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Molecular imaging and radioimmunoguided surgery

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Molecular imaging comprises a series of diagnostic modalities that provide information on the physiology and molecular composition of cells and tissues. One of these modalities, radioimmunodetection, uses radiolabeled monoclonal antibodies (mAbs) to image tissues. Two radioimmunodetection modalities are described in this article: immunoscintigraphy and radioimmunoguided surgery (RIGS). In immunoscintigraphy, the radioactivity is measured with the use of an external gamma camera and used to create images. In RIGS, the radioactivity is detected intraoperatively with the use of a handheld gamma probe to help the surgeon detect foci of otherwise occult disease. Both techniques have the potential to improve the preoperative and intraoperative localization of cancer. Multiple studies have been performed on the efficacy of RIGS on different malignancies, especially colorectal cancer. Despite the good sensitivity of the technique, some concerns revolve around the high rate of false positives and the real significance of leaving RIGS-positive tissue behind in terms of long-term outcomes and survival. More studies are warranted to further develop the technique and determine the specific role it will play on the diagnosis and management of surgical disease. Surgeons should actively participate in these studies and in expanding the applications of this promising technology.

The history of surgery is intimately tied to the technological evolution of surgical instruments and imaging techniques. New surgical instruments permit a more precise and discrete manipulation of tissues while improved imaging techniques enhance the discriminatory ability of the surgeon to allow for a more accurate and even targeted therapy.

Visualization and imaging, in any of its forms (including direct visualization), is pivotal for the conduction of an operation. Recent advances in the field of diagnostic radiology (eg, computerized tomography and magnetic resonance imaging) have facilitated the preoperative planning of procedures. Similarly, techniques such as laparoscopy and intraoperative ultrasound have helped the surgeon acquire information that is usually invisible to simple sight and touch.

Conventional imaging modalities, such as X-rays, ultrasound, computerized tomography, and magnetic resonance imaging, allow better characterization of the *morphology* of organs. However, they provide no information on the *physiological processes* of cells and tissues, characteristics that may be important in guiding the therapy for some patients. Molecular imaging is a diagnostic modality that exploits these particular processes and the molecular composition of tissues to allow a more specific localization and quantification of their biologic activity. Molecular imaging can use different techniques, including nuclear medicine, optics, and magnetic imaging, among others.

Some nuclear medicine modalities have already gained widespread use, such as Technetium-99m (^{99m}Tc)-MIBI single photon emission tomography (SPECT) for assessment of myocardial ischemia and Fluorine-18 (^{18}F) fluoro-

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deoxyglucose (FDG) positron emission tomography (PET) for detection of highly-metabolic tissues (eg, malignancies). Other nuclear medicine modalities like radioimmunodetection, a technique where antibodies are combined with radioisotopes to image tissues, are in earlier phases of development and will likely become important tools in the preoperative and intraoperative imaging of patients. For that reason, it is imperative for surgeons to understand the basic tenets of these technologies and their potential applications. This article will explore the principles and clinical evidence of radioimmunodetection techniques, in particular of radioimmunoguided surgery (RIGS).

Principles of radioimmunodetection and RIGS

Since the development of techniques to allow the production of large numbers of monoclonal antibodies (mAbs) from the hybridization of myeloma cells with antibody-secreting cells in the 1970s,[1] antibodies have been increasingly used for diagnostic and therapeutic purposes. Radioimmunodetection entails the administration of an *antibody* targeted to a particular *antigen*; the antibody is labeled with a *radioisotope* and detected with the use of a *camera or probe*. In immunoscintigraphy, the distribution of radioactivity is identified with a detection camera (eg, gamma camera, SPECT, PET) and an image is produced. In RIGS, the radioisotope is measured with a handheld probe that allows its intraoperative detection. Due to the nature of the technique and the over-expression of particular antigens in malignant tissue, radioimmunodetection has been mainly used in oncologic processes. Even though most of the discussion below will refer to malignant diseases, further development of this modality will likely expand its utility.

Antibodies

Antibodies, or immunoglobulins, are proteins made up of four polypeptide chains: two heavy (H) and two light (L) (Figure 1).[2] Both heavy and light chains have an area that remains relatively constant within the same type of antibody (C), and an area that varies depending on the particular antigen specificity of the antibody (V). The variable domains of a light and a heavy chain form together the area of antigen recognition. Heavy chains generally have four domains: three constant and one variable. The constant domains are numbered C_H1 to C_H3, and the variable domain is called V_H. Similarly, the light chains have a constant (C_L) and a variable (V_L) domains. The four chains are arranged in a Y-shaped conformation that can be divided in two parts by the hinge region: the antibody-binding fragment (Fab) and the crystallizable fragment (Fc). Since each antibody contains two identical areas for antigen recognition, they are said to be *bivalent*.

Desirable characteristics[3,4] of mAbs used for radioimmunodetection include: (1) high affinity and specificity for the antigen, (2) adequate circulation and distribution, (3) effective tumor penetration, (4) high avidity for the antigen (ability to remain bound to the antigen), (5) rapid clearance from blood and normal tissues, and (6) minimal immunogenicity. Even though the ideal mAb is far from being developed, antibodies can be selected and modified accordingly to meet the most desired characteristics for a particular application.

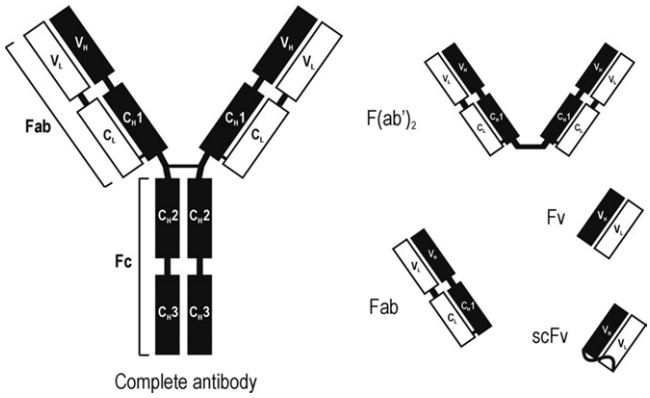

Figure 1 Schematic structure of an antibody. An antibody is formed by four separate chains: two heavy (H; dark) and two light (L; white). Each heavy chain is formed by four domains (C_H1, C_H2, C_H3, and V_H), and each light chain is formed by two domains (C_L and V_L). The antibody can be divided in two: an antibody-binding fragment (Fab) and a crystallizable fragment (Fc). Engineered antibody fragments being used in radioimmunodetection include $F(ab')_2$ (the two Fab fragments joined by the hinge region), Fab (individual Fab fragments), Fv (only the variable domains of the heavy [V_H] and light [V_L] chains) and scFv (the V_H and V_L domains joined by a linker peptide to increase their stability).

Affinity of a particular mAb for an antigen plays an important role in the differentiation between tumor and normal tissue in radioimmunodetection (tumor-to-nontumor targeting ratio). Affinity is determined by the area of antigen recognition. Attempts to increase the affinity of mAbs are being made by inducing mutations or substituting whole gene segments in the DNA that encodes for the mAb and selecting the ones that have the optimal profile.[5]

Two other important determinants of the tumor-to-nontumor ratio are the distribution and clearance of mAbs from blood, and their ability to penetrate tumor. Imaging should be performed after the unbound mAbs in blood and other tissues have been cleared. Smaller antibody fragments tend to have shorter half-lives (ie, faster clearance) and are also able to penetrate tumor better. These fragments include Fab, $F(ab')_2$ (the two Fab fractions of an antibody with the hinge region), Fv (fragment with only the variable domains V_H and V_L), and scFv (single-chain Fv; Fv fragment with a peptide linker between V_H and V_L to increase its stability) (Figure 1).[5] Some of these fragments, such as Fab, Fv, and scFv, are *monovalent* (they only have one area of antigen recognition) and as such may have less affinity for tumor tissue. Clinical experimentation is currently underway to define the best mAb or mAb fragment for specific future applications of radioimmunodetection.