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MOLECULAR IMAGING: UP-AND-COMING TECHNIQUE IN RADIATION ONCOLOGY

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ABSTRACT Over the past few years, a tremendous progress has been made in the field of oncology pertaining to advanced treatment planning, cancer imaging and treatment techniques. The limitation of conventional imaging in radiation oncology prompted the development of molecular imaging; which allows identifying or describing living biological process at cellular and molecular level and is especially addressed to reveal abnormalities in cells and molecules which cause the cancer. The techniques used include radiotracer imaging like PET scan & SPECT, MRI & MR spectroscopy, optical imaging, ultrasonography, and others. The early detection of cancer cells by molecular imaging and prompt treatment leads to better outcomes, which can potentially be assessed in early stage thorough molecular imaging. Different pathways, where this technique can be acted are cell metabolism & proliferation, hypoxia, apoptosis, angiogenesis. In the last decade, diagnostic methods involving molecular imaging have made advancement in the field of diagnosis and staging, target definition and response assessment of cancer. The most common used imaging technique is 18-FDG PET-CT Scan, which has immense value in cancers of lung, breast, prostate, lymphoma and many more with increased sensitivity and specificity. Advancement in the understanding of the pathophysiology of cancer has led to developments of multimodality treatment concepts comprising surgery, radiotherapy, chemotherapy and molecularly targeted anti-cancer agents. It can be concluded, combined use of molecular imaging with these treatment modalities can prove to be a boon for cancer management.

KEYWORDS : Molecular imaging, PET scan, radiation oncology

INTRODUCTION:

Over the past few years a tremendous progress has been made in the field of oncology pertaining to advanced treatment planning, cancer imaging and treatment techniques. Conventional imaging techniques such as CT scan, X-rays only provide the anatomical information about cancer but lacks in giving information about the molecular pathways such as glycolysis, angiogenesis and apoptosis happening in cancer cells.[1] Even the most advanced radiation treatment techniques, do not consider the specific molecular characteristics of tumors pertaining to various alterations occurring during their evolution and progression. Also, there are limitations of radiation delivery to maintain the therapeutic ratio. Hence, further improvement in radiation treatment will only provide minimal benefits. For these reasons, to further increase the tumor control, understanding of the biological mechanisms behind tumor is required so that specific pathways can be targeted in addition to conventional treatment.

The limitation of conventional imaging in radiation treatment planning and delivery prompted the development of molecular imaging. Molecular imaging can be defined as the direct or indirect noninvasive monitoring and recording of the spatial and temporal distribution of in vivo molecular, genetic, and/or cellular processes for biochemical, biological, diagnostic, or therapeutic applications. Molecular imaging allows identifying or describing living biological process at cellular and molecular level and is especially addressed to reveal abnormalities in cells and molecules which cause the disease.[2]

A descriptive definition was presented by the SNM (Society of Nuclear Medicine; in 2007). "Molecular imaging is the visualization, characterization, and measurement of biological processes at the molecular and cellular levels in humans and other living systems. To elaborate, molecular imaging typically includes 2- or 3-dimensional imaging as well as quantification over time. The techniques used include radiotracer imaging under nuclear medicine, MR (magnetic resonance) imaging, MR spectroscopy, optical imaging, ultrasonography, and others."[3]

The rationale behind molecular imaging is that we can study the biological processes taking place in the body or more precisely in cancer cells much before they would be seen on conventional imaging like CT (computed tomography) or MRI (magnetic resonance imaging). This enables us to identify the disease at an early stage, as a result necessary action can be taken at a much earlier stage of the disease leading to better outcomes in terms of tumor control. CT or MRI assess treatment response based on anatomic changes, which typically occur slowly over the course of weeks and months after the treatment; whereas with molecular imaging techniques treatment response can potentially be assessed during treatment and to no doubt after treatment completion sooner before CT or MRI. Characterizing the changes in tumor microenvironment before, during and after treatment helps to anticipate the response to treatment.

Molecular Pathways

Cell metabolism, cell proliferation, hypoxia, apoptosis, angiogenesis and certain receptors are the pathways in molecular imaging (table-1). Imaging probes are designed that target these specific pathways which are then detected by various molecular imaging techniques. The basic principle of targeting the biological processes with molecular imaging is depicted in figure-1.

Table 1: Commonly targeted pathways in molecular imaging

Targeted pathway	Mechanism
Metabolism	Cancer cells have an increased glucose metabolism (glycolysis), protein metabolism, upregulated amino acid transport due to increased cell proliferation and turn-over which can be detected by detection of radiolabeled glucose, amino acids and proteins respectively.[4]
Cell Proliferation	Uncontrolled cell proliferation is indicated by increased lipid precursors (choline for cell membrane synthesis) and increased nucleosides (particularly thymidine- for DNA synthesis). It can be assessed by detecting radiolabeled lipid precursors and nucleosides and measuring choline concentration.[5,6]
Hypoxia	Due to imperfect vasculature, solid tumors develop low oxygen concentrated hypoxic regions. Hypoxia is the basis for angiogenesis, metastasis and radiotherapy resistance. Hypoxia can be detected by use of radio-tracers that accumulates in regions with $pO2 < 10$ mm Hg.[7]
Apoptosis	Apoptosis is programmed cell death and externalization of phosphatidylserine (PS) to the cell surface is a hallmark of the apoptotic process.

	PS is normally present on inner surface of cell membrane. Vesicle protein 'Annexin' binds with externalized phosphatidylserine. Apoptosis can be detected by radiolabeling Annexin and its detection by molecular imaging.[8,9]
Angiogenesis	Angiogenesis is the development of new blood vessels from pre-existing blood vessels. It is fundamental to tumor growth and its transition from benign to malignant state. Angiogenesis results in up regulation of VEGFR (Vascular Endothelium Growth Factor Receptor) and Integrin αvβ3 which is a cell adhesion molecule and plays a key role in endothelial cell migration and survival. Glycosylated penta-peptides labeled with radionuclide are used to image αvβ3 integrin expression. VEGFR can be detected by gadolinium enhanced MRI.[10]

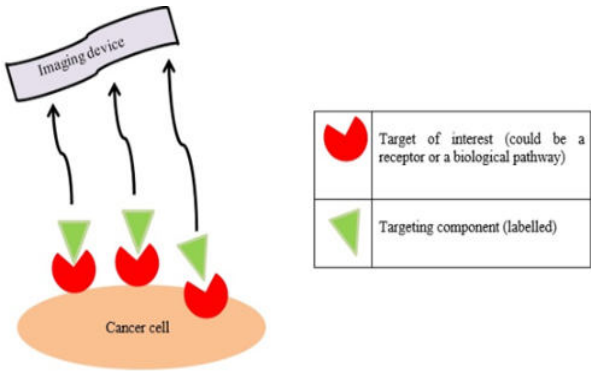

Figure 1: Basic principles of targeting the biological process

Molecular Imaging Modalities and Techniques

At present, molecular imaging modalities mainly include PET (positron emission tomography) scan, SPECT (single photon emission computed tomography), MRI (magnetic resonance imaging) and MRS (magnetic resonance spectroscopy), CEUS (contrast enhanced ultrasound), optical imaging and CT (computed tomography).[11-16]

Positron Emission Tomography (PET)

In this process, a proton in the nucleus of the isotope is converted into a positron that is ejected from the atom. The ejected positron then travels some distance, generally in the range of 1 to 10 mm depending on its energy, before annihilating with its opposite particle - the electron. In this annihilation, the positron and electron energy are converted into a pair of 511 keV photons, emitted in opposite directions which is then detected by paired detectors.[17] The radioisotope is attached to a targeting agent forming a radiotracer which is designed to localize specific biological properties in vivo. PET radioisotopes are produced in a cyclotron and are administered intravenously in dose range of approximately 15mCi.[18]

The various radioisotopes used in PET scan are 18-FDG (fluoro-2-deoxyglucose) to study glucose metabolism, 18-FLT (fluorothymidine) for cellular proliferation, 18-FMISO (fluoromisonidazole) and 18-FAZA (fluoroazomycin-arabinoside) for hypoxia, 18-F galacto-RGD (arginine-glycine-aspartic acid) for angiogenesis, 18-FMT [fluoro-alpha-methyl-tyrosine] for amino acid metabolism, choline (11C) for lipid metabolism and acetate (11C) for fatty acid metabolism and few more.

The classical PET radiotracer used is [18F] 2-fluoro-2-deoxy-D-glucose (FDG). Many tumors are known to have high glycolysis rate as compared to normal tissue.[4] FDG exploits this abnormal increase in glucose metabolism to image tumors. FDG is transported into tumor cells (figure-2) in increased amounts as a result of the up-regulation of glucose transport proteins, i.e. GLUT1 and rate-limiting enzymes like hexokinase. Inside the cell, FDG is phosphorylated into FDG-6-phosphate which cannot undergo glycolysis due to the presence of a fluorine substitution in the molecule. FDG-6-phosphate is metabolically "trapped" within the cell and accumulates over time which is detected by PET imaging. FDG is routinely used in the clinic for diagnosis, staging, detection of disease recurrence and monitoring treatment response in cancer.[19]

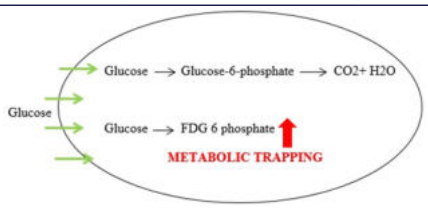

Figure 2: Increase transport of glucose in cancer cells and metabolic trapping of FDG glucose

PET allows quantitatively accurate detection of molecularly targeted radioisotopes to determine tissue biological properties or processes, such as commonly performed imaging of glycolysis and cell proliferation. Standardized uptake value (SUV) is the standard method of quantitating the degree of radiotracer uptake observed in a PET scan. Typically, either the maximum or mean value of the SUV in a region of interest is used clinically; SUV of 2.5 or greater is commonly taken as the diagnostic threshold indicating malignancy.[20]

Use of PET scan for imaging hypoxia, gene expression, cell surface receptors and proteins, tumor angiogenesis, and apoptosis is rapidly advancing. During tumor cell proliferation, there are elevation of choline metabolites such as phosphocholine and phosphatidylcholine which are required for cell membrane synthesis. Radiolabeled choline [11C] is taken up in cancer cells which is detected by PET Scan; example- prostate and brain cancer.[21]

Due to increased proliferation, malignant cells show increased nucleoside uptake which is required for DNA (deoxy-ribo nucleic acid) synthesis. Radiotracer used for detecting increased nucleoside uptake is FLT (18-fluorothymidine). FLT is resistant to degradation and is trapped intra-cellularly after phosphorylation by thymidine kinase (figure-3); example- colorectal, brain, lung and breast cancer.[22]

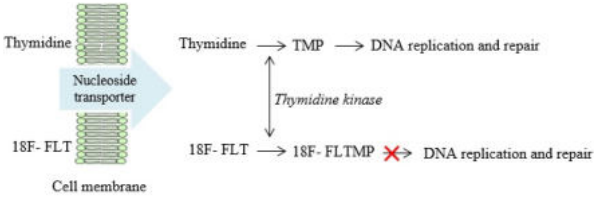

Figure 3: PET scan to detect the increased nucleoside uptake

[DNA: Deoxy-ribonucleic acid, FLTMP: Fluorothymidine mono phosphate, TMP: Thymidine mono phosphate]

[18F] Fluoromisonidazole (FMISO) is used to detect hypoxia. Nitro group of FMISO undergoes reduction reaction and is trapped in hypoxic cells with pO2<10 mm of Hg. With time, it accumulates in hypoxic cells leading to signal amplification and detection by PET scan. Example- head and neck cancer, lung cancer and soft tissue sarcoma.[23]

Single Photon Emission Computed Tomography (SPECT)

Gamma-emitting radioisotopes are used for SPECT scan.(table-2) Radiolabeled tracers are injected intravenously in the human body which concentrates in an organ or structure of interest. The main detector component of a clinical SPECT system is the Anger gamma camera which measures the gamma radiation emitted by the radiotracer directly and there is no annihilation reaction.[24]

When the gamma camera is rotated around the object to be imaged, projection data are obtained and tomographic SPECT images are mathematically reconstructed. Typical quantity of radiotracer administered varies from 11 to 30 mCi.[25]

Table 2: Radioisotopes frequently used in SPECT

Radionuclide	Half life	Energy	Application
99mTc (Technetium)	6 hours	140 KeV	Somatostatin receptor detection in neuroendocrine tumor, hypoxia and apoptosis.
111In (Indium)	2.8 days	171.3 KeV and 245.4 KeV	PSMA (prostate specific membrane antigen) in prostate cancer.

99mTc-HYNIC (hydrazino-nicotinamide) detects somatostatin receptor expression in neuroendocrine tumors. It can also detect apoptosis by binding to externalized phosphatidylserine (PS). Annexin V has an affinity to bind with phosphatidylserine; annexin V is radiolabeled with 99mTc forming a radiolabeled annexin V- PS complex which can be detected by SPECT.[26] Prostate-specific membrane antigen (PSMA) is a tumor marker associated with prostate cancer. J591 is a humanized monoclonal antibody which is radiolabeled with Indium-111 and it targets the external domain of PSMA and can detect both skeletal and soft tissue diseases. SPECT is indicated in rising level of prostate-specific antigen (PSA) in a patient who has had prostatectomy but who has no obvious location for a metastatic focus as determined by CT or MRI.

Magnetic Resonance Imaging (MRI)

MRI is an imaging modality that uses a powerful magnet and radiofrequency energy to visualize the internal structure and soft tissue morphology of the body. In MRI, the signals of interest are mainly from 1H nuclei in water. MRI signals from other nuclei (e.g. 31P, 23Na, 13C) are much smaller than 1H nuclei (P = Phosphorus, Na = Sodium, C = Carbon, H = Hydrogen).

Hydrogen nuclei have a property called “spin” which is in random direction in absence of magnetic field. When strong magnetic field is applied, it makes the spins of the hydrogen nuclei line up along the magnetic field. Some hydrogen nuclei line up in the direction of the magnetic field (low energy) and others line up opposite to the direction of the magnetic field (high energy). The RF coil produces energy in the form of a rapidly changing magnetic field. The hydrogen nuclei with low energy absorb the energy sent from the RF coil. Once the low energy nuclei absorb the energy, they change their spin direction and become high energy nuclei. When the RF coil is turned off, these 'high energy' hydrogen nuclei go back to their previous 'low energy' state releasing energy in the form of waves which are detected by 'receiver coils' which finally converts the energy waves into an electrical signal which forms the image.[27]

Gadolinium (Gd) complexes or SPIO (Super-Paramagnetic Iron Oxide) particles are used to visualize the biochemical processes. The most commonly used contrast agents are based on gadolinium. SPIOs contrast agents are not currently in widespread use. To achieve highly specific binding properties, Gd-chelates or SPIOs are conjugated to antibodies, peptides or peptide-mimetics for visualization of specific molecular processes (targeted contrast agents). Free gadolinium ions are very toxic (can cause cardiovascular collapse and respiratory paralysis) and therefore they are combined with a chelate such as diethylene tri-amine penta-acetic acid (DTPA). Gadolinium is a positive contrast agent which causes increased signal intensity on T1 weighted images. It is given intravenously in a dose of 0.2 ml/kg. Targeted gadolinium contrast agents can detect apoptosis by binding to externalized phosphatidylserine. Angiogenesis is detected by targeting and binding to VEGFR (Vascular Endothelium Growth Factor Receptor). Targeted MRI contrast agent based on iron oxides enables imaging of VCAM-1 (Vascular Cell Adhesion Molecule-1) endothelium by upregulating vessels of cerebral metastases and it is used to detect brain metastases earlier than gadolinium-based contrast media.[28]

Magnetic Resonance Spectroscopy (MRS)

MR spectroscopy uses selective radiofrequency pulses for the investigation of the molecular composition of tissues. It is used to detect metabolic changes in cancerous as well as in normal tissues by using different metabolic markers.[29] 1-H is the most common nuclide imaged using MRS methods due to its relative abundance compared to other nuclei and has been demonstrated to detect, quantify, and differentiate neoplastic disease processes in the brain, breast, and prostate.[30]

Metabolites that are used to map spectroscopic images are-

- Choline (Cho) (raised in cancer)
- Citrate (Cr) (normally found in prostate tissues)
- N-acetyl aspartate (NAA) (normally found in brain tissues)

The concentration of each of these metabolites can be mapped on spectroscopic images. The 1H signal from total choline (Cho) is significantly elevated in cancer tissue, which is correlated with increased lipid synthesis due to cellular over proliferation. A decrease may be seen following successful treatment. Brain tumors are characterized by a loss of N-acetyl aspartate (NAA), elevated levels of choline and reduced levels of creatine.[30] MRS of brain cancers has been used for the purposes of diagnosis & tumor grading, radiation treatment planning and response assessment, following administration of therapy as well as information on prognosis and survival.[31-33] Classical spectral signature of prostate cancer consists of increased choline and decreased citrate levels.[30] In breast cancer, increase in the levels of choline and its metabolites associated with cell membrane synthesis and turn over resulting from malignant cell growth can be monitored using MRS.[34] Advantages of MRS are that there is no need for ionizing radiation and it has high spatial resolution but poor sensitivity.

Contrast Enhanced Ultrasound (CEUS)

Conventional ultrasound imaging provides only structural information. CEUS for molecular imaging includes the use of specialized contrast agents (microbubbles). Microbubble shell is composed of albumin, lipid or polymers and it has a gas filled core. CEUS can be targeted or non-targeted. Non targeted CEUS is used for a clearer picture of organ of interest and to study its blood perfusion. Targeted CEUS is more important in cancer. Contrast agents are designed that bind to a specific molecule. Most common targeted pathway is angiogenesis.[35] Microbubbles specifically adhere to their molecular target which can be detected by ultrasound. Released microbubbles agents binds to vascular endothelium molecules (markers for angiogenesis) like VCAM and VEGFR (figure-4). The microbubble contrast is given intravenously and volume administered is 0.1–5 ml (concentration $\approx 10^9$ bubbles/ mL).[36] Ultrasound transducer is fixed in position over region of interest then targeted contrast agent is administered into peripheral vasculature followed by a waiting period of 4-30 min. Targeted contrast agent first accumulates in micro vasculature after which imaging is done to detect the accumulated contrast. When contrast agent is cleared from microvasculature imaging is done again to detect retained contrast agents. Advantages of CEUS are that it is a real-time imaging, uses nonionizing radiation, easy portability and low cost, high spatial and temporal resolution (50 μm to 0.5 mm). Ultrasound does not depict lungs and bone owing to poor ultrasound propagation; it is mainly used for liver.[37]

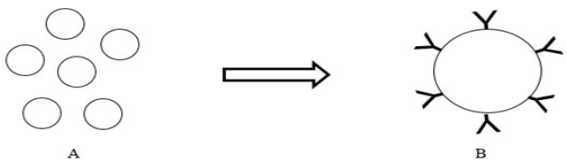

Figure 4: A. Gas-filled microbubbles; B. Gas-filled microbubbles coated with antibody

Optical Imaging

In the process of optical imaging, light interaction with biological tissues leads to a number of events such as absorption, scattering, and emission of light. Optical molecular imaging is based on detecting visible and infrared photons after they are transmitted through biological tissues. Optical imaging is used for imaging gene expression, tumor cell tracking and imaging of receptors which helps in selecting targeted therapy. Major optical imaging techniques are- fluorescence and bioluminescence.

Fluorescence is the emission of light by a substance that has absorbed light. Targeted probes are designed which typically consists of a fluorescent dye (e.g. cyanine dye, Cy 5.5) attached to a binding agent (antibody or peptide) and is given intravenously. These probes target the specific molecular pathways or receptors. Upon excitation with externally applied light, fluorescent tracers (targeted probes attached to binding agent) emit light that can be detected by a CCD (charged coupled device) camera. Example- fluorescent dyes can be conjugated with Trastuzumab to image HER-2-positive tumors in breast cancer.[38]

In bioluminescence (figure-5), an in vivo enzymatic reaction is responsible for the emission of light. The most common enzymatic tool is the firefly luciferase system. Tumor cells or DNA are labeled with luciferase gene, which are then detected by optical imaging to monitor cell activity and gene behavior in living subjects.[39]

Advantages of optical imaging are that it allows real time observation and acquisition of image, easy to use and minimal toxicity to biological systems, but it has poor specificity.

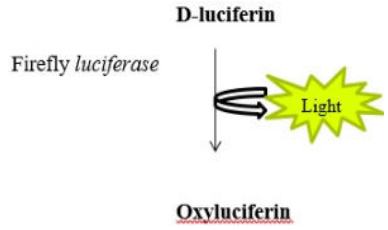

Figure 5: Enzymatic reaction in bioluminescence (Emission of yellow-green light can be used to visualize luciferase-expressing cells in vivo following intravascular injection of D-luciferine)

Computed Tomography (CT)

Contrast agents for CT based molecular imaging are under research.[40] CT molecular imaging abilities have not yet been completely explored.

Application of Molecular Imaging in Radiation Oncology

In the last decade, diagnostic methods such as positron emission tomography (PET), computed tomography (CT), magnetic resonance imaging and spectroscopy (MRI/MRS), contrast enhanced ultrasound (CEUS) and optical imaging have made advancement in the course of treatment in oncology. Molecular imaging is useful in diagnosis and staging, target definition and response assessment (early and late) of cancer.[41]

For diagnosis and staging FDG PET-CT Scan is used most commonly. Integrated PET/CT systems are used for target definition in esophageal, cervical, pancreatic, non-small cell and small cell lung cancer. PET-CT produces anatomic and metabolic images of the patient during a single procedure in the treatment position. Compared to using CT alone, the inclusion of PET data changes the size of delineated gross tumor volumes by approximately 20% to 40% in half of non-small cell lung cancer patients.[42] The literature indicates 84% to 87% specificity and 88% to 93 % sensitivity of FDG-PET in various oncologic applications.[18]

Molecular imaging can also be used to assess the treatment response. Early response assessment is done during the course of radiotherapy and it serves as an early predictor of treatment outcome and potentially allows the therapy to be adapted in order to maximize its benefit known as biologically adaptive radiotherapy (BiART). Late response assessment is done after the radiotherapy has been completed and it serves as a late predictor of treatment outcome. Anatomic changes visible on CT or MRI, typically occur slowly over the course of weeks and months after the therapy; whereas with molecular imaging treatment response can potentially be assessed much earlier.[43-44] The ability of molecular imaging to detect altered molecular pathways very early in the progression of disease has the potential to detect and cure disease in its most treatable phase.

CONCLUSION

Incidence of cancer has been rising from a few decades all over the world. Development of cancer is multifactorial; genetic changes and altered molecular pathways have a strong influence on development of cancer. Advancement in the understanding of the pathophysiology of cancer has led to developments of multimodality treatment concepts comprising surgery, radiotherapy and molecularly targeted anti-cancer agents. Combined use of molecular imaging with these treatment modalities can prove to be a boon for cancer management. Without a doubt, it can be said that molecular imaging has opened up a new dimension of cancer management. A strong collaboration is required between radiation, surgical and medical oncologist, pathologist, nuclear medicine specialist, and medical physicist team for far-reaching understanding and finest use of this nascent technique.

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