Published in final edited form as:
J Control Release. 2009 January 5; 133(1): 2–3. doi:10.1016/j.jconrel.2008.09.074.

Drug Targeting and Tumor Heterogeneity

You Han Bae
Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, 421 Wakara Way, Suite 315, Salt Lake City, Utah 84108, e-mail address: you.bae@utah.edu

1. The myth of drug targeting to solid tumors

"Missile drug," "Magic bullet" or similar words found in newspaper articles or public magazines have often fascinated general readers and scientists for the reason that these words in some way implicate 'remote,' 'seeking' and 'selective destruction.' "Drug targeting" was initially used to describe the possible *in vivo* action of an anticancer drug conjugated to a monoclonal antibody (mAb) which is specific to a surface antigen on target cancer cells. Since then, a range of targeting systems were devised and appeared in scientific papers. These targeting systems exploit over-expressed receptors of nutrients or specific chemistry on cancer cells. The 'targeting' has also given an impression that such conjugates find target cells similar to a smart missile, which may actively chase its target from miles away. This is certainly not the case.

It is seemingly not possible that any water-soluble or nano-sized construct, designed as a drug carrier and introduced into the blood stream, will actively seek target cells that are remotely sitting in solid tumors. It is likely that the drug carriers stumble on floating cells such as leukemic cells in the blood compartment by a probability of collision. To reach its target solid tumor site, a drug conjugate or drug carrier must find the fenestraes (openings) on the tumor blood vessels by chance through convectional flow and random diffusional process. This process, which is linked to probability issues, is called 'passive targeting' coupled with 'enhanced permeability (EP)' [1]. The EP effect on carrier accumulation at tumor sites may be dominated by an opposite force; hydrostatic pressure, which is slightly higher in tumor extracellular space than in normal tissues/organs. Traditionally, tumor targeting approaches are classified into 'passive targeting' and 'active targeting'; however, the active targeting process cannot be separated from the passive because it occurs only after passive accumulation in tumors. Long-circulating property has always been valuable. A longer circulation time definitely improves the probability for a carrier to find large openings on the blood vessels. During circulation in the blood stream, the incorporated drug should remain in the carrier to give high enough concentrations at the target sites. If premature release of drug occurs before the carrier reaches therapeutic sites, it may not help in drug accumulation at the tumor sites.

2. The EPR effect is only a part of the drug targeting

The role of active targeting (interactions) is to promote the entry of drug carriers into target cells. When the retention effect caused by a poorly developed lymphatic system in a solid tumor is significant for macromolecules and nanocarriers in the tumor extracellular space [2], simply sticking to cell surfaces by interactions may not give noticeable contribution to the delivery.

Stand-by effect will only be obvious for conjugate molecules which are small in size (MW<10,000) because the interactions prevent diffusional loss after reaching the target sites and could become an effective mode for imaging. The relatively large carriers (macromolecules and nanocarriers) that adhere to cell surfaces without intracellular translocalization may not give any additional benefit by 'retention' effect. When the drug release occurs in the extracellular space it would be effective on sensitive cells. If the target cells are resistant to anticancer agents by drug efflux mechanisms, it will greatly limit the bioavailability in the cells. Another issue for a nanocarrier after extravasation is that the carriers do not travel far away from the vessels. Most nano-sized particles may locate juxtaposition to the leaky sites due to limited permeability or mobility in extracellular space and non-specific interactions with extracellular matrix. This can block the leaky sites (road block) and hinder extravasation of drug carriers arriving subsequently. It is noted that the tumor blood vessels are not homogeneous in its structure, distribution and density (peripheral region *vs.* central core). EP and tumor physiology are heterogeneous. This greatly influences the supply of oxygen and nutrients. And also the size of fenestraes and its distribution look very much different from location to location even in the same capillary blood vessel.

Thus, the cell internalization is essential for nanocarriers for effective drug delivery besides enhanced permeation and retention (EPR) effect. This may help clearing the carriers from the road, deliver drug intracellulary, avoid Pgp efflux function, and thus kill the cells. This will also help high accumulation of the drug in the target sites. The drug accumulation in the tumor site varies from a few % to around 10%, depending on tumor size, degree of tumor maturation, and vascular structure, while about 1% for the drug without any carriers in small animal models. In other words, the delivery approach can greatly increase drug concentration under favorable conditions, while the rest of drug (for example, which is slightly decreased from 99% to 90%) will distribute in healthy organs. However, the biodistribution profile of a drug carried by soluble macromolecules or nanocarriers will deviate from that of low molecular weight counterpart due to limited permeability. This results in smaller distribution volume in the body, when the loaded agent remains in the carriers. More accumulation of the drug carriers in organs having mononuclear phagocyte system (MPS) cannot be avoided. One of most important functions of drug carriers is altering the toxicity profiles of an anticancer drug.

3. Is active targeting really effective?

Most scientists working in this area love to use 'cell-specificity,' induced by short-range interactions in the interaction pairs of antigen/mAb, ligand/receptor, aptamer/counter part, and a peptide selected from phase display method/counterpart, etc. Specific interactions accompany the words of 'target cell-unique expression' or 'over-expression' when compared with other organs/cells. However, there are seldom quantitative analyses of such specific interactions for active endocytosis in clinical settings. Numerous unanswered questions persist in this regard. For example, what fraction of tumor cells from patients expresses a specific antigen or receptor? What is the expression level in each individual cell? Is the expression level sufficient for active endocytosis? How does the binding affinity between a carrier and a target cell influence the internalization? Is this a matter of kinetics? After endocytosis, does any recycling occur before the release of the payload? If so, what degree?

The cells in a tumor definitely present their diversity: cell types, genomics, proteomics and many others [3]. They are even in a dynamic developing state. Now there are more accumulating evidences in support of the cancer cells that include the side population of stem-like cells, which are characterized by a set of different surface markers from bulk. The cancer stem cells are quiescent under normal conditions. It is claimed that the stem-like cells are sitting on the summit of multidrug resistance and primarily responsible for metastasis and recurrence. After considering all these factors, and if the targeting approach can only kill a fraction of the

cell population, which is located on the accessible part of tumor to the carriers that expresses specific antigen or receptors and high enough for interactions and active endocytosis, how could the tumor be eradicated by current active targeting approaches? In breast cancer patients, less than 30 % is diagnosed positive for Her-2/*neu* antigen. The antigen expression level is graded by the extent of immunostaining. The 'positive' is judged by the staining level which exceeds above a certain standard (but never 100%) and does not guarantee Herceptin therapy [4]. Very recently there have been many papers published which used Her-2 targeting approach. The analysis of folate receptor expression by immunoassay on clinical breast tumors did not show a better situation [5]. A question is whether the results of active targeting based on in vitro or small animal results can really be translated clinically. Despite a long history and many investigators still working on it, the question has remained unanswered.

4. A new paradigm in drug targeting to solid tumors

EPR effect is the single most commonly used term to explain drug targeting. The porosity and pore size of tumor blood vessels has made all kinds of nanocarriers attractive for tumor targeting therapy, imaging, and diagnosis. The porosity is developed in tumors to timely supply enough nutrients to the newly developing area. However, when the tumor stops growing or when its mass starts reducing by any therapy, does the porosity still remain unchanged and thus allow continuous intervention with nanocarriers? It can be postulated that when a growing tumor changes its phase to shrinking, the blood vessel may change its mode from a rapid to a normal supply with a matured structure [6]. In this case, what will be the role of nanocarriers?

It is now the time to re-think tumor specific targeting approaches and cancer nanotechnology, a long-lasting paradigm. Current targeting approaches may need a paradigm shift. The key words in the new paradigm are 'heterogeneity' and 'dynamic state' of cancer cells and tumor physiology.

Acknowledgements

This article was in part supported by National Institute of Health through grant CA122356.

References

1. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics. J Control Rel 2000;65:271–284.
2. Hwang HY, Kim IS, Kwon IC, Kim YH. Tumor targetability and antitumor effect of docetaxel-loaded hydrophobically modified glycol chitosan nanoparticles. J Control Rel 2008;128:23–31.
3. Greller LD, Tobin FL, Poste G. Tumor heterogeneity and progression: Conceptual foundations for modeling. Invasion Metastasis 1996;16:177–208. [PubMed: 9311385]
4. http://www.medscape.com/viewarticle/424719.
5. Hartmann LC, Keeney GL, Lingle WL, Christianson TJ, Varghese B, Hillman D, Oberg AL, Low PS. Folate Receptor overexpression is associated with poor outcome in breast cancer. Int J Cancer 2007;121:938–942. [PubMed: 17487842]
6. Hobbs SK, Monsky WL, Yuan F, Roberts WG, Griffith L, Torchilin VP, Jain RK. Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment. Proc Natl Acad Sci USA 1998;95:4607–4612. [PubMed: 9539785]