Improved Tumor Targeting With Radiolabeled, Recombinant, Single-Chain, Antigen-Binding Protein

Steven M. Larson*

"The imperial messenger sets out on his journey; a powerful, an indefatigable man, now pushing with his right arm, now with his left, he cleaves a way for himself through the throng; if he encounters resistance he points to his breast, where the symbol of the sun glitters; the way is made easier for him than it would be for any other man..."—Franz Kafka, "The Imperial Messenger."

"ROBOCOP... part man, part machine"—ROBOCOP, MGM.

Franz Kafka would have found the microenvironment of a tumor compatible with his strange visions—a region where ominous transformations occur that change the natural and orderly into something progressively more unnatural, chaotic, and dangerous.

We wish to send some messengers of our own in the form of radioactivity-laden antitumor antibodies into the dark world of the tumor to discover or to destroy the cancerous cell. Special qualities of the antibody are sought that will make the way easier for passage and uptake of the radiolabeled antibody into the tumor. In this issue of the Journal, Colcher and colleagues have introduced the use of a unique form of a radiolabeled antibody that could be a superior messenger molecule for carrying radioactivity to cancer deposits in vivo.

In the last 10 years, there has been steady progress toward developing clinically useful diagnostic and therapeutic applications of radiolabeled antibodies in oncology. And yet, despite certain progress, it is becoming increasingly clear that, for most tumor types, antibody uptake in tumor is not as great as would be expected. (4)

Perhaps the major limitation of antibody localization lies within the microenvironment of the tumor (5). The facility of antibody penetration of tumors depends on a combination of two factors: diffusion (i.e., antibody movement resulting from concentration gradients) and bulk fluid flow. There are physiologic barriers to the free interaction of radiolabeled antibodies from the blood with their target antigens on tumor cells deep within a tumor mass in vivo. The problems appear to be (a) slow passage of antibodies through capillary endothelium; (b) heterogeneous blood supply, which limits the delivery of antibodies in more poorly perfused regions of tumor; (c) an elevated interstitial pressure within the center of the tumor, so that bulk flow of interstitial fluid tends to carry the antibody into the normal tissues surrounding the tumor; and (d) relatively large transport distances within the tumor interstitium, which is particularly important for large molecules, since the rate of diffusion along a concentration gradient is inversely proportional to molecular weight.

Several approaches have demonstrated improved tumor targeting in experimental and clinical situations, including the use of immune fragments to increase tumor penetration and to make the time of optimal tumor targeting more rapid (6,7) and the use of physical factors to increase the tumor perfusion with heat (8) or to increase the leakage of tumor capillaries with radiation (9).

Use of recombinant tumor antigen-binding protein is based on the rationale that the lower the molecular weight, the more rapidly one can achieve diffusion into the tumor interstitium and an optimal contrast of tumor to normal tissue for imaging. Therapeutic index may also be improved because of the more rapid clearance from the normal tissue versus tumor.

The ability to manipulate the genetic code to produce a novel single-chain, antigen-binding protein patterned after a biologically active antibody is a major advance (10). The single-chain, antigen-binding protein consists of the variable region of the heavy chain (V_H) and the variable region of the heavy chain (V_H) connected by a unique peptide piece. The work of Colcher and associates is important because it is the first in vivo application of a radiolabeled, single-chain, antigen-binding protein in vivo and demonstrates (a) that tumor antigen-binding affinities are sufficiently well maintained to make in vivo tumor targeting possible; (b) that absolute uptake is comparable to uptake of larger antibody fragments such as Fab; (c) that the time to development of optimal tumor-to-normal tissue contrast is very short; and (d) that normal tissues, including kidney, do not retain the single-chain, antigen-binding protein to any large extent.

It should be emphasized that this work is preliminary and that the B6.2 antibody system is unlikely to be useful clinically because the target antigen is widely expressed on normal tissues in humans. Nonetheless, the findings are encouraging and suggest that if single-chain, antigen-binding proteins can be patterned after more generally useful antibodies, these proteins may have advantages over conventionally produced monoclonal antibodies or antibody fragments. First, the rate of tumor uptake of single-chain, antigen-binding proteins will be considerably more rapid, because the diffusion across capillary barriers and interstitial distances will be more rapid. Second, these proteins should be considerably less immunogenic, due to lower molecular weight. In addition, since the development of human anti-mouse antibody (HAMA) binding reduces the ability of antibodies to target tumor antigen (11), reduction of immunogenicity would result in increased ease of use and lowered risk of allergic reaction. Third, it is likely that production will be facilitated because the antibody can be produced in bacterial cell systems, rather than in mammalian cells. Thus, implementation should be less expensive on a large scale, and some of the important concerns that have retarded Food and Drug Administration approval of monoclonal antibodies produced by hybridoma methods are eliminated. One of these concerns is the potential contamination of the radiopharmaceutical...
cal agent with oncogenes or viruses. Finally, the rapid clearance and metabolism of the antigen-binding proteins will reduce radiation exposure to normal organs, particularly the kidney and bone marrow, and will permit the use of additional radioisotopes with short half-lives and favorable properties for imaging, such as technetium 99m (half-life, 6 hr).

If the tumor targeting is as rapid as these early experiments appear to show, it may even be possible to use positron-emitting radioisotopes such as fluoride 18 (half-life, 110 min) as labels for these preparations. This method would permit use of positron-emission tomography (PET) for tumor detection, which is likely to be superior to that possible with conventional nuclear imaging. The advantage of PET is based on extremely high resolution and excellent contrast imaging (12).

The in vivo stability of the single-chain, antigen-binding proteins used by Colcher et al. is encouraging, but it is unlikely that these agents will solve all our targeting problems (13). The special qualities of these antibodies may not be enough to overcome the intrinsic limitations of delivery or the problem posed by expression of the antigen on some normal tissues (14). Both of these factors have sometimes limited the effectiveness of the targeting of tumor antigens. Antibody affinity appears to play an important role in targeting, and it may be difficult to develop single-chain, antigen-binding proteins that achieve the objectives of low molecular weight and optimal affinity of antigen binding.

One problem with “designer” molecules like these proteins is that we do not quite know what to make. Discovery of the best targeting has been completely serendipitous, and why some radiolabeled antibodies are so much better than others is mysterious. For example, the GD2 antigen-binding antibody 3F8m (an IgG3) concentrates to a remarkable extent in the solid tumor neuroblastoma, with peak uptakes of 0.1% of the injected dose per gram (15). This is much greater than the usual concentration of radiolabeled antibodies in solid tumors, which is 0.005% of the injected dose per gram (3).

Kafka’s messenger got through tight spaces with finesse. But maybe what we need is something more like ROBOCOP, who in the movie of the same name blasts his way through obstacles and is equipped to do some real damage when he gets to the bad guys!

Thus, we are tempted to let our imaginations loose to consider the potential benefits of genetically engineered constructs of immune proteins for the future of antibody-directed delivery of radioactivity. Apparently, we don’t have to be satisfied with what nature has provided us in the way of immune proteins for the purpose of antibody-directed delivery to tumors. Through genetic engineering, we can make a tailored molecule that has more desirable features. Perhaps in the future, it will be possible to improve on nature still further by adding other utilities to a targeting molecule, such as a functional group for rapid radio-labeling or a site for attachment of biologic modifiers that could locally dilute tumor blood vessels and reduce unfavorably high interstitial pressures in tumors. In this way, we could turn our antibody into something beyond a simple messenger/carrier—a totally novel kind of genetically engineered entity more like ROBOCOP—a molecule as fully equipped as our knowledge can make it to function optimally in the hostile environment of the tumor, seeking out and destroying the bad cells while minimizing injury to the host.

References

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