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EDITORIAL

Biologic Characterization of Melanoma Tumors by Antigen-Specific Targeting of Radiolabeled Anti-Tumor Antibodies

Over the past decade, considerable progress has been made in the use of anti-tumor monoclonal antibodies for targeting human tumors in vivo, both for the purpose of diagnosis (*1*) and for therapy, particularly of radiosensitive tumors such as lymphoma (*2*, *3*). In a brief summary of the clinical aspects of this work, it can be stated that "proof of principle" has been achieved: namely, that otherwise clinically occult tumors are detectable by these methods (*4*, *5*) and that durable remissions in chemotherapy-resistant human tumors (lymphomas) (*6*) and neuroblastoma (*7*) have been produced. The use of Technetium-99m-labeled antibodies promises to make diagnostic applications more and more practical in the future (*8*). The increasing availability of stem cell stimulating factors and autologous marrow rescue procedures promises to be effective in reducing marrow toxicity, which is the critical organ for current radioimmunotherapy regimens (*9*).

Thus, the reader is probably already familiar with the use of monoclonal antibodies (Mabs) for the purpose of diagnosis and therapy of human tumors. I would like to use this occasion to discuss an additional in-vivo use for radiolabeled antibodies; namely, to characterize, based on external imaging of radioactivity uptake, the biologic features of tumors in terms of the expression of specific antigens as markers for known stages of cell differentiation, or as structural components related to specific functions of the tumor cell.

In this issue of *The Journal of Nuclear Medicine* (*10*), Murray et al. focus upon a component of the multi-factorial problem of the mechanisms of radiolabeled antibody targeting to human xenograft tumors in animal models by focusing on "host-factors," which may alter uptake. The principal finding of the paper is that tumor site can influence in vivo tumor uptake, in a manner that is dependent on the antibody used. Four out of the five antibodies used, showed altered uptake when melanoma tumors were in the lung or liver site, in comparison to subcutaneous sites.

It is clear that certain biologic features of tumors and characteristics of currently available anti-tumor antibodies may limit the amount of uptake of the radiolabeled monoclonal antibodies (*11–31*). Table 1 summarizes factors that are known to influence the uptake of radiolabeled antibodies into human tumor xenografts in animal models. Some of these have been confirmed in human tumors.

A review of Table 1 should convince even the most casual reader that the localization of radiolabeled monoclonal antibodies to human cancers is a complicated process that is influenced by diverse biologic and technical features. Improved understanding is important, however,

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TABLE 1
Factors Affecting Tumor Targeting with Radiolabeled Monoclonal Antibodies

Factor	Key finding
Tumor-related factors	
Antigen expression	Uptake increases with increasing antigen concentration in animals (*10*) and in vivo in human tumors (*11*)*.
Tumor size	Uptake inversely proportional to tumor size in animal models (*12*) and in human tumors (*13*).
Tumor capillary permeability	Uptake increased with radiation induced changes (*14*) or in tumors with freely permeable capillary networks (*15*).
Host-related factors	
Lymphokines	Alpha interferon induces increases in uptake in animals (*16*, *17*) and in human tumors (*18*)*.
Tumor site	Uptake greater in more "vascular" tumor beds, such as renal capsule (*15*), although this varies for different antigen/antibody systems (*8*).
Cross-reacting antigens	Uptake is reduced by competition with normal tissue antigens, in animals (*19*), and also in human tumors in vivo (*20**, *21**).
Human anti-mouse ab.s	Uptake is reduced by development of human antimouse antibodies and clearance from the blood is accelerated (*20**, *22**).
Radiolabeled antibody-related factors	
Antibody affinity	Higher affinity leads to greater localization at optimal doses of antibody in human xenograft systems (*23**, *24**).
Radionuclide label	Intracellular retention in tumor and normal tissue is much greater for radiometals such as indium-111 (*25**, *26**) as related to differential metabolism of the tracer.
Fragment size	Larger fragments, such as IgG, have higher absolute uptake, but smaller fragments, such as Fab'$_2$ (*27*) Fab (*28**) or antigen-binding peptides (*29*) may have greater tumor penetration and improved selectivity of uptake in comparison to normal tissues.
Immunoreactivity	Increasing immunoreactivity increases tumor uptake in human tumor xenografts (*30**, *31**).
MAB dose	Increasing antibody dose may increase tumor uptake in human melanoma tumors (*20**, *21**). This feature is not observed in animal model systems (*8*) and depends on the presence of cross-reacting human antigen.

* Human melanoma tumors.

since new knowledge in this area is likely to lead to better antibodies and improved applicability to human cancer diagnosis and therapy. Melanoma tumor is used frequently in animal models as xenografts as well as in human studies.

Despite this complexity, there is evidence that the concentration of the antigen target on the tumor is a major determining factor in the degree of tumor uptake. Evidence for this viewpoint comes from many sources. Certainly, in the test tube, radioimmunoassay is based on the quantitative relationship between antibody binding as expressed in the familiar binding equation (*32*):

$$B/F = K[AG] - b[AB^*], \quad (1)$$

where

B/F is the bound to free ratio associated with antigen; K is the affinity constant for antigen-antibody binding; [AG] is the concentration of tumor-associated antigen; [AB*] is the total concentration of tracer antibody; and b is the fraction of free antibody which is bound.

Because the concentration of trace

radiolabeled antibody is small, the term b[AB*] can be ignored, and Equation 1 becomes

$$B/F = K[AG]. \quad (2)$$

This equation states that specific tumor uptake is directly proportional to the concentration of the specific antigen target. Numerous experimental studies support this relationship as a basis for radiolabeled antibody uptake in vivo. For example, in a study of three cell lines expressing three levels of EGF receptor, low, medium, and high anti-epidermal growth factor receptor antibody uptake correlated with in vivo antigen concentration (*33*). In a study of human melanoma tumors biopsied at 48 and 72 h after injection of iodine-131-Fab 96.5, there was a linear correlation with antigen concentration measured with a radioimmunoassay (*12*). Many of the features affecting antibody uptake in Table 1 are consistent with the dependence between B/F, K, the affinity of binding, and [AG], the antigen concentration.

Frankly, although this study gives us new information, it does not go far enough. In using multiple antibodies targeting different but relatively well-characterized antigens on several tumor types, Murray et al. had the opportunity to better define the role of differing antigen expression on uptake and also relating changes in uptake to in vivo shifts in antigen expression. As Murray et al. well know, there is evidence that such antigenic expression has important biologic implications. The main strong point of the paper is the unequivocal demonstration that in metastatic models the growth site influences the degree of uptake. However, the chief deficiencies of the paper are the lack of detail about the description of the relevant antibody/antigen binding, especially under experimental conditions and site-by-site variations.

Since I am learning to be a New Yorker (and New Yorkers are supposed to be brash), I would like to suggest a starting point for Murray et al. in the soon to be started "next study": that they measure the antigen concentration of their tumors in vivo and correlate these changes with the uptakes observed.

In melanoma tumor lesions in patients, there is great heterogeneity from cell to cell even in the same lesion (Fig. 1). Variability in cell size and shape, the degree of pigmentation, and antigen expression are seen in what amounts to mixed populations of cells at various stages of progression from a more primitive unpigmented cell toward a better differentiated pigmented cell. The expression of specific antigens on melanoma tumor cells is likely to be related to the differentiation program of such tumor cells, as assessed by a panel of differentiation traits, including expression of EGF receptor on early cells and high levels of tyrosinase, an enzyme involved in melanin synthesis, in more differentiated cells. Many individual cell clones can be derived from individual melanoma tumor lesions in patients and these clones can be stimulated to differentiate by specific promoters, such as cholera toxin and PMA. As the cells differentiate toward a more mature pigmented cell, the antigen profile on the cell also shifts.

Given this background, let us consider how we might characterize the tumor cell lines used in the Murray paper and determine what their relative uptake and changes in uptake suggest about the expression of tumor-specific antigens on different cell lines and at different body sites within the animal models used. First, the antibody NR-LU-05, recognizes the chondroitin sulfate proteoglycan (*35*), a marker of more primitive cells. Second, the antibody 96.5, a marker of the p97 membrane iron-binding protein, is widely known as melanotransferrin (*36*), and is expressed at all stages of differentiation. Third, CL507, which binds to the immunoglobulin supergene gene product, and ICAM, a cell adhesion molecule, are also a marker of cells at an earlier stage of differentiation (*37*) and (Houghton A., *personal communication*).

The model that I propose here, namely, that antigen concentration is directly proportional to the degree of uptake of the radiolabeled antibody targeting that antigen, makes some predictions as to what Dr. Murray and his collegues will find if they analyze the antigen content of the tissue removed from the tumor-bearing animals in this series. The chondroitin sulfate proteoglycan antigen was expressed in all cell

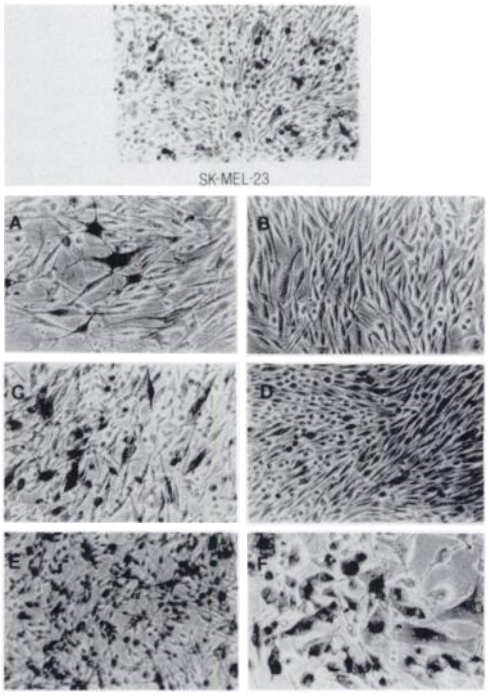

FIGURE 1
SK-MEL-23, a melanoma metastasis, shows a heterogeneous appearance on histologic examination (Top Center). Individual clones derived from this single metastasis (A-F) express different antigens corresponding to different stages of differentiation. (See Reference *32* for details; reprinted with permission).

lines, suggesting that the cells were at an early to intermediate stage of differentiation and that this particular antigen was little affected by metastasic site. The p97 antigen was markedly affected by site of tumor metastases and, especially in terms of the lung site, showed marked increase in concentration in comparison to the subcutaneous site. On the other hand, in the liver the p97 antigen was markedly reduced in uptake under the experimental conditions. The ICAM antigen was really loaded on the DX3 tumors which were likely to be much less differentiated than, for example, HS294T, which expresses a modest amount of this substance in vivo. ICAM is known to be responsive to cytokines, such as gamma interferon that are available in vivo, and so it is perhaps not surprising that in the paper by Murray et al. (*10*) CL207 in vitro uptake was much less, in comparison to in vivo uptake, under the right growth circumstances.

One final thought regarding antigen distribution and antibody localization: if antigen is the major determinant to uptake then factors that reduce access to antigen play a major role in diminishing uptake. In a previous study, we found that after i.v. injection of radiolabeled antibody (^{131}I-9.2.27 targeting the chondroitin sulfate proteoglycan) in human patients with melanoma (*38*) that there was a good general correspondence between the total concentration of tumor-associated antigen and the concentration of radiolabeled antibody when one tumor was compared to another. The highest uptakes were in tumors with 3+ and 4+ expressors of the antigen and the lowest uptakes were in tumors with minimal antigen. However, at a microscopic level, there was a great deal of heterogeneity in radiolabeled antibody uptake, and even in areas of high antigen expression some tumors had only modest uptake of the antibody. In these tumors, the antibody tended to accumulate around the vessels. Thus, antigen content is clearly a necessary and, perhaps, predominating factor in localization, but some of the variability in tumor uptake in vivo must also reflect incompletely understood barriers to the penetration of radioantibody into human tumors and reduced access to antigen. Current clinical research addresses tissue microenvironments as they relate to antibody localization. However, we should not lose sight of the central role of antigen expression to the uptake of radiolabeled antibody which we have observed in vivo (*12*).

In summary, I suggest that the findings in the paper by Murray et al. may be explained by the fascinating in vivo heterogeneity of human melanoma antigen expression and the ability of at least some of these antigens to respond to host factors, such as the presence of lymphokines and the presence of altered growth state of the tumor at different sites. Perhaps I have exaggerated the interpretation of these data, but only to emphasize that in the future we should think about using radiolabeled antibodies to characterize tumors. As we learn more about the biologic functions of the targeted antigens, we may at the same time learn about the detailed biologic status of tumors. Perhaps one day, we will find out clues to the behavior of melanoma tumors based on such antigenic characterization that will improve our ability to predict the aggressiveness of melanoma tumor and devise better regimens for treating it.

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