] One line of evidence has implicated an activation and stabilization of the hypoxia inducible factor 1 (HIF-1), either as the consequence of intratumoral hypoxia or due to altered gene expression.[21] The HIF-1α subunit of HIF acts as a critical modulator of glucose metabolism in growth factor-dependent cancer cells: HIF-1α induces a switch from predominant aerobic glycolysis (favoring cell growth and proliferation) to anaerobic glycolysis (favoring survival in an oxygen-deprived microenvironment).[22]

Another, more recent line of evidence suggests that an activation of the oncogene Akt and its gene product, the serine/threonine kinase Akt, may be sufficient to stimulate the switch to aerobic glycolysis.[23] Of note, aerobic glycolysis is not just an epiphenomenon but is indeed necessary for growth and survival of the cancer cell. Among the postulated reasons for increased aerobic glycolysis in cancer is the fact that glycolysis can provide ATP faster than oxidative phosphorylation, that the products of glycolysis are required for fatty acid synthesis and the maintenance of nonessential amino acid pool during cell growth,[24] and that glycolysis provides the nucleotide precursors for RNA and DNA synthesis.[18,19] To some degree, the increased glucose metabolism and FDG accumulation in cancer cells may also reflect an adaptation to intermittent hypoxia.[12] However, although glycolysis is frequently activated in areas of hypoxia, high glucose metabolism is also observed in normoxic tumor zones and vice versa. Therefore, a high degree of hypoxia is not necessarily a surrogate marker for high glucose metabolism: tumor glucose metabolism is up-regulated for many reasons and, vice versa, high levels of hypoxia may result from high interstitial pressure within the tumor, thereby restricting substrate (glucose) delivery to the hypoxic cell.

FDG-PET has been applied in radiation oncology for the extent of disease evaluation and staging of many malignancies: detection of advanced disease may preclude irradiation with curative intent[25,26] and FDG-PET may delineate the locoregional disease (size and shape of primary tumor, nodal involvement) better than structural imaging in many circumstances,[25,27-30] leading to changes in GTV and/or PTV in up to two-thirds of cases.[26,29,31-36] Dose escalation to FDG-avid tumor subvolumes has been performed in pilot trials, based on the belief that these may represent the most aggressive cell populations that are less likely to respond to standard doses and may thus give rise to clinical recurrences.[37] The feasibility of this concept has been proven, but conclusive results are not yet available. Several studies have also shown that the incorporation of FDG-PET data improves the interobserver agreement among radiation oncologists for defining the GTV, which is generally recognized to be rather poor when using CT alone.[30,38] Residual abnormal FDG uptake after the end of radiation or concurrent chemoradiation therapy is suspicious for residual disease and thus an indicator of poor progno-

sis.[39,40] Many of these issues will be addressed in separate contributions in this issue of the Seminars.

Fatty Acid Metabolism

Many malignancies (including prostate, breast, head and neck, esophageal, gastric, hepatocellular, and colorectal cancers) are characterized by alterations in fatty acid metabolism, which can be summarized as the "lipogenic phenotype" of cancer.[13,41,42] Although glucose and fatty acid metabolism are interrelated, increased de novo fatty acid synthesis is also an independent mechanism in cancer pathogenesis, in particular through upregulation of the critical enzyme fatty acid synthase (FAS).[42-45] This upregulation of FAS occurs in response to growth factor receptor activation[46] or direct (ie, growth-factor independent) activation of receptor tyrosine kinases, which initiate or enhance signal transduction cascades, such as the Akt/PI-3-kinase pathway.[47-49] The common element through which these pathways induce transcription (and thus increased synthesis) of FAS is the sterol regulatory element binding protein: this protein binds to the sterol regulatory element in the promoter region of FAS on the DNA.[50] In addition to increased de novo synthesis, greater levels of FAS in cancers cells also can be the result of decreased enzyme degradation due to removing ubiquitin from FAS, thus preventing FAS from proteasomal degradation.[51]

As a practical clinical consequence, the imaging of fatty acid synthesis and, thus, indirectly the activity of the FAS enzyme, should enable us to study cancer development, aggressiveness, and its response to therapies aimed at FAS inhibition or degradation.[52,53] On the basis of the rationale stated previously, one can imagine that this class of drugs should be useful in the combination therapy of malignancies with documented FAS over-expression, such as prostate cancer.

Increased fatty acid synthesis ultimately leads to increased membrane lipid biosynthesis, for which choline kinase (ChoK) is a critical enzyme in cancer development and progression.[54-56] ChoK enables the conversion of choline to phosphatidylcholine, which is a major component of all membranes.[57] This has been exploited for cancer imaging with either MR spectroscopy[58] or labeled choline compounds,[59,60] which revealed an elevated choline peak as well as increased choline uptake and retention in cancer cells.

Fatty acid synthesis and membrane lipid synthesis can be imaged using radiolabeled acetate or choline.[61] (An increased choline peak in MR spectroscopy is similarly an indicator of malignancy.[62]) Beyond the investigation of tumor biology, imaging with agents tracing fatty acid synthesis may be of particular interest in malignancies that are not imaged well (eg, because of low uptake or urinary excretion) with the standard clinical PET tracer FDG. This malignancies might include prostate and bladder cancer, for which the clinical utility of ^{11}C acetate and ^{11}C or ^{18}F choline has been demonstrated or at least suggested. It might potentially also include scenarios in which FDG cannot reliably distinguish between inflammation/infection and cancer or between sequelae of treatment and residual malignancy (eg, radiation necrosis in the brain). Accordingly, clinical applications for imaging with labeled acetate or choline, of interest to radiation oncology, may included lymph node staging in primary or recurrent prostate cancer,[63,64] the localization of sites of recurrence in patients with prostate-specific antigen relapse,[65-68] the detection of bladder cancer and its nodal metastases,[69,70] the detection of hepatocellular carcinoma with acetate,[71] the detection characterization of malignant brain lesions with choline,[72] and the potential for differentiating between radiation necrosis and tumor recurrence in the brain.[72]

In contrast with FDG, for which an abundance of data are available, it is currently unclear whether the degree of acetate or choline uptake in cancer correlates indeed with the expression levels of critical enzymes and if it has any prognostic value. However, in one recent study, ChoK expression in lung cancer was an independent prognostic marker for disease-specific survival.[73]

Proliferation

Tumor cell proliferation can be imaged with labeled thymidine or thymidine derivatives. ^{11}C thymidine was long considered the gold standard for PET imaging because this agent is integrated into the DNA of proliferating tumor cells and because the degree of uptake accurately reflects DNA synthesis.[74] However, the use of ^{11}C thymidine generates images of inferior quality and requires complex modeling for image interpretation. ^{18}F-fluorothymidine (FLT) has therefore emerged as promising radiotracer for clinical use.[75] In vitro, in vivo, and in most clinical studies, tumor cell uptake of FLT shows excellent correlation with thymidine kinase-1 (TK-1) activity[76-78] and cellular or tissue markers of proliferation, such as the proliferating cell nuclear antigen or ki-67.[78-81] Phosphorylated FLT is trapped intracellularly but is not integrated into the DNA. The agent has been studied in a variety of cancers, including breast, lung, gastrointestinal, and head and neck cancers, sarcomas, malignant brain tumors, and lymphoma.[80-87] Although FLT uptake is lower than FDG uptake in many tumors, it shows better correlation with tumor cell proliferation.[81,84,87-89] It is expected that FLT will be of major impact in the response assessment of malignant tumors. To prove its clinical utility, FLT would have to be superior to FDG for this purpose, for instance by demonstrating treatment response earlier,[90] and/or more reliably, and by accurately distinguishing between treatment-induced inflammatory changes and tumor recurrence.[84] Initial clinical studies suggest that FLT can indeed be applied for the response assessment, for instance in breast cancer,[82] lymphoma,[91] or malignant brain tumors.[92]

In a small number of breast cancer patients, a 20% change in FLT standard uptake value (SUV) as compared with baseline was defined as significant, based on the fact that this was outside the 95% confidence interval for repeated measurements in the same patient.[93] However, more data are needed to establish the reproducibility of FLT measurements in larger groups of patients and a variety of tumor histologies. A potentially limiting factor may be the relatively low FLT uptake in many malignant tumors: A 20% change in SUV may

be more difficult to determine accurately in lower activity ranges, in particular when assessing the activity in a single pixel within a given region of interest, which is prone to image noise (the current standard clinical approach by measuring SUVmax). Two recent studies also suggest caution against the premature acceptance of FLT for imaging treatment response. In these studies, changes in FLT uptake in the early post treatment stage occurred slower than the reduction in FDG uptake or did not correlate with clinical tumor regression.[94,95] One potential explanation for major discrepancies between treatment-induced changes in FDG and FLT uptake might be the activation of the salvage pathway and the enzyme thymidylate synthase (which is not traced by FLT) in response to therapy or, vice versa, preferential treatment-induced inhibition of the salvage pathway with consecutive upregulation TK-1 for nucleotide synthesis or a redistribution of nucleoside transporters from the cytosol to the plasma membrane.[96]

The clinical utility of FLT may be limited for assessment of bone and liver lesions because of high normal uptake in bone marrow and glucuronidation and accumulation of radioactive metabolites in the liver. False positive uptake can occur as well. Nevertheless, FLT appears to be most promising agent for immediate application in larger clinical research studies. ^{18}F-1-(2′-deoxy-2′-fluoro-beta-d-arabinofuranosyl)thymine (FMAU) is another promising agent for measuring proliferation. In contrast to FLT, it is incorporated into the DNA and its uptake pattern resembles that of thymidine.[97,98] FMAU may find complementary use to FLT and may be particularly useful in tumors whose proliferation is not dependent on TK-1 activity. FMAU has been used in patients[99] but not in any clinical studies.

Hypoxia and Angiogenesis

Hypoxia generally refers to a deficiency in the amount of oxygen reaching body tissues. It occurs in tumors as a consequence of tumor cell proliferation exceeding the rate of angiogenesis, ie, tumor cells are growing beyond the maximum range of oxygen diffusion in tissue. In malignant tumors, hypoxia is an indicator of poor prognosis, regardless of the treatment modality used.[100] It is believed to be one of the leading causes of radiation and chemotherapy treatment failure.[101-105] Hypoxic cells are resistant to the cytotoxic effects of ionizing radiation[106-109] and require radiation doses up to 3 times greater than for the same level of cell inactivation to the same cells under normoxic conditions. Hypoxia in tumor cells leads to amplification and overexpression of various signaling factors, such as HIF-1α or HIF-2α, which promote tumor growth, invasion, metastasis, and resistance to apoptosis.[21] HIF-1α also activates the vascular endothelial growth factor, which confers radiation resistance to endothelial cells and increases the proliferation and regrowth of tumor blood vessels.[110] Experimentally, the eradication of HIF-1α positive hypoxic cells leads to a suppression of angiogenesis and tumor growth.[111] HIF-2α can induce overexpression of the endothelial growth factor receptor, which is then available for autocrine signaling (from the cancer cell to its own cell surface receptor; recall that cancer cells are autonomous and do not depend on external growth signals), thereby promoting tumor growth.[112]

The use of selective hypoxia targeting PET tracers provides a noninvasive way of measuring regions of low partial oxygen pressure within the tumor tissue. A number of compounds are available for the imaging of hypoxia (reviewed in[109]). The earliest hypoxia tracers used in man were ^{18}F-FMISO for PET,[113] and ^{123}I-IAZA for SPECT studies.[114] The ideal hypoxia tracer should show high specific uptake and essentially irreversible retention in hypoxic cells, low background activity in normoxic tissues, chemical stability against enzymatic cleavage in blood, rapid blood clearance enabling imaging as early as possible after injection, and the scan findings should be reproducible. Quantitative (rather than just qualitative) assessment of the extent and severity of hypoxia is necessary for radiotherapy applications, and several groups are engaged in validating hypoxia tracers for application in radiobiological modeling. To assure application outside the research environment, the radiosynthesis should be reasonably simple or automated. None of the currently available agents, which vary in their degree of lipophilicity, plasma half life time, and route of excretion, meets all of these requirements. It is currently unclear whether there will ever be one optimal hypoxia imaging agent, but the suitability of various hypoxia radiotracers is presently under investigation, including ^{18}F EF-5,[115] ^{60}Cu-ATSM,[116] ^{18}F-FETNIM, and ^{18}F-FAZA.[117]

In considering the advantages and disadvantages of these agents, the location of the cancer under study may be important: For instance, imaging at delayed time points may be acceptable in head and neck or lung cancer as long as an accurate and reproducible information can be obtained but may not be acceptable in intestinal tumors when hepatobiliary clearance of the tracer or its radioactive metabolites interferes with the detection of hypoxia-specific uptake. Moreover, it is important to note that the use of hypoxia tracers to define tumor hypoxic fraction, ie, the ratio of the volume of hypoxia to that of tumor, is highly contingent on the threshold selected for the radiotracer uptake relative to blood or background. Rajendran and coworkers[119] at the University of Washington have selected an operational threshold to identify intratumoral hypoxia regions as those PET image voxels for which the ^{18}F-FMISO concentration is 1.2 times greater than the activity concentration measured in blood at the time of the PET scan. However, any change in this threshold value can make a significant alteration in the measured tumor hypoxic fraction. This threshold sensitivity impacts on the use of the hypoxic fraction as a prognostic variable or as a target for dose painting. Another limitation for the imaging of hypoxia is the fact that hypoxia distribution is not static over time; whereas some tumor regions may exhibit chronic (static) hypoxia, other regions may be subject to transient, acute (dynamic) hypoxia,[120] depending on changes in tumor vessel vasomotion and red cell flux,[121] changing rheologic conditions, presence or absence of anemia, changing rates of metabolism and proliferation[122] and changes in intratumoral interstitial pressure.[123] Chronic hypoxia is thought to result from limited oxygen diffusion ($>$150 μm distance between blood vessel and cell), whereas acute hypoxia may result

from transient blood flow fluctuations in the intratumoral vascular network that occur in cycles of minutes to hours.[121,124] This may have implications for the clinical utility of hypoxia imaging. It has been suggested that dose escalation to hypoxic tumor subvolumes may improve locoregional cancer control rates. If "dose painting" to hypoxic tumor subregions were to be based on PET imaging data, the reproducibility of these imaging data (both in terms of spatial distribution and intensity of hypoxia) would have to be proven first for a given radiotracer. Finally, recent studies that used immunohistochemical staining methods to detect hypoxia in clinical tumor biopsy samples[125] have shown that normoxic-hypoxic oxygen gradients occur in vivo over very small distances (150-200 μm), which are far beyond the spatial resolution of any macroscopic imaging technique (including micro-PET). Therefore, the intensity of PET hypoxia marker uptake within any PET image voxel depends on the number of hypoxia cells combined with their depth of hypoxia.

The most commonly used agents for PET imaging of hypoxia are nitroimidazole derivatives. For many years, ^{18}F fluoromisonidazole (FMISO) has been the standard PET imaging test for measuring tumor hypoxia.[113,119] FMISO is relatively hydrophilic, shows a suboptimal signal-to-background ration, and may require dynamic and dual-time point imaging for meaningful conclusions.[126] More recently, other agents including ^{18}F fluoroerythronitroimidazole and ^{18}F fluoroazomycin arabinoside with potentially more favorable pharmacokinetics (less background activity) have been developed.[117,118] ^{124}I-IAZGP is another nitroimidazole derivative, labeled with the long lived isotope iodine-124 (half life time 4 days), thus permitting imaging at later time points with potentially superior target-to-background ratio.[127] Other nitroimidazoles, which are more lipophilic than the aforementioned compounds, include ^{18}F EF3 and ^{18}F EF5.[128,129] Few data are available on the intrapatient comparison in the biodistribution of these agents. All nitroimidazoles diffuse freely through the plasma membrane. Once inside the cells, they are reduced by reductase enzymes to nitro radical metabolites that can bind covalently to intracellular macromolecules such as proteins, DNA or RNA, causing their retention within the hypoxic cells. In normal, nonhypoxic cells, this reaction is reversed by oxidation, so that the compounds can not bind effectively and are not retained in the cell.

Another novel hypoxia tracer, with entirely different chemistry, is the metal complex ^{64}Cu methylthiosemicarbazone (Cu-ATSM).[130,131] Again, this lipophilic ATSM complex is reduced in hypoxic cell and remains trapped. It differs from the nitroimidazole compounds by its faster washout from normoxic cells, leading to higher contrast and potentially better image quality. Although initial clinical research studies appeared very promising, showing prognostic value of Cu-ATSM uptake in predicting radiotherapy treatment response,[116] there is now evidence that the behavior of Cu-ATSM may vary between different tumor histologies.[132] Researchers investigating the comparative cellular uptake of ^{64}Cu-ATSM versus ^{18}F-FMISO[133] demonstrated considerable variability in the uptake profile of the ^{64}Cu-ATSM compound between different cell lines, suggesting a possible tumor dependence of the intensity of PET signal. If confirmed, this would preclude the value of Cu-ATSM as a hypoxia radiotracer. For a detailed review of the radiosynthesis and validation of the various hypoxia tracers we refer to reference.[109]

Potential clinical applications for hypoxia imaging include:

- The selection of patients with poor prognosis for inclusion in adjuvant protocols and/or closer surveillance after initial treatment with curative intent.
- The identification of patients with hypoxic tumors who may benefit from a combination therapy of irradiation with (1) radiation sensitizers[134]; (2) vasodilators or carbogen breathing as was employed in the ARCON (accelerated RT with carbogen and nicotinamid) trial in advanced head and neck cancer[135]; and (3) hypoxic cell cytotoxins such as tirapazamine. In a randomized phase II trial, the addition of tirapazamine to standard therapy with cisplatin and radiation therapy provided better results than the combination of 5FU, cisplatin and RT.[136] This was recently confirmed by preliminary data from an ongoing phase III study.[137] Because tirapazamine is relatively toxic to normal tissues (in particular those with suboptimal oxygenation), second generation agents are being developed now.
- The identification of hypoxic subvolumes in malignant tumors or metastases that could be targeted with higher radiation doses in an attempt to overcome radioresistance in hypoxic cells.[138-140]

The ultimate test for hypoxia PET imaging will be whether such images can be employed for hypoxia-directed treatment strategies that improve patient outcome.

Malignant tumors are characterized by the development of chaotic and leaky blood vessels, leading to a disturbed microcirculation.[123] Disturbed microcirculation may give rise to 2 prognostically negative features: low oxygen tension and high interstitial pressure precluding the delivery of chemotherapy in sufficient concentrations. Experimentally, the latter can be reversed by treatment with the vascular endothelial growth factor-specific antibody bevacizumab.[141] Treatment with angiogenesis inhibitors has also shown initial promising results in clinical studies,[142] but it is currently unclear which subgroup of patients may particularly benefit from this new class of drugs. Because the inhibition of angiogenesis per se may not cause cell death and tumor shrinkage (but instead "only" prevent further growth), it is also unclear how the efficacy of angiogenesis inhibitors could best be shown and monitored in the clinical setting (rather than inferring efficacy indirectly from achieving stable disease). Changes in tumor hyperemia can obviously be documented on contrast-enhanced CT/magnetic resonance imaging (MRI) or MRI perfusion sequences, but these studies are nonspecific. In contrast, PET can specifically image the process of angiogenesis, for instance, by using ^{18}F RGD peptide,[143-145] which binds specifically to $\alpha_v\beta_3$ integrins; these are expressed at the surface of activated endothelial cells during angiogenesis.[146]

Tumor hypoxia and angiogenesis are intimately related and HIF-1α is probably the single most important factor promoting the expression of proangiogenic proteins.[147-149] Interestingly, hypoxia seems to be an early and dynamic phenomenon in the development of metastases. Microscopic clusters of metastatic cells are avascular and hypoxic. Only the development of (hypoxia-induced) vascularity permits further growth, eventually again leading to macroscopic hypoxia once these metastases outgrow their blood supply.[150]

Apoptosis

^{18}F annexin is one potential imaging agent to visualize apoptotic cell death.[151] Annexin-V is a 36-kDa molecular weight protein that binds to phosphatidylserine (PS) lipid residues; these residues are only present on the inner cellular membrane of the healthy cell. However, during the process of cell death by apoptosis, parts of the cell membrane undergo an inversion resulting in the transient exposure of PS to compounds present in the interstitium (including, for example, annexin-V that was injected intravenously). Therefore, labeled annexin-V should be an ideal tracer for the imaging of apoptosis in vivo. Whereas extremely promising images of myocardial apoptosis have been obtained in nuclear cardiology[152] using ^{99m}Tc-annexin-V, similar high contrast images have not been observed in cancer response to either chemotherapy or radiation therapy. A plausible hypothesis for this difference is that apoptotic cell death in response to cancer therapy probably occurs over a much longer, protracted time interval, rendering a smaller window of synchronous events to build a potent annexin-V imaging signal. This may perhaps change with the clinical introduction of new high radiation dose techniques (eg, hypofractionation or single dose irradiation), which might result in a higher number of imageable apoptotic bodies in a shorter time frame. The potential of annexin-V imaging in these scenarios has yet to be investigated.

Historically, radiation oncologists and biologists have focused on the detection of residual surviving tumor cells (rather than cell death), because it is still widely believed that sterilizing the last tumor cell done is necessary to cure a patient. Whether cell death markers will find a role in cancer therapies is therefore not known, but it is conceivable that apoptosis imaging might find a niche as part of early response assessment. Of note, radiochemists have provided a variety of annexin-V tracers in addition to the fluorinated form. For example, a form of ^{124}I-labeled annexin-V is under investigation,[153] as well as multistep targeting approaches of annexin-V imaging, for instance by using ^{64}Cu-labeled streptavidin that is administered after pretargeting of apoptotic cells with biotinylated annexin-V.[154] Overall, however, the utility of annexin-V PET imaging in cancer has yet to be proven.

Conclusion

Hallmarks of cancer, important for cancer development, progression and resistance to therapy, can be imaged with currently available PET probes. We have highlighted abnormalities in cancer metabolism and microenvironment that provide the rationale for the clinical application of these radiotracers. Functional and metabolic imaging has certainly improved our understanding of cancer biology, but it will be important to show that this also translates into a measurable improvement in diagnosis, therapy design and ultimately patient outcome.

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