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Review Article

Targeted therapies directed to tumor-associated antigens

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Abstract

Despite advances in cancer therapies in recent years, side effects of systemically administered drugs often remain a dose-limiting factor in therapeutic protocols. To overcome this problem, self-directed localization of therapeutic agents to the disease-affected target tissue has been proposed. For tumor targeting, disease-specific cellular tumor-associated antigens have been exploited for this purpose. This review describes the tumor-associated antigens currently used in the clinic, as well as methods to identify new and more useful ones for future use. Subsequently, ligands that are being used for the recognition of these targets are described, including antibodies and their derivatives, peptides, as well as natural ligands. Furthermore, carrier molecules that will be able to transport the therapeutic moiety to the tumor are discussed, such as liposomes, nanoparticles, viruses, proteins and cells. Finally, various classes of therapeutic moieties that can be employed in such an approach are listed, including toxins, cytokines, chemical compounds, radionuclides and therapeutic genes. Consideration of the advantages and disadvantages of each component in a tumor-targeted therapeutic agent will allow the rational design of such a construct for maximum clinical efficacy, tailored to the individual needs of the patient.

Introduction

In recent years, considerable progress has been made in the development of targeted therapeutics for the treatment of cancer. Several tumor-targeted therapies have been approved for clinical use, and others have shown remarkable progress in preclinical animal models. The aim of this review is to describe the current status of tumor-assocated antigen (TAA)-targeted therapies, first describing which TAAs have been employed to date, followed by a description of ligands, carriers and therapeutic moieties that have been investigated.

Tumor-associated antigens

An ideal TAA should have several characteristics (1): 1) the expression of the antigen should be homogeneously distributed throughout the tumor to ensure targeting and eradication of all cancer cells; 2) the expression of the antigen should be high enough to ensure the accumulation of a sufficient dose of the targeted agent to achieve a therapeutic effect; 3) the expression of the antigen should be absent or limited in normal tissues; and 4) the antigen should be membrane-bound to avoid the creation of an unwanted 'sink' for the targeted agent in the bloodstream or interstitial fluid. The great majority of the antigens that have been described exhibit most but not all of these characteristics. An example is carcinoembryonic antigen (CEA), which was first identified in tissues from colon cancer patients in 1965 (2). Since then, it has become apparent that this molecule is overexpressed in several other carcinomas as well, including lung and breast cancer (3). In these cancers, loss of polarity of cancerous cells leads to shedding of plasma membrane-derived vesicles with CEA expressed on their surface (4), which accumulate in draining lymph nodes and blood vessels. CEA thus ends up in the bloodstream, where it can be exploited as a serum marker to monitor potential recurring disease after surgery for colon cancer (3). Despite its presence in the bloodstream —mentioned above as an unwanted property of TAAs—, CEA is utilized as a target in cancer patients, with an antibody frag-

ment demonstrating selective tumor accumulation in a pilot clinical trial (5). This illustrates that not all criteria for TAAs must be met for successful clinical application directed towards these molecules. Rather, these criteria should be viewed as guidelines for the identification of potential new markers in neoplastic disease.

Currently exploited tumor-associated antigens

Tumor antigens include growth factors and their receptors, oncogenes and their products, hormones and glycoconjugates including glycoproteins and glycolipids (6). Only very few truly tumor-specific antigens exist, the surface Ig idiotype expressed in B-cell lymphomas being a prime example (7). Several vaccine strategies targeting this idiotype are currently in phase III clinical trials for non-Hodgkin's lymphoma, and several other diseases are being explored as well (8). Most antigens, however, are also expressed in normal adult tissues or during developmental stages of the embryo. An example is the aforementioned CEA, which is normally expressed in the colon, stomach, tongue, esophagus, cervix, sweat glands and prostate (3).

Epidermal growth factor receptor (EGFR, c-ErbB-1), from the group of growth factor receptors, is overexpressed in squamous cell carcinoma of the head and neck (9), breast cancer (10) and ovarian cancer (11), among others. An antibody directed to EGFR, Erbitux™ (cituximab), was recently granted accelerated approval by the U.S. Food and Drug Administration (FDA) for the treatment of metastatic colorectal cancer based on its therapeutic effects in phase II clinical trials. Another growth factor receptor family member, c-ErbB-2 (HER2/neu), is overexpressed in several types of cancer as well, with breast cancer as a prominent example (12). An antibody directed to c-ErbB-2, Herceptin™ (trastuzumab), was one of the first antibodies to be successfully used in cancer patients and more than 35,000 patients have been treated since 1998, when the antibody was approved by the FDA. It is important to note that Herceptin™ only has therapeutic effects in patients overexpressing HER2, mandating proper screening for receptor expression before treatment protocols are initiated. Other examples of growth factor TAAs include the folate receptor, which is overexpressed in ovarian cancer and mesothelioma, and c-Met, the receptor for hepatocyte growth factor (HGF), which was recently identified as a new target for tumor therapy (13).

In addition to overexpression of TAAs in neoplastic cells, cell-surface glycosylation patterns can differ from healthy tissue, and these aberrant patterns can be utilized for tumor-selective targeting. As an example, the mucin-1 molecule is heavily glycosylated in normal tissues, whereas in neoplastic cells the molecule is underglycosylated (uMUC-1). This has led to the development of antibodies specifically directed to the underglycosylated epitopes on this protein, as well as cancer vaccines based on this premise (14). Furthermore, a synthetic peptide derived from an antibody to uMUC-1 has been incorporated into a multimodal imaging probe and used successfully to image uMUC-1-positive tumors in mice (15), demonstrating the feasibility of targeting based on glycosylation patterns.

Identification of novel tumor-associated antigens

Given the lack of TAAs, as described above, ongoing efforts are aimed at identifying new antigens for a variety of cancers, utilizing advanced technologies such as mRNA microarrays, proteomics and serological identification of antigens by recombinant expression cloning (SEREX).

The identification of more than 20,000 genes by the human genome project has provided a pool of possible new targets in cancer, and screening for these has become significantly more facile in recent years due to the commercialization and increased availability of mRNA microarray technology. Several new targets have been identified using this technology, such as the carcinoma-assocated antigen GA733-2, and hepsin, a transmembrane serine protease found in prostate cancer (16). Another example is the high expression of the preferentially expressed antigen of melanoma (PRAME) gene detected by microarray in ovarian cancer samples (17).

Compared to the number of genes present in the human genome, the number of possible protein targets is increased severalfold due to the intermediate steps between mRNA and protein expression, and the protein variability potentially introduced with each of these steps. These variabilities include mRNA splicing, post-translational processing and glycosylation, among others. The Human Proteome Organization (HUPO), formed in 2001, aims to systematically characterize protein expression in health and disease, and its Plasma Proteome Project involves the participation of several laboratories throughout the world to identify tumor markers that can be used both for screening and as therapeutic targets (18). The mRNA and protein array technologies can also be combined in order to discover new biomarkers, as was demonstrated by Nishizuka *et al.,* who identified villin as a new marker for colon cancer using this combination of technologies (19).

Another method used to identify new tumor targets is SEREX, which was first desribed by Sahin *et al.* in 1995 and is based on the presence of TAA-recognizing antibodies in the serum of cancer patients (20). In this approach, a cDNA library is constructed from tumor specimens and cloned into expression vectors. Clones are then screened for reactivity with the serum of the patient, and the nucleotide sequence of the cDNA insert is determined. In recent years, several antigens have been identified that can be classified into different groups, including cancer testis antigens, differentiation antigens, overexpressed gene products, mutated gene products, splice variants and cancer-related autoantigens. Examples of antigens discovered using this technology are

melanoma-associated antigen 1 (MAGE1) in melanoma and metastasis-associated protein 1 (MTA1) in prostate cancer (21).

Therapies directed to tumor-associated antigens

The basic design of a tumor-targeted therapeutic agent consists of a targeting ligand mediating recognition of the TAA, a carrier molecule to which the targeting moiety can be attached or incorporated, and a therapeutic entity mediating the antitumor effects (Fig. 1). However, in many targeting constructs these entities cannot be readily distinguished due to overlapping functions of the incorporated entities. For example, in the case of antibody-based immunotoxins or radioimmunoconjugates, the antibody serves simultaneously as the targeting ligand and the carrier molecule. Therapeutic moieties can be either covalently attached to the carrier molecule, as is the case in immunoconjugates, or incorporated into the carrier, as is the case in immunoliposomes.

Ligands used in targeting tumor-associated antigens

Several types of ligands that recognize TAAs have been described; the most widely used include antibodies and their derivatives, peptides and natural ligands, which will be described in more detail below. In addition to these protein-based ligands, various sugars and even DNA sequences have been utilized. As an example of the latter, systematic evolution of ligands by exponential enrichment (SELEX) identified an oligonucleotide that interacts with tenascin-C, an extracellular protein found in tumor matrix (22). This aptamer has been utilized to target human glioblastoma cells in nude mice after systemic administration (23).

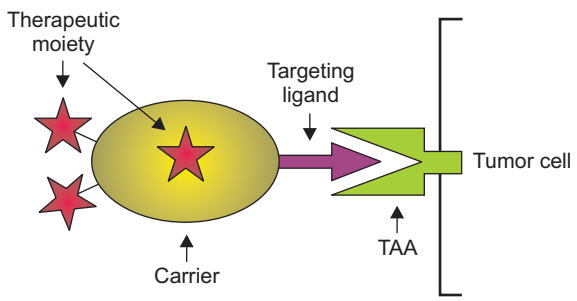

Fig. 1. Schematic representation of a targeted therapeutic recognizing a tumor-associated antigen. A targeted anticancer construct usually consists of three moieties: a targeting ligand (purple) mediating recognition of a tumor-associated antigen (TAA, green), a carrier (yellow) to which the targeting ligand is attached, and a therapeutic moiety (red) which can either be attached to the carrier surface or be incorporated inside the carrier, depending on the carrier type.

1. Antibodies and their derivatives

Since their discovery, antibodies have been heralded as 'magic bullets', able to target any cell type of choice. When Kohler and Milstein described the creation of monoclonal antibody-secreting cells (24), antibody production became trivial, fostering their application for the treatment of disease. However, initial optimism was tempered when mouse-derived antibodies evoked strong human anti-mouse antibody (HAMA) responses in patients, thus limiting their efficacy and resulting in toxicity. Humanization of antibodies can partially alleviate this shortcoming, although antigenicity can never be completely eliminated (25). These hurdles have not stopped the development of therapeutic antibodies for the treatment of cancer, with Herceptin™, Erbitux™ and Rituxan™ (rituximab) as examples for the treatment of breast cancer, colon cancer and lymphoma, respectively.

Whole antibodies cannot penetrate tumor tissue very efficiently due to their high molecular weight (approximately 150 kD). This sparked the genetic engineering of antibody fragments, such as single-chain variable antibody fragment (scFv) proteins (26). Although a monovalent scFv molecule has a lower avidity than a divalent IgG molecule, genetic engineering can increase the affinity of an scFv for its target antigen, as demonstrated by Kuan *et al.*, who increased the binding affinity of an anti-EGFRvIII scFv by approximately 15-fold, resulting in improved targeting to glioma cells in tumor-bearing mice (27).

2. Peptides

Tumor-associated antigens have been used to screen phage display libraries for the presence of high-affinity peptides that can subsequently be used for targeting purposes. This technique was first pioneered by Smith in 1985 (28), and by the early '90s the first random peptide libraries were constructed. As an example, Peletskaya *et al.* screened a 15-mer library for peptides able to bind the Thomson-Friedenreich (T) antigen, a glycoantigen present on several carcinomas, and identified peptides with an affinity of around 1 μM (29, 30). One of the most well-known examples of a peptide identified by phage display is the RGD amino acid sequence, which has high affinity for integrins that are expressed in tumor vasculature. This peptide, identified by the group of Ruoslathi in 1993 (31), has been used extensively in drug- or gene-targeting approaches, either in linear or cyclic format. For example, Schiffelers *et al.* coupled this peptide to a liposome carrier and demonstrated selective localization of this drug-targeting construct in angiogenic vasculature in a dorsal skin chamber model (32). Furthermore, incorporation of RGD in both adenoviral and adeno-associated viral vectors led to a dramatic enhancement in infectivity of these vectors in a variety of cancer cells (33, 34).

3. Natural ligands

In addition to antibodies and peptides, natural ligands can be utilized to target therapeutic moieties to their desired site of action due to their interaction with growth factor receptors overexpressed on tumors or tumor endothelium. As an example, the vitamin folate has been used in numerous constructs to target the folate receptor, which, as mentioned above, is overexpressed in a wide range of tumors. For example, adenoviral vectors have been redirected to cancer cells using an antibody fragment directed against the adenoviral knob protein chemically conjugated to folate (35). In addition, folate has been incorporated into protein-based constructs and liposomes and administered as low-molecular-weight prodrugs (36).

Another illustration of a natural ligand is EGF, which is the ligand for the EGFR, overexpressed in breast and ovarian cancer (see above). It has been used to redirect liposomes to human glioma cells *in vitro* (37), and a genetic fusion protein consisting of the soluble domain of the coxsackie and adenovirus receptor (CAR) and EGF has successfully been used to retarget adenoviral vectors to EGFR-overexpressing cancer cells (38).

Carrier molecules

The choice of carrier will depend on the particular payload of the therapeutic construct –whether for instance a gene, a protein or a low-molecular-weight compound needs to be delivered. Carriers can be divided into particle-type carriers, soluble carriers and cell carriers (39). The first category includes, among others, liposomes, nanoparticles and viruses, the latter being extremely efficient in the delivery of gene constructs. Soluble-type carriers include antibodies or fragments thereof, which have intrinsic targeting and therapeutic capacities, and relatively inert proteins such as albumin, which might be more suitable for the delivery of low-molecular-weight compounds. Cellular carriers have been exploited in recent years for the delivery of immunotoxins and even viruses, and this area of research will likely continue to grow due to the surge in stem cell research. The different types of carriers are briefly discussed below.

1. Liposomes

Liposomes are small vesicles comprised of a phospholipid bilayer enclosing an aqueous compartment. They are regarded as useful drug-targeting vehicles because of their high drug-loading capacity, their structural versatility and the harmless nature of their components (40). One of the disadvantages of liposomes is the accumulation of these particle carriers in cells of the reticuloendothelial system after intravenous administration, but the use of molecules such as polyethylene glycol (PEG) as a polymeric coating of the lipid bilayer can prevent this uptake to a large extent (41). Targeting moieties such as antibodies and peptides can be readily incorporated into liposomes, and such targeted constructs have successfully been applied in animal models of cancer, such as neuroblastoma (42, 43).

2. Nanoparticles

The term nanoparticle is applied to a wide variety of particle-type carriers, but in general is considered to be devices smaller than 100 nm. Research into these types of drug or gene carriers is blossoming, although most particles are still in the preclinical stage of development. An example is the use of dendrimers, which are well-characterized, highly branched synthetic macromolecules that can simultaneously incorporate targeting, imaging and therapeutic motifs. Dendrimers targeted to the folate receptor are able to selectively deliver methotrexate to human tumor cells implanted in nude mice, resulting in enhanced therapeutic effects and decreased toxicity compared to the free drug (44). Another example where the nanoparticle is not only the carrier molecule but also has intrinsic therapeutic properties is the use of gold nanoshells, which are comprised of a silica core covered by a thin metal shell. Targeting of these nanoshells to c-ErbB-2-expressing cells, followed by irradiation with near-infrared laser light and subsequent hyperthermia induction, results in tumor cell death (45), although experiments *in vivo* need to further clarify their therapeutic potential.

3. Viruses

For gene therapy applications in cancer, viral vector systems still have a higher gene delivery efficiency compared to naked DNA- or polymer-based vector systems, although significant progress with the latter is under way. In the last few years, targeting of viral vectors to TAAs has finally moved from cell culture test systems to *in vivo* models in mice, with adenoviral and measles virus vectors as prime examples. With respect to the former, we have recently demonstrated retargeting of adenovirus serotype 5 (Ad5) to human CEA in a transient transgenic mouse model using a bispecific adapter molecule incorporating an anti-CEA scFv (46). Hedley *et al*. have elegantly improved upon this targeting system, demonstrating direct genetic incorporation of scFv antibodies into the adenoviral capsid, although targeting capacities still need to be assessed *in vivo* (47). With respect to the measles virus, Nakamura *et al.* demonstrated that the native tropism of this virus can be ablated while simultaneously introducing tumor-targeted tropism via the incorporation of scFv antibodies into the H protein of the virus (48). Subsequent replication of this virus in human tumor xenografts in nude mice demonstrated specific, receptor-mediated antitumor activity due to the oncolytic nature of this virus, illustrating the concept that the carrier can

simultaneously serve as the therapeutic agent and tumor-targeting construct.

4. Antibodies and their derivatives

An illustration of a carrier that simultaneously serves as the targeting moiety is the use of antibodies and their derivatives, wherein a therapeutic molecule is directly conjugated to the antibody. Incorporation of toxins (49), cytokines (50), radioisotopes (51) or drugs (52), via either chemical conjugation or recombinant technology approaches, has demonstrated some efficacy. However, the drawback of chemical conjugation strategies is the generation of heterologous products due to variations in the number of therapeutic molecules coupled per antibody, as well as the localization of the attached moiety on the carrier. In addition, there is a risk of conjugating a therapeutic moiety to the antigen-binding site of the antibody, thereby eliminating antigen recognition and -targeting characteristics. Therefore, when the therapeutic moiety is a peptide or protein, such as a cytokine or a toxin, recombinant DNA approaches are the method of choice, resulting in a uniform end product with an optimized configuration of all the components involved (53). Although antibodies have a reduced loading capacity for drugs compared to particle-type carriers, this may be compensated for by their superior biodistribution and pharmacokinetics (54). Therapeutic studies comparing both types of drug carrier systems are required to clarify their possible applications in the treatment of cancer.

In addition to their function as targeting motifs and carrier molecule, antibodies also have intrinsic therapeutic effects due to their interaction with the immune system. Their constant domain (Fc) interacts with Fc receptors on the surface of natural killer (NK) cells, which can release perforins and granzyme, which will kill the cell to which the antibody is attached –a process known as antibody-dependent cellular toxicity (ADCC). Antibodies also bind components of the complement system, leading to direct cell toxicity –a process known as complement-dependent cytotoxicity (CDC). These processes, together with intracellular signaling cascades triggered by receptor interaction with the antibody, mediate the therapeutic effects observed in clinical trials employing antibodies such as Herceptin™ and Rituxan™, which are not directly conjugated with separate therapeutic moieties (55).

5. Modified (plasma) proteins

Plasma proteins such as albumin (56) are attractive carriers because of their small molecular weight and easy conjugation with homing peptides (57), sugars (58) and drugs of interest (39, 59). However, extensive modification with targeting or therapeutic motifs can lead to changes in protein charge and hydrophobicity, resulting in a rapid accumulation in the liver (60). Therefore, only minor modifications can be allowed for tumor-targeted therapeutics, where uptake in the liver should be avoided.

6. Cellular carriers

The presence of tumor-infiltrating lymphocytes in tumors prompted the idea of recovering such autologous cells from the patient, followed by *ex vivo* modification and subsequent reintroduction to the host (61). Such autologous cells would have the advantage of not being recognized by the immune system, and would thus be able to localize to the tumors more efficiently. Even if nonautologous cells are used, most likely they could circulate long enough to have a therapeutic effect (61). Most strategies explored to date employing cell carriers have utilized cells of the immune system, such as T-cells or macrophages. For example, a T-cell line expressing a diphtheria toxin-IL-4 fusion protein significantly inhibited tumor growth in a mouse model of leukemia, due to the innate recognition of tumor cells by this cell line (62). Another elegant example is the engineering of T-cells recognizing CEA, which after signaling release a cytotoxic retroviral vector (63). There are numerous examples of cells other than T-cells that home to tumors, such as tumor cells (64) or osteoclasts (65), and more recent research involving stem cells has started to expand (66). Further work is needed to clarify the therapeutic potential of such cell carrier-based systems in clinical settings.

Therapeutic moieties

Although some targeting or carrier moieties possess intrinsic therapeutic effects –antibodies are a prime example—, in most cases the inclusion of a separate therapeutic moiety is required in the design of targeting constructs. Examples of these are toxins, cytokines, drugs, radionuclides or therapeutic genes, which will be described in this section.

1. Toxins

The toxins most widely used for conjugation to antibodies are diphtheria toxin (DT) and *Pseudomonas* exotoxin (PE), both of which are derived from bacteria. Several DT fusion proteins have been constructed, such as with IL-2, a fusion protein that is currently approved by the FDA for the treatment of lymphoid cancers (denileukin diftitox, Ontak®, Onzar®) (67). Other constructs include fusions with a single-chain antibody against CD19 and CD22 (68) or granulocyte-macrophage colony-stimulating factor (GM-CSF) (69). In comparison with DT, PE-derived proteins have been more extensively explored in the clinical setting (70). As an example, a fusion protein of PE with a single-chain antibody against c-ErbB-2 was administered intratumorally to patients with cutaneous tumor lesions originating from melanoma or metastasis of breast

or colon cancer, leading to several partial responses and even some complete regressions (71). A fusion protein of PE with an anti-CD25 single-chain antibody has also been clinically investigated, with a reported 23% response rate in hematological malignancies (72). For further information, the reader is referred to a recent review by Johannes *et al.* (70).

2. Cytokines

A variety of cytokines have been explored for potent immunostimulating effects in the context of neoplastic disease, including IL-2 (73), GM-CSF (74) and IL-12 (75). In order to concentrate these potent immunomodulators into tumor tissue and avoid systemic side effects, multiple antibody-cytokine fusion proteins have been developed. A few examples will be described here. One of these is a genetic fusion between an anti-c-ErbB-2 antibody and IL-2, which was able to significantly decrease tumor progression in an immunocompetent murine tumor model (76). In an analogous approach, GM-CSF fused to the same antibody produced similar therapeutic effects (77). Unfortunately, although tumor growth was delayed, neither of these constructs induced complete tumor remission. However, in addition to their use as direct therapeutic agents, their recent application as enhancers for vaccines against tumor antigens holds significant promise in the treatment of cancer (78). As a final example, the targeting of IL-12 to tumor vasculature using an antibody specific for the oncofetal ED-B domain of fibronectin resulted in significant antitumor effects in three different murine models (79). Overall, the described antibodies fused with cytokines demonstrate the utility of these immunostimulating molecules as therapeutic moieties in targeted therapeutics for the treatment of cancer.

3. Drugs

Classical cytotoxic drugs used in chemotherapeutic approaches for cancer have been conjugated to antibodies or incorporated into liposomes to reduce their side effects when systemically administered to patients. Classes of antineoplastic drugs that have been conjugated include antimetabolites such as methotrexate (44), alkylating agents such as cisplatin (80), anthracyclines such as doxorubicin (81), antimitotic agents such as vincristine (82), as well as other antitumor agents. There are several considerations with respect to the choice of drug and the type of coupling chemistry employed for construct preparation. For instance, most drug-targeting constructs will enter the target cell via a lysosomal pathway, where the drug is released from its carrier. The drug has to subsequently traverse the lysosomal membrane to reach its pharmacological target, and drug hydrophobicity and charge are key determinants in this process (53). With respect to conjugation procedures, most strategies employ conjugation of drugs to proteins via the presence of a carboxylic acid or amino group in their chemical structure. When no such groups are present in the structure of candidate drugs for targeted delivery, incorporation into targeted constructs may be problematic. Fortunately, the advent of combinatorial chemistry libraries of pharmacologically active compounds enables researchers to tailor the chemical structure of a drug with a desired therapeutic effect to their specific needs.

4. Radionuclides

Radionuclides excert their therapeutic effect by emitting radiation and subsequent ionizing effects. Radiation is effective over a distance of several cell diameters, thus resulting in a beneficial bystander effect with respect to antigen-negative tumor cells in the vicinity of the targeted cell. An added advantage is the possibility to measure the delivered dose to a tumor by noninvasive imaging techniques (83). Table I lists the isotopes used for radioimmunotherapy in cancer (1). β-Emitters have a low energy and a longer path length, and are therefore suitable for the treatment of solid tumors. α-Emitters on the other hand have a high energy and a shorter path length, and are therefore used to target hematological malignancies (84). An example of radioimmunoconjugates used in the clinical setting is yttrium-90-labeled ibritumomab tiuxetan (Zevalin®), an anti-CD20 antibody used for the treatment of lymphoma (85).

5. Therapeutic genes

A wide variety of therapeutic gene approaches have been utilized in the context of cancer, such as immunomodulatory genes, mutant gene correction or enzymes for prodrug activation. Examples of immunomodulatory genes are HLA-B7, which has been tested in melanoma (86), or genes encoding cytokines such as IL-2 (87). Many cancers harbor mutations of the tumor suppressor gene p53, and it is therefore not surprising that strategies to express wild-type p53 have been tried to correct this problem. For instance, a replication-incompetent adenoviral vector expressing wild-type p53 has been tested in numerous clinical trials, including in

Table I: Isotopes commonly used for radioimmunotherapy in cancer (based on Ref. 1).

	Radioisotope
β-Emitters	Iodine-131 (94)
	Yttrium-90 (95)
	Rhenium-188 (96)
	Rhenium-186 (97)
	Copper-67 (98)
α-Emitters	Bismuth-212 (99)
	Astatine-211 (100)

combination with conventional chemotherapy in head and neck cancer (88). Enzyme/prodrug systems, also known as 'suicide gene therapy', are based on the expression of enzymes able to convert prodrugs to their toxic metabolites, leading to tumor cell death. Compared to systemic chemotherapy, the localization of toxic effects to cells expressing the enzyme is a significant advantage. A well-known example is the herpes simplex virus thymidine kinase (HSV-TK) enzyme which phosphorylates the prodrug ganciclovir to its toxic metabolite ganciclovir triphosphate. This system has been tested in various cancers, including prostate (89), brain (90) and colon cancer (91). Other suicide gene systems include cytosine deaminase/5-fluorocytosine and nitroreductase/CB-1954. Further studies utilizing these systems are exploring combinations of multiple systems such as a thymidine kinase and cytosine deaminase fusion gene (92), or combination of suicide gene therapy with other therapeutic modalities such as cytokines (93).

Conclusions

Targeted therapies to TAAs offer tremendous potential for the treatment of cancer without the occurrence of side effects, which are so often a dose-limiting factor for conventional therapies. The development of techniques such as mRNA microarrays and proteomic approaches will greatly facilitate screening for new TAAs, which will broaden the spectrum of available targets and increase the types of tumors that can be treated with targeted therapies. These techniques will also facilitate the screening of tumors for the expression of a wide variety of TAAs, providing the ability to tailor targeted therapy to the individual patient.

Recent years have witnessed the clinical application of several monoclonal antibodies with intrinsic therapeutic effects, such as Herceptin™, Erbitux™ and Rituxan™. However, as described in this review, their application as targeting moieties in complex carrier systems offers additional potential for therapeutic application in neoplastic disease, although most of these constructs are still at the preclinical stage of development. Many other targeting ligands have been identified, and it is anticipated that screening methods such as phage display will identify many more in the coming years.

Although coupling methods to attach targeting ligands to a carrier used to involve relatively unspecific chemical approaches, the advent of molecular engineering has made feasible the genetic fusion of different components of the targeting system. This latter coupling method has greatly reduced the variability in the resultant constructs –a clear advantage for clinical development. A wide range of carriers and therapeutic agents have been utilized in targeted constructs, and the combination of both will result in an even greater number of possible therapeutic agents. This will further advance the individualization of future therapies, making the realization of tailor-made medicine feasible in the future.

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