Assessments the Binding Affinity of the Corresponding Ligands of Tumor Specific Antigen of Prostate Cancer, Opportunity for Immunotoxin Development

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ABSTRACT

Prostate cancer is the third major cause of death in men worldwide after lung cancer, cardiovascular disease and is known in two decades. Since 1987, the first treatment for prostate cancer was initiated in the wake of the global health. So far safer and effective methods to treat this life threatening condition is better. During the past two decades' scientists to develop drugs targeted therapies in cancer research priorities were. These therapies primarily based on a specific antigen in tumor tissues will be deployed. Prostate cancer-specific antigens of the most valuable PSMA (Prostate specific membrane antigen) is. In this study, to evaluate the PSMA binding affinity ligands can be connected to evaluate and recommend the best ligand for use in targeted drugs.

Keywords: Prostate cancer, PSMA, Targeted drugs

Cancer is characterized by uncontrolled and invasive growth of cells. These cells may spread to other parts of the body, and this is called metastasis (1). Tumorigenesis is a multistep process that requires several integrated events to allow a cell to grow rapidly without the input of extraneous growth-stimulating signals, and to overcome growth-inhibitory signals and host immune responses. Tumour cells must also be able to circumvent APOPTOSIS, replicate indefinitely and sustain growth and survival by maintaining a sustainable oxygen and nutrient supply. Mutations

References

that result in constitutive activation of oncogenes or functional inactivation of Tumour-Suppressor Genes are important tumorigenic events. Although these mutations can affect several cellular pathways in the absence of de novo protein synthesis, aberrant gene expression — which is often downstream of these mutations — also has an important role in tumour initiation and progression. In addition, mutations within gene promoter or enhancer regions, or epigenetic changes, can induce abnormal expression of genes that regulate cellular differentiation, the cell cycle and apoptosis, thereby enhancing the potential for cellular transformation. Moreover, aberrant transcription of the genes that are needed to initiate the host antitumour immune response and induce neovascularization can result in tumour immune escape and angiogenesis — events that are essential for cancer progression (2). The hallmarks of cancer are properties such as constitutive proliferative signaling, de-regulation of cellular energetics, resistance to cell death, evasion of growth suppressors, avoidance of immune destruction, enabling of replicative immortality, tumor-promoting inflammation, activation of invasion and metastasis, genomic instability and mutation, as well as induction of tumor angiogenesis (3). Prostate specific membrane antigen (PSMA), is a unique membrane bound glycoprotein, which is overexpressed manifold on prostate cancer as well as neovascularization of most of the solid tumors, but not in the vasculature of the normal tissues. This unique expression of PSMA makes it an important marker as well as a large extracellular target of imaging agents. PSMA can serve as target for delivery of therapeutic agents such as cytotoxins or radionuclides. PSMA has two unique enzymatic functions, folate hydrolase and NAALADase and found to be recycled like other membrane bound receptors through clathrin coated pits. These capabilities to target PSMA for prostate cancer-specific therapy has become. PSMA is a type II membrane glycoprotein, Mr 100,000 dalton with an intracellular segment (amino acids 1–18), a transmembrane domain (amino acids 19–43), and an extensive extracellular domain (amino acids 44–750). Human PSMA gene was first cloned in Dr. Heston’s laboratory from LNCaP cells and was found to be located in chromosome 11p11-12, which encodes for PSMA transcript expression in prostate. Another gene, highly homologous to PSMA was found to be located at the loci 11q14.3 is called PSM-like. The PSM like gene is expressed in different tissues, such as kidney and liver, but not in prostate. These capabilities to target PSMA for prostate cancer-specific therapy has become. This should be connected to different ligands to the receptor cells. These ligands can act as carriers of drugs to cancer cells and also help to deliver the drug into the target cell (4-6).

References

Material and method:

Sequence extraction: To study the receptor ligand interactions and gene-ligand protein sequence will be needed. Refer to the database to obtain this sequence with NCBI Gene database and search can be achieved PSMA protein structural sequence. The conventional structure of a protein called PSMA FOLH1 in these databases is retrieved.

Ligand assays:

To determine the PSMA protein ligands can be connected to refer to the database String. The database can also be connected to a particular receptor called ligands, to study the biochemical properties and their performance in vivo as well.

3D refinement:

Many three-dimensional structure of molecules extracted from the site String Swiss model be derived from the database. In fact, the same structures polypeptide sequence and correspond to 90% will be suitable for us as structures. Due to the PDB code, the structure of the ligands in the base string to three-dimensional structure of the format PDB achieved, but many ligands three-dimensional structure not have a clear and we obtain three-dimensional structure they need to use the software Modeler we have.

Docking:

PSMA binding affinity between ligands and receptors to study the exact process used Dokhing. In this method, the receptor binding epitopes between the ligand and receptor binding specified then the various states will be investigated. Finally, it is possible for researchers to be the best and truest obtain a connection between the ligand and antigen. Also according to the energy level and stability of the connection can be correct comparison between different ligands to determine the best bridging ligand did. There Doking different software for online and offline software Molegro were used in this study.

Results:

The molecular pathway of antigens

In addition to its role as a tumor marker, PSMA contains a binuclear zinc site and is active as a glutamate carboxypeptidase, catalyzing the hydrolytic cleavage of α- or γ-linked glutamates from peptides or small molecules. Its substrates include poly-γ-glutamated folates, which are essential nutrients, and the poly-γ-glutamated form of the anticancer drug methotrexate, in which case cleavage renders it less efficacious. The enzymatic activity of PSMA can be exploited for the design of prodrugs, in which an inactive glutamated form of the drug is selectively cleaved and thereby activated only at cells that express PSMA.

PSMA also cleaves and inactivates the abundant neuropeptide N-acetyl-L-aspartyl-L-glutamate (α-
-NAAG), which is an inhibitor of the NMDA ionotropic receptor and an agonist of the type II metabotropic glutamate receptor subtype 3. A breakdown of the regulation of glutamatergic neurotransmission by α-NAAG is implicated in schizophrenia, seizure disorders, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis. Thus, inhibition of PSMA potentially confers neuroprotection both by reducing glutamate and increasing α-NAAG.

Indeed, the subnanomolar inhibitor 2-(phosphonomethyl) pentanedioc acid has been shown to provide neuroprotection in cell culture and/or animal models of ischemia, diabetic neuropathy, drug abuse, chronic pain, and amyotrophic lateral sclerosis.

### 3D characterization of Antigen

#### Overall Structure of PSMA.

The structure reveals a symmetric dimer with each polypeptide chain containing three domains analogous to the three TfR1 domains: a protease domain (residues 56-116 and 352-591), an apical domain (residues 117-351), and a helical domain (residues 592-750) (Fig. 1 and Table 1). A large cavity (∼1,100 Å²) at the interface between the three domains includes a binuclear zinc site and predominantly polar residues (66% of 70 residues) (supporting information). The observation of two zinc ions and conservation of many of the cavity-forming residues among PSMA orthologs and homologs identify the cavity as the substrate-binding site.

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**Fig. 1.** Structural comparison of PSMA and related proteins. The common fold of the protease or protease-like domain consists of a central seven- to eight-stranded mixed β-sheet and 6-11 flanking α-helices. Ribbon diagrams of side and top view of PSMA (A), side and top view of TfR1 (PDB ID code 1CX8) (B), AAP (PDB ID code 1RTQ) (C), CPG2 (PDB ID code 1CG2) (D). The protease (or protease-like) domains are shown in blue, the apical domains of PSMA and TfR1 in green, the helical domains in red, and the CPG2 dimerization domain in pink. Water molecules are red, zinc ions are orange, zinc-binding residues are yellow sticks, and carbohydrate residues are shown in a color-coded ball-and-stick representation. Representative substrates are shown below the appropriate molecule. R1 = OH or NH2, and R2 = H or CH3 for folate and methotrexate, respectively. Note: A mono-γ-glutamated folate is shown for clarity; in poly-γ-glutamated folate/methotrexate, the additional glutamates (up to seven) are attached to the glutamate shown via a γ-peptide linkage.
Corresponding ligands of the antigen

Using the Software string of 10 ligands can be connected to the PSMA antigen obvious that this between us three ligands AIMP1(aminoacyl tRNA synthetase complex-interacting multifunctional protein 1), ASPA (aspartoacylase) and DPP4 (dipeptidyl-peptidase 4) because of higher binding affinity and specific 3D structure chose. In addition, both ends of the variable heavy and light chains of monoclonal antibody J591 as the antibody to PSMA antigen were explored.

Assessment the binding affinity of ligands to the antigen

By studying the connections made between ligands with binding epitopes involved in binding epitopes of PSMA was assessed on the basis of J591vL epitope with 197 amino acids, J591vH the epitope with 161 amino acids, DPP4 epitope with 44 amino acids, ASPA the epitope with 88 amino acids and AIMP1 epitope with 62 amino acids present on PSMA for connection altogether.

The stable connection Compared with the level of minimum energy center and energy per connection was assessed on the basis of stable, J591vL by -398 binding energy, J591vH by -399 binding energy, DPP4 by -893 binding energy, ASPA by -788 binding energy and AIMP1 by -706 binding energy level was determined.

Fig. 2. A surface rendering in which the helical domain is light red, the apical domain is light green, the protease domain is light blue, and zinc ions are orange. The residues lining the substrate-binding cavity are highlighted in a darker version of the color corresponding to the domain from which the residue originates.
Fig. 3. 3D structure of PSMA ligands. A (AIMP1), B (ASPA), C (DPP4), D (J591vH), E (J591vL)

Fig. 4. 3D picture of docking with PSMA and ligands. A (AIMP1), B (ASPA), C (DPP4), D (J591vH), E (J591vL)
Discussion:

Cancer is a leading cause of death in the world (about 7.6 million people died of cancer in 2008) (Globocan 2008 (IACR) - globocan.iarc.fr; section of Cancer Information). Based on World Health Organization projections, in 2030, the number of people expected to die of cancer will be around 11.4 million (7, 8).

Despite the enormous amount of resources devoted to the area of drug development, cancer treatment remains one of the biggest challenges in public health. Many drugs have been developed to treat cancer. Most of them come from high-throughput chemical library screening and are designed to target specific oncogene products involved in cancer progression. However, after exposing tumor cells to those anticancer drugs, both toxicity and the development of resistance are major reasons for failure in cancer therapy. Therefore, new paradigms for cancer drug development are urgently required. It is interesting and somewhat surprising that bacteria may hold the key for finding new therapeutic approaches against cancer (9). Cancer therapy is often challenged with secondary effects caused by standard therapies and frequently faces tumor cell resistance and the inability to eliminate micrometastases. Therefore, new therapies are urgently needed. Recently, there has been renewed interest in the development of new therapeutic anticancer modalities based on the use of live bacteria and their purified products. Specific targeting of cancer cells would then allow the use of more cytotoxic products without undesired toxicity to normal tissues. Not only live attenuated bacterial strains have been proposed as anticancer agents but also products derived from them such as enzymes, secondary metabolites, proteins, or derived peptides and toxins. Bacterial toxins are among the most cytotoxic products in nature. New strategies, by genetic and protein engineering, offer the possibility to fuse these toxins with monoclonal antibodies termed immunotoxins, creating new powerful chimeric proteins that specifically target cancer cells (1, 9).

Conventional anticancer therapeutics often suffer from lack of specificity, resulting in toxicities to normal healthy tissues and poor therapeutic index. Antibody-mediated delivery of anticancer drugs or toxins to tumor cells through tumor selective or overexpressed antigens is progressively being recognized as an effective strategy for increasing the therapeutic index of anticancer drugs. Chemotherapy remains an important and widely used treatment option for many types of cancers, but the toxic side effects often limit optimal dosing of anticancer drugs, thus leading to disease relapse, development of drug resistance and poor quality of life of cancer patients. Since tumor cells

References

share many common features with the normal cells, designing a drug that would selectively destroy cancer cells, bypassing normal healthy cells represents a major challenge of oncology drug development. One possible strategy to overcome the collateral toxicity of conventional anticancer agents is to harness the power of “precision-guided” monoclonal antibodies (mAbs) that can deliver the drugs to the selectively-expressed or overexpressed antigens present on cell surface of malignant cells. Development of a number of actively pursued antibody (Ab)-based technologies, including immunotoxins (ITs). Tumor-targeting ITs are chimeric molecules composed of a protein toxin moiety that is either chemically conjugated or genetically fused to mAbs or Ab fragments (10). Selective delivery of active agents, such as chemotherapeutics that are ubiquitously toxic to cancer cells and normal tissues, to the organs in question. It is ideal that those toxic chemicals are only delivered to tumor and/or tumor-related tissues to kill tumor cells with minimal side effects. Actively targeted drug delivery enables targeted and intracellular delivery of therapeutics to specified cells by active ligands. In addition, naturally occurring biological barriers always preclude the access of drug to target destinations. Targeted drug delivery may expedite traversal over such barriers. For example, gene drugs for intracellular targets or most of the drugs for central nervous system (CNS) diseases may need particularly targeting strategies to penetrate the lipid membrane of target cells and the blood–brain barrier (BBB), respectively. Molecularly targeted therapeutics block tumor growth by antagonizing specific targets (extracellular or intracellular) needed for carcinogenesis. Targeted therapy is expected to be more effective than conventional chemotherapy (11). The idea of targeted therapy, whereby drug or protein molecules are delivered to specific cells, is a compelling approach to treating disease. Immunotoxins are one such targeted therapeutic, consisting of an antibody domain for binding target cells and molecules of a toxin that inhibits the proliferation of the targeted cell. Immunotoxins are antibodies that are either chemically or genetically coupled to eukaryotic toxins. These chimeric proteins are used to deliver the toxin to a specific cancer cell to initiate apoptosis (12). Although conventional anticancer therapies, consisting of surgical resection, radiotherapy and chemotherapy, are effective in the management of many patients but for about half of cancer sufferers these are ineffective, so alternative techniques are being developed to target their tumours. Experimental cancer treatments are medical therapies intended or claimed to treat cancer by improving, supplementing or replacing conventional methods. These include photodynamic therapy, HAMLET (human

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alphalactalbumin made lethal to tumor cells), gene therapy, telomerase therapy, hyperthermia therapy, dichloroacetate (DCA), non-invasive RF cancer treatment, complementary and alternative therapy, diet therapy, insulin potentiating therapy and bacterial treatment. But many of these therapies are controversial due to lack of evidence, efficacy, feasibility, availability, specificity and selectivity (1). Immunotherapy for cancer offers great promise as an emerging and effective approach. Since tumors are immunogenic, the immunotherapeutic strategy employs stimulation of the immune system to destroy cancerous cells. But the major hurdle is the ability of tumors to escape the immune system due to development of tolerance as they are weakly immunogenic and sometimes body takes them as self antigens. Thus one of the novel immunotherapeutic strategies employs bacteria to enhance the antigenicity of tumor cells. Protein toxins such as Pseudomonas exotoxin, diphtheria toxin, and ricin may be useful in cancer therapy because they are among the most potent cell-killing agents. Although they are very lethal yet for therapeutic efficacy these toxins need to be targeted to specific sites on the surface of cancer cells. This process is accomplished by eliminating binding to toxin receptors by conjugating the toxins to cell-binding proteins such as monoclonal antibodies or growth factors. These conjugates bind and kill cancer cells selectively thus sparing normal cells, which don't bind the conjugates. A wide variety of DT ligands such as IL-3, IL-4, granulocyte colony stimulating factor (G-CSF), transferrin (Tf), EGF and vascular endothelial growth factor (VEGF) have been studied for targeted tumors. The transferrin-DT conjugate (Tf-CRM 107) and DT-EGF have reached the stage of clinical trials in patients of brain tumor and metastatic carcinomas respectively. Similarly a large variety of antibodies and ligands to surface antigens overexpressed in different tumors have been conjugated to PE. Important ones tested in clinical trials are IL-4, IL-13, monoclonal antibody - recognizing a carbohydrate antigen Lewis Y, reacting with metastatic adenocarcinoma cells (Mab B3) and transforming growth factor (TGF-α) (1). Management of unresectable or metastatic tumors is based on the cytotoxic effect of radiotherapy and/or chemotherapy. Numerous toxin-based therapies have been developed in this field for their potent cell-killing activity. However, because of the nonspecific effects of toxins on normal and tumor cells, targeting of cancer cells generally remains a prerequisite for such therapeutic applications. Targeting of toxins to cancer cells has used a variety of modalities, namely a direct interaction between the toxin and its natural receptor expressed on tumor cells; vectorization of the toxin by a natural ligand or a monoclonal antibody that specifically recognizes cancer cells that express their natural receptors; and gene therapy allowing cellular penetration of a viral vector expressing the toxin. In addition to these direct cancer cell cytotoxic approaches, a few publications have reported anticancer immune activation via T-lymphocytes, natural killer cells, or dendritic cells with tumor-directed targeting by toxin compounds.
Conclusion

The most developed class of targeted cytotoxic treatments is constituted by immunotoxins. The immunotoxin approach is based on the use of tumor targeting ligands or antibodies that are linked to the catalytic moieties of bacterial or plant protein toxins.