Role of antibodies in cancer targeting

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Abstract

The development of chemotherapeutic agents capable of specifically eliminating tumor cells has been a great challenge since these agents cannot differentiate between normal body cells and tumor cells. Enhanced elimination of cancer cells without affecting normal body cells can be achieved by developing strategies which can enable drug targeting. With recent advances in antibody engineering strategies, the development of different antibody-associated tumor-targeted delivery systems for chemotherapy, chemoprevention, and early cancer diagnosis has become possible. In this review, the role of antibodies for cancer diagnosis, chemoprevention, and chemotherapy will be discussed with an emphasis on recent advances in antibody engineering.

Key words: Antibody engineering, cancer diagnosis, cancer targeting, chemoprevention, chemotherapy

INTRODUCTION

Cancer cells share many common features with the cells from which they are derived. In the case of bacterial and viral infections, well-defined discriminate molecular targets specific to the parasite can be identified. Chemotherapy against these unique targets can lead to the eradication of these parasites. In the case of cancer, there is a lack of such unique targets since they are derived from normal body cells of an individual. Chemotherapeutic agents like paclitaxel and doxorubicin cannot distinguish between normal cells and cancer cells leading to indiscriminate cytotoxicity of almost every cell they penetrate. Because of the nonselectivity of these drugs, the dose has to be often restricted to lower levels in order to avoid toxic side effects of these drugs on normal cells. This low dose is often not enough for complete eradication of all cancer cells. Prolonged low-dose exposure of these cytotoxic drugs to cancer cells gives them ample time to generate mutations and henceforth they generate mechanisms such as production of transmembrane efflux pumps in order to evade killing through these cytotoxic agents. This often leads to relapse and drug resistance and ultimately failure of chemotherapy. If the concentration of a cytotoxic agent reaching tumor cells can be increased compared to that reaching normal body cells, selective killing of cancer cells can be achieved.[1]

The identification of antigens specific to cancer cells called tumor-specific antigens or the antigens overexpressed by cancer cells called tumor-associated antigens has enabled researchers to develop therapies that can specifically target these antigens. Antibodies or ligands can be used to target these antigens which can lead to specific accumulation of a cytotoxic molecule at the tumor site. Targeting with nonantibody ligands such as arginine–glycine–aspartate (RGD), folate, trasferin, etc., has the disadvantage that they are abundantly present even on normal cells and hence the cytotoxic agent targeted through these ligands can bind to nontarget tissue. In the case of ligands such as folate present abundantly in diet, a free form of ligand can compete with the ligand-modified targeted delivery. Toward this end, antibodies serve much better target specificity and selectivity.[1]

ANTIBODY ENGINEERING

Among the antibodies circulating in serum (IgG, IgA, IgM, IgE), IgG is the major antibody used for therapeutics and diagnostics. It has a Y-shaped structure. The two arms of the Y structure contain antigen recognition sites and the stem structure below brings about effector functions. Monoclonal antibodies themselves[2] or their fragments can be used for cancer therapy. Monoclonal antibodies used, such as recently approved Herceptin or Rituxumab, cause selective cellular toxicity first by binding to specific target antigen followed by cell lysis either by antibody-

dependent cellular cytotoxicity, complement activation, complement-dependent cytotoxicity, or by inhibition of signal transduction (e.g. the inhibition of dimerization of a receptor by receptor blocking through a monoclonal antibody).[2] The antibody taken as a whole has a high affinity to antigens and owing to two antigen recognition sites per antibody molecule, avidity is also high. The entire antibody molecule has a disadvantage of higher immunogenicity in humans. While for imaging their long half lives can result in delayed clearance resulting in high background noise. For use of antibodies in imaging clearance of an antibody from the body should be quick in order to reduce the background noise during imaging. While using antibodies for cancer therapeutics, immunogenicity of murine antibodies in humans may cause degradation of the antibody by immune system before it reaches targeted tissue or in worst case may cause hypersensitivity reactions like anaphylactic shock or massive cytokine release. With recent advances in antibody engineering techniques, chimeric, humanized, or human antibodies can be produced which may elicit little or no immunogenic response. Immunogenicity and serum half life can be reduced by the use of antibody fragments rather than using a full antibody molecule. The F_c fragment of an antibody molecule can bind to F_c receptors on macrophages and elicit immunogenic response by its complement activating effect. Hence, using an antibody fragment devoid of the F_c fragment, since it lacks the F_c region, elicits less immunogenic response. The disadvantage of using antibody fragments such as Fab or scFv is that it reduces antibody avidity. The strategy to overcome the problem of reduced avidity includes engineering of antibody fragments which have multivalent binding sites or delivering many such fragments through a common carrier, e.g., immunoliposomes. First, antibody fragments are cleaved by using enzymes such as papain or pepsin. Then the cleaved fragments are joined by genetic engineering techniques to form monovalent or bivalent fragments. To increase the avidity of engineered fragments, dimeric, trimeric, or tetrameric conjugates of Fab or scFv antibody fragments can be engineered by using either chemical or gentic cross-links between individual fragments. Multivalent antibody fragments can even be engineered wherein they have binding sites for more than one different antigen; this way more than one cellular target can be identfied by a single multivalent antibody conjugate.[3]

IMMUNOCONJUGATES

Tumor-targeted immunoconjugates consist of an antibody and an effector moiety bonded together by either covalent cross-links or genetic fusion. If the effector moiety is a cytotoxic drug, it is called antibody–drug conjugate; if the effector moiety is a protein toxin, it is called an immunotoxin; if the effector moiety is a radionuclide (toxic radioactive isotope), it is called a radioimmunoconjugate. [4,5] Antibody drug conjugates consist of a cytotoxic drug conjugated to an antibody for targeting a specific antigen. The antibody binds to the specific antigen and is internalized by receptor-mediated endocytosis. Free drug is released in the intracellular compartment by lysozomal enzyme-mediated degradation of the drug–antibody conjugate whereby the drug restores its cell-killing potential. As the drug is directly available in the intracellular compartment, it evades efflux by transmembrane efflux pumps belonging to the ABC transporter family found in multidrug-resistant (MDR) cancer cells. Hence drugs delivered through targeted antibody–drug conjugates can effectively kill MDR cancer cells by escaping efflux pumps. The site of conjugation should be specific because any variation in the structure of the binding site of the antibody may lead to loss of its binding activity. Cystein residues can be used for antibody drug conjugation or additional cystein residues can be introduced for site-specific conjugation. An example of an immunoconjugate approved by FDA is Gemtuxumab which consists of a conjugate of a humanized anti-CD33 antibody and a cytotoxic drug calicheamicin joined together by two cleavable bonds.[6,7] Generally, a higher number of drug molecules are attached to a single targeting antibody. But as the number of drug molecules attached per antibody molecule increases, the target binding activity of the antibody decreases. To overcome this problem, carriers like dextran, hydroxymethylpropylamineacrylamine (HPMA), and serum albumin can be used to attach more number of drug molecules per targeting antibody.[1]

IMMUNOTOXINS

Immunotoxins are another class of antibody drugs used for targeted killing of cancer cells. Here a protein toxin is attached to either a full antibody, or scFv, or a targeting ligand. The conjugation is either through chemical cross-links or through genetic fusion.[5] Toxins are mainly cytotoxic enzymes. The antibody recognizes and binds to the specific tumor antigen and gets internalized. After the conjugate is internalized, the attached toxin begins its cytotoxic function and causes cell death.[6] Toxins used are primarily plant toxins or bacterial toxins. These sometimes show immunogenicity. To avoid this, toxins of human origin have been tried. They include proapoptotic proteins which can cause apoptosis or RNAses.[8]

BISPECIFIC ANTIBODIES

Bispecific antibodies have binding sites for two different epitopes of same or different antigens. These have a variety of applications in cancer therapy and diagnosis. When

a bispecific antibody has binding sites for two different epitopes for the same antigen, it increases both affinity and avidity of the antibody for the antigen. Bispecific antibodies having binding sites for epitopes for two different antigens can be used for the recruitment of toxins, cytotoxic agents, or radionuclides to a specific targeted site. A bispecific *diabody* consists of two scFv fragments fused with two different Fv domains for the recognition of two distinct antigenic epitopes. A bispecific *minibody* consists of two scFv fragments again fused with two distinct Fv binding domains connected by a six-carbon heavy chain. These bispecific antibody structures can be administered first whereby target-specific binding of antibody occurs, followed by infusion of the radionuclide or cytotoxic drug. A cytotoxic drug or radionuclide can bind to the second binding site on the bispecific antibody. scFv–Fc are another class of antibody fragments where a scFv fragment is attached to a Fc fragment. Mutation in the Fc domain leads to loss of the binding property of the Fc receptor.[7] Exposure of radionuclide for longer duration can lead to enhanced toxicity; hence it is ideal to have quick blood clearance and higher tumor uptake in short duration. This can be achieved by using smaller sized antibody fragments. MicroPET image in mice infused with diabody, minibody, and scFv–Fc (^{124}I-labeled anti-CEA [carcinoembryonic antigen]) showed least background noise with a diabody followed by a minibody and scFv–Fc fragments because of increase in clearance rates which is associated with their size.[3]

ANTIBODY-DIRECTED ENZYME PRODRUG THERAPY

This includes the activation of a weakly toxic prodrug by an enzyme attached to the antibody at the tumor site to an active toxic drug. This strategy includes the use of enzymes which are either mammalian, nonmammalian with human homologs or nonmammalian enzymes. The problem with nonmammalian enzymes is immunogenicity while that with mammalian enzymes is lesser target specificity.[6]

ANTIBODY CYTOKINE FUSION PROTEINS

One of the factors that modulate the survival of tumor cells is the immune tolerance in tumor microenvironment. This is related to levels of cytokines in the tumor microenvironment which can modulate the activity of regulatory T-cells leading to the protection of tumor cells from immune surveillance.[9] If proinflammatory cytokines are administered without targeting the tumor site, they may cause massive damage to normal cells; hence the targeting of cytokines to tumor cells is very essential. Antibodies against specific tumor antigens and proinflammatory cytokines like IL-2 or TNF-alpha can be fused and administered causing an accumulation of the complex at tumor mass resulting in the immune activation of CD8+ T-cells which can cause the destruction of tumor cells. Even here, engineered antibody fragments serve similar advantages mentioned before compared to the whole antibody molecule.[6]

ANTIBODIES AND CANCER VACCINE TARGETING

The concept of cancer vaccine is under active development. Many peptide and DNA vaccines are being tested in human subjects through clinical trials. Most vaccine antigens including recombinant protein-based vaccine antigens or plasmid-based vaccine antigens elicit only a weak immunogenic response when administered alone. The immunogenicity of these antigens can be increased by their association with an adjuvant. Traditional live attenuated vaccines are highly immunogenic because they contain many epitopes and several pathogen-specific molecular patterns which can be identified by the immune system. Newer recombinant protein antigens, peptide antigens, or protein antigens expressed through plasmid DNA do not contain as many antigenic epitopes which can be presented to antigen-presenting cells (APCs) and hence they do not elicit a strong immune response.[10] Antibodies can be used for specific targeting of antigens to APCs. Monoclonal antibodies against MHC-II determinants on APCs can be conjugated with tumor-specific or tumor-associated antigens for immunotargeting of antigens toward APCs. Through this strategy, a significant increase in IgG responses were observed in different animal models.[11]

ANTIBODY LIGAND-FUSED PROTEINS

Apoptosis which is programmed cell death is one of the cellular mechanisms which does not function normally in most tumor cells.[12] Apoptosis can be triggered through death receptors by extracellular factors such as Fas ligand, tumor necrosis-related apoptosis-inducing ligand, and tumor necrosis factor. Antibody ligand-fused proteins can be used to trigger apoptotic pathways in tumor cells. Toward this end, recombinant multimeric Fas ligand or anti-Fas antibodies and tumor necrosis-related apoptosis-inducing ligand were developed to evaluate their efficacy. Although efficacious, when recombinant multimeric FasL was administered systemically, systemic toxicities were observed with high incidence of hepatic toxicity. In order to avoid these systemic toxicities caused by recombinant multimeric FasL, a soluble FasL(sFasL) was used. sFasL is

inactive in its non-aggregated form, but when aggregated, mimics the biological FasL activity. In order to target sFasL to tumor cells, antibodies for specific antigens on tumor cells were attached to sFasL. Attachment of antibody-sFasL complex on tumor cell surface was followed by recruitment of more sFasL molecules resulting in formation of an active trimeric Fas ligand which was then able to trigger the apoptotic pathway resulting in cell death specific to cell types for which antibody-sFasL was targeted with reduced systemic toxicities.[6]

ANTIBODIES FOR TARGETED SIRNA DELIVERY

The natural phenomenon of gene silencing by small interfering ribonucleic acid (siRNA) can be exploited for therapy of various diseases including cancer and HIV. For siRNA to find its way to clinics, its targeted delivery is essential. The targeted delivery of siRNA to the desired cell type increases its therapeutic index, decreases unwanted systemic effects, and increases the proportion of the payload delivered at the desired site of action. Antibodies are potential agents which can be used for targeted siRNA delivery. Song *et al*. showed targeting siRNA specifically to Her2+ breast cancer cells using a siRNA condensed through a fused protein complex. The fusion protein consisted of a fragment of small-chain M59 antibody which can recognize Her2 conjugated at the C terminus of protamine. A constant region of the antibody was discarded in order to avoid immunogenic reactions. When this siRNA–anitbody–protamine complex was tested *in vitro* and *in vivo*, the gene silencing effect was observed only in Her2+ breast cancer cells and not in Her2- breast cancer cells. A similar strategy can be applied for targeting specific cell types by using the siRNA–protamine–antibody complex with antibody fragments against the surface antigen specific to a particular cell type.[13]

CANCER STEM CELLS

Increasing knowledge and evidence about the existence of a small population of cancer cells called cancer stem cells which differentiate to form cancer cells may require targeting through stem cell specific antigen for specific eradication of these subtypes of cancer cells.[14] Relapse of tumor cells after chemotherapy is associated with a small population of stem cells left behind unaffected by chemotherapy. These cells differentiate later to become normal cancer cells which are often resistant to previous therapy. Since stem cells are not rapidly dividing cells, conventional chemotherapy targeting rapidly dividing cells remains ineffective against cancer stem cells. Hence for a complete eradication of cancer, it is important to eradicate cancer cells along with cancer stem cells. This requires the identification of specific targets or exclusive pathways nonexistent in normal cells but prevalent in cancer stem cells. This is still a developing concept and needs more research.[15]

In conclusion, monoclonal antibodies can be effectively used for cancer therapy. They can help in specific targeting of other relatively less selective anticancer agents. To enhance the selectivity of antibody therapy for tumors, the identification of more tumor-specific antigens is necessary. Although many strategies have been proven to be useful in preclinical models, these findings need to be confirmed through human trials.

REFERENCES

1. Allen TM. Ligand-targeted therapeutics in anticancer therapy. Nat Rev Cancer 2002;2:750-63.
2. Adams GP, Weiner LM. Monoclonal antibody therapy of cancer. Nat Biotechnol 2005;23:1147-57.
3. Holliger P, Hudson PJ. Engineered antibody fragments and the rise of single domains. Nat Biotechnol 2005;23:1126-36.
4. Milenic DE, Brady ED, Brechbiel MW. Antibody-targeted radiation cancer therapy. Nat Rev Drug Discov 2004;3:488-99.
5. Payne G. Progress in immunoconjugate cancer therapeutics. Cancer Cell 2003;3:207-12.
6. Schrama D, Reisfeld RA, Becker JC. Antibody targeted drugs as cancer therapeutics. Nat Rev Drug Discov 2006;5:147-59.
7. Wu AM, Senter PD. Arming antibodies: Prospects and challenges for immunoconjugates. Nat Biotechnol 2005;23:1137-46.
8. Mathew M, Verma RS. Humanized immunotoxins: A new generation of immunotoxins for targeted cancer therapy. Cancer Sci 2009;100:1359-65.
9. Rice J, Ottensmeier CH, Stevenson FK. DNA vaccines: Precision tools for activating effective immunity against cancer. Nat Rev Cancer 2008;8:108-20.
10. Singh M, O'Hagan D. Advances in vaccine adjuvants. Nat Biotechnol 1999;17:1075-81.
11. Barber BH. The immunotargeting approach to adjuvant-independent subunit vaccine design. Semin Immunol 1997;9:293-301.
12. Evan GI, Vousden KH. Proliferation, cell cycle and apoptosis in cancer. Nature 2001;411:342-8.
13. Song E, Zhu P, Lee SK, Chowdhury D, Kussman S, Dykxhoorn DM, *et al*. Antibody mediated *in vivo* delivery of small interfering RNAs via cell-surface receptors. Nat Biotechnol 2005;23:709-17.
14. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001;414:105-11.
15. Sawyers C. Targeted cancer therapy. Nature 2004;432:294-7.

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