Conventional cancer treatments include surgery, chemotherapy, and radiotherapy. Chemotherapy, also known as drug therapy, plays an important role in cancer therapy. However, its efficacy is affected by dose-dependent toxicity and side effects. At present, the efficacy of drug therapy has hit a plateau. Targeted therapy refers to the cancer-related molecules as targets, drugs, antibodies, and other active agents that efficiently locate cancer cells and achieve the goals of cancer treatment. Targeted therapy has the advantages of orientation and location, which can reduce drug dosage, improve treatment effect, and decrease toxicity, becoming a research focus throughout the world. In targeted therapy, most important is defining the target molecules, which can supply the theoretical and practical basis for targeted therapy. Depending on the location between the target molecules and the cancer cells, there are two types of target molecules, cancer-cell-specific targets and cancer-related targets. The former refers to the target molecules located in cancer cells; the latter refers to target molecules do not exist in cancer cells but closely relate to the state of the cancer cells.

Target molecules in cancer cells

Depending on the anatomical location of the target molecule, target molecules are divided to membrane target molecules, cytoplasm target molecules and nucleus target molecules of cancer cells respectively.

Membrane-targeted molecules of cancer cells

The cell membrane is the door for transforming material and energy between the cell and its environment, but is also the first place that drugs act on the cells. Thus, the outer surface of the membrane is an ideal location for selecting target molecules. **Membrane-receptor target molecules** The epidermal growth factor receptor (EGFR) family contains ErbB1 (EGFR), ErbB2 (HER2), ErbB3 (HER3), and ErbB4 (HER4), and is composed of extracellular, transmembrane, and intracellular parts. Extracellular parts bind to ligands, while ATP and tyrosine kinase binding sites exist in intracellular parts. After being activated by binding its specific ligand, EGFR activates many downstream pathways and takes part in tumor cell proliferation, adhesion, invasion, metastasis, apoptosis, and tumor angiogenesis. EGFR is overexpressed in many tumor cells, such as head and neck, ovarian, cervical, bladder, and esophageal cancers \(^1\). At present, intensive study of targeted drugs for the extracellular domains of EGFR have been made, and many have been market-oriented, such as Herceptin (Trastuzumab), Erbitux, and Nimotuzomb. Herceptin is a humanized monoclonal antibody that selectively acts on extracellular sites of HER2. HER2 is overexpressed in many types of tumor tissue, including breast, ovarian, colon, lung, gastric, prostate, and cervical cancers \(^2\). Herceptin was authorized by the US Food and Drug Administration (FDA) for treating patients with metastatic breast cancer in 1998, with a significantly higher treatment effect than other anti-breast cancer drugs. Now, Herceptin is the drug of choice for anti-breast cancer in patients with high HER2 expression. In 2007, the FDA approved Cetuximab for the treatment of patients with colon cancer, and later, it was approved for head and neck squamous cell carcinoma. Nimotuzomb, a recombinant humanized anti-EGFR...
monoclonal antibody that was approved by the State Food and Drug Administration (SFDA) of China in April 2006, is the first humanized monoclonal antibody drug. Nimotuzumab combined with radiotherapy is used in EGFR positive III/IV stage nasopharyngeal carcinoma treatment.

**Cell adhesion target molecules** CD20 is an important differentiation antigen in B-cells, expressing in more than 95% normal and deteriorated B-cell surface and has been considered a specific marker of the B-cell surface. Internalization following CD20 and anti-CD20 antibody binding is not obvious and CD20 does not shed from the cell surface obviously either, which makes CD20 an ideal target for B-cell lymphoma. The FDA authorized Mabthera, Zevalin, Bexxar and 131I-Tositumomab in 1997, 2002, and 2003, respectively. They have shown good therapeutic effect on 50% of patients with non-Hodgkin’s lymphoma.

CD52 is a common antigen, which distributes in lymphocytes, monocytes, eosinophils of the hematopoietic system, and monocytic differentiated-dendritic cells. CD52 is also expressed in many malignant tumors and certain lymphoid cells at different levels. The anti-CD52 monoclonal antibody Alemtuzumab is a humanized antibody, which was authorized by the FDA in 2001 for the treatment of patients with refractory recurrent B-cell chronic lymphocytic leukemia with remission rate of 19%.

As the membrane-targeted molecules locate on the tumor cell surface, with the advantages of being accessible and easy to identify, targeted molecules on the tumor cell surface become the first choice for targeted therapy. At present, targets on the cell surface, such as CD147, CD82, and CD137, are in the experimental phase. However, due to the complexity of tumorigenesis and how easily they mutate, specific targets on the surface of cancer cells are difficult to determine. Many methods for targets on the cancer cell surface have proven inefficient. For example, using antibodies and special inhibitors to detect the function of tumorigenesis and to confirm the possibility of target molecules, genomic and proteomic research has examined that some normal and mutated membrane proteins are overexpressed partially on the cancer cell surface. In recent years, surface display techniques have emerged, which supply novel thinking for the determination of molecular targets on the cancer cell surface. Surface display techniques are new genetic engineering technologies, which display exogenous peptides (or protein domain) in the form of fusion proteins on the microbial surface. The displayed peptides or proteins are maintained relatively independently of spatial structure and biologic activity. Unknowing target molecules, functional target molecules are selected from a library of random proteins or peptides to get a specific binding ligand. This technology shows great prospects for research for specific targeted therapies.

At present, phage display, bacterial surface display, yeast display, and in vitro ribosome display are the commonly used techniques. Surface display technology has made great process in cancer targeted therapy. However, the relatively mature phage display or the newly developed ribosome display has some defects. For example, phage display cannot achieve high throughput, bacterial display cannot control levels of protein expression well, and ribosome display has problems with mRNA stability. Solving these problems is the key to determine whether surface display can be widely used in targeted treatment for cancer, but as a new means for targeted research, surface display is worth the attention.

**Cytoplasm-targeted molecules of cancer cells**

Since material synthesis, signal transduction and many other processes are completed in the cytoplasm of cells, and cytoplasm-targeted molecules are mostly studied.

**Cytoskeletal proteins** Cytoskeletal proteins play essential roles in cancer cell proliferation, expansion, and metastasis. Thus, inhibiting the function of cytoskeletal proteins has become an important direction in research.

Microtubules are important for maintaining cell shape, cell division and proliferation, organelle composition, and transport and signal transduction. Drugs targeted to microtubules take advantage of their dynamic characteristics that increase depolymerization or decrease polymerization, thus directly affect cell mitosis and many physiologic functions of cells, and cells are arrested at M phase. Available in France since 1989, Navelbine inhibits microtubule depolymerization. In addition, Taxol was approved as a new drug for advanced ovarian cancer therapy by the FDA in 1992, and its semi-synthetic derivative product Docetaxel was marketed in 1995. Taxol can induce and enhance microtubule polymerization and assembly, prevent microtubule depolymerization, and stabilize microtubules, thus inhibiting tumor cell growth. Taxol has a good therapeutic effect not only for patients with ovarian, uterine, and breast cancers, but has good effects in a variety of other cancers.

Vimentin is a type of intermediate filament protein, which is involved in cytoskeleton formation and contact with the cell membrane, and mainly distributes in mesenchymal tissue and cells. It is served as a specific marker for tumors of mesenchymal origin. However, a large number of reports in recent years showed that vimentin was expressed in many types of epithelial tumor cells, and was related to the malignancy of the tumor cells. Vimentin as a target molecule is now being research, and it has been proved that vimentin promoted tumor metastasis in patients with prostate cancer through the Src kinase pathway.

**Functional proteins** Types of kinases in cytoplasm, like tyrosine kinase, serine/threonine kinase, signal transducer and phosphatase, are involved in variety of signal transduction pathways and play important physiologic roles. These proteins are abnormally expressed in tumor cells, and have been selected as target molecules. Except for a few molecules, most of them are under research (Table 1).

Most functional molecular targets belong to signal transduction pathways. During the process of tumor initiation and development, abnormal events in signal transduction often occur; thus, signal transduction has become an important research area for targeted therapy. Many proteins in signaling pathways are possible target molecules. However, at present, the mechanisms of signal transduction are not completely understood, nor are the networks between the signaling pathways, or cross-cutting or compensation signals between various pathways. Drugs that target single pathways have not shown effective treatment for...
patients with cancer. Many targeted drugs are still being tested on animals, and very few drugs are marketed. Recent years, researchers have focused on a single drug for multi-targets, which means one drug can inhibit several signal transduction pathways simultaneously. But these drugs may have more toxic side effects, so further researches are needed. To enhance efficient treatment for targeted signal transduction pathways, the key point is to understand the mechanism of the signal transduction pathway, which needs further basic research.

**Heat shock proteins (HSP)** Heat shock proteins are a group of conservative proteins whose synthesis increases significantly under physiologic stress or pathologic state. HSPs are composed with many proteins, forming a complex in vivo, and participate in protein folding and stretching, poly-complex assembly, and kinase/transcriptional factor formation. Synthesis of HSP is needed for regulating and stabilizing the amount of proteins caused by abnormal tumor proliferation. Thus, HSP is constantly overexpressed in many tumor cells. 17-(Allylamino)-17-demethoxygeldanamycin (17-AAG), an inhibitor of HSP90, which has the combination capacity with HSP90 secreted by tumor cells is 100-fold higher than HSP90 secreted by normal cells, could kill tumor cells selectively. 17-AAG is currently in clinical phase II, but the latest research shows that it is easy to produce drug resistance. More clinical data are required for to determine drug efficacy.

**Nucleus-targeted molecules of cancer cells**

**DNA topoisomerase** In cancer cells, both the amount and the activity of DNA topoisomerases are much higher than normal cells. Inhibiting the activity of DNA topoisomerase may abolish the rapid proliferation of cancer cells and further repress or kill cancer cells. Thus, DNA topoisomerase is considered as a target for anti-cancer drugs. DNA topoisomerases include Topo I and Topo II, the representative drugs targeted the two isomerase are showed in Table 2.

### Table 1 Molecular targets related to the functional proteins and drug development

<table>
<thead>
<tr>
<th>Protein</th>
<th>Target molecules</th>
<th>Mechanism of action</th>
<th>Drugs in investigation or on the market</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine protein</td>
<td>Bcr-Ab1</td>
<td>Inhibit Bcr-Ab1 expression</td>
<td>Imatinib 2001[17]</td>
</tr>
<tr>
<td>kinase</td>
<td>Intracellular region of EGFR</td>
<td>Ras/Raf/MAPK pathway</td>
<td>Gefitinib 2003[18]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VEGFR/PDGFR pathway</td>
<td>Sradfenib 2005[19]</td>
</tr>
<tr>
<td>Protein kinase</td>
<td>Extracellular signal-regulated kinase</td>
<td>Ras/Raf/MAPK pathway</td>
<td>PD98059[21], U0126[22]</td>
</tr>
<tr>
<td></td>
<td>Phosphoinositide 3-kinase</td>
<td>P3K-AKT-mTOR pathway</td>
<td>Wortmannin, LY294002[23]</td>
</tr>
<tr>
<td></td>
<td>Akt protein</td>
<td>P3K-AKT-mTOR pathway</td>
<td>Celecoxib[24]</td>
</tr>
<tr>
<td></td>
<td>Protein kinase C</td>
<td>Participate in many signal conduction pathways</td>
<td>Bryostatin-1, PKC412[25,26]</td>
</tr>
<tr>
<td>Signal conduction</td>
<td>Ras protein</td>
<td>Ras/Raf/MAPK pathway</td>
<td>Zanemstra (rejected by FDA)</td>
</tr>
<tr>
<td>molecules</td>
<td>mTOR protein</td>
<td>P3K-AKT-mTOR pathway</td>
<td>Temsirolimus 2007, everolimus 2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>deforolimus (phase III)</td>
</tr>
<tr>
<td>Protein phosphatase</td>
<td>PTEN protein</td>
<td>Abnormal expression in many cancer cells</td>
<td>NEDD4-1 protein[27]</td>
</tr>
</tbody>
</table>

**Histone deacetylase (HDAC) and p53** HDAC is involved in regulating histone acetylation and changing chromosomal structure, and therefore mediating gene expression. Low levels of histone acetylation were found in many hematologic and solid tumors. Inhibitors of HDAC showed antitumor activity in many tumor cells, making HDAC a target for cancer therapy. In 2006, the HDAC inhibitor Vorinostat was approved by the FDA for the treatment of patients with cutaneous T-cell lymphoma. At present, a variety of HDAC inhibitors are involved in clinical trials.

p53 is an important tumor suppressor gene in vivo. Studies show that abnormal expressions of p53 are present in more than 50% of tumor cells. In 2004, recombinant human p53 adenovirus injection (Gendicine) has approved by the SFDA for the treatment of patients with head and neck carcinomas, which

### Table 2 DNA topoisomerase Inhibitors and drug development

<table>
<thead>
<tr>
<th>Target molecules</th>
<th>Classification</th>
<th>Mechanism of action</th>
<th>Drugs in investigation or on the market</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topo I</td>
<td>alkaoids</td>
<td>Bind to the Topo I and DNA complex</td>
<td>Camptothecin, Irinotecan</td>
</tr>
<tr>
<td></td>
<td>plug-in type</td>
<td>Plug-in the combination of Topo II and DNA double strands</td>
<td>Anthracene nucleus: Adriacin, Dauorubicin</td>
</tr>
<tr>
<td></td>
<td>non-plug-in type</td>
<td>Effect the enzymatic function by acting on DNA single strand</td>
<td>Podophyllotoxin: Etoposide, Teniposide[28]</td>
</tr>
</tbody>
</table>

**Telomerase** The main biologic function of telomerase is to stabilize the length of chromosomal telomeric DNA and extend the shortened telomeres by its reverse-transcriptase activity for replicating and extending telomeres. Therefore, telomerase increases the capacity of cell proliferation in vitro. In 1991, Harley proposed a telomere-telomerase theory that telomerase played an important role in cell regeneration. In 1995, Hiyma et al. published a number of papers showing that telomerase activity was enhanced in many types of tumors, indicating that telomerase takes part in cell immortalization, and making telomerase a novel method of targeted therapy. GRN163L, a inhibitor of telomerase, has been studied in clinical trials and its efficacy is being observed.

**Histone acetyltransferase (HAT)** HAT is involved in regulating histone acetylation and changing chromosomal structure, and therefore mediating gene expression. Low levels of histone acetylation were found in many hematologic and solid tumors. Inhibitors of HAT showed antitumor activity in many tumor cells, making HAT a target for cancer therapy. In 2006, the HAT inhibitor Vorinostat was approved by the FDA for the treatment of patients with cutaneous T-cell lymphoma. At present, a variety of HAT inhibitors are involved in clinical trials.

**Drug resistance** The mechanisms of drug resistance are complex and diverse. The most common mechanism is overexpression of some drug resistance proteins. These proteins are usually overexpressed in tumor cells and confer drug resistance.

**Clinical trials** The clinical trials of many targeted drugs have been performed worldwide. The results of these trials are promising, and many targeted drugs have been approved by the FDA for clinical use.
was the first gene therapy drug marketed in the world. Clinical studies of patients with nasopharyngeal carcinoma showed that the 5-year survival rate was 66.7% compared with 59.2% in the control group.\(^\text{33}\)

## Cancer-related target molecules

Cancer-related target molecules are not located in tumor cells, but are extracellular molecules that affect the functions of tumor cell differentiation, proliferation, and metastasis, which suppress and treat cancer by regulating their function. Depending on the location between the target molecules and the tumor cells, there are two types of target molecules: target molecules of angiogenesis, which provide nutrition for the tumor cells, and extracellular matrix targeted molecules, which relate to tumor cell metastasis.

### Target molecules of angiogenesis

In the early 1970s, Folkman first proposed a theory that tumor cell growth depends on blood vessels, which if the tumor volume is larger than 2 mm\(^3\), oxygen and nutrients supplied by new blood vessels are needed. Not only tumor cell growth depended on angiogenesis, but tumor metastasis also needed nutrient support.\(^\text{34}\) Based on this theory, inhibiting angiogenesis became a new tumor therapy, blocking the blood supply for tumor cells to depress tumor cell proliferation, invasion, and metastasis. Currently, anti-angiogenesis therapy focuses on following aspects.

**Vascular endothelial growth factor (VEGF)** As the well-known agent for blood vessel penetration and the cause of endothelial cell mitosis, VEGF plays some important roles in endothelial cell proliferation, migration, and blood vessel construction. The VEGF signaling pathway is the key rate-limiting step for tumor angiogenesis, growth, and metastasis. The main strategies for targeting the VEGF pathway include the following. (1) Blocking the VEGF function by antibodies: Recombinant human VEGF monoclonal antibody Avastin, which is a representative drug and was approved by the FDA in Feb 2004, is the first marketed VEGF inhibitor in the world. Combining Avastin with fluorouracil is recommended as the first-line antibody drug for patients with advanced colorectal cancer.\(^\text{35}\). (2) VEGF receptor (VEGFR) tyrosine kinase activity is inhibited by small molecular drugs. Representative drugs are Vandetanib, Sunitinib, and so on, and clinical experiments have proved that as second-line drugs for patients with non-small cell lung cancer have better efficacy. Phase III trials are ongoing.\(^\text{36}\).

**Direct inhibition of endothelial cell growth** TNP-470 is the first drug for inhibiting angiogenesis that has entered clinical trials. It has a strong inhibitory role in endothelial cell proliferation and angiogenesis. In clinical trials, the combination with other antitumor drugs showed efficient antitumor activity in patients with cervical, gastric, breast, and lung cancers.\(^\text{37}\). Endogenous angiogenesis inhibitors angiotatin and endostatin have strong inhibitory effects on tumor angiogenesis, but at present, better results have only been achieved in animal experiments. In 2000, canstatin was founded as an endogenous angiogenesis inhibitor, and it can significantly suppress endothelial cell proliferation and migration and induce cell apoptosis. Magno et al.\(^\text{38}\) proved that combining electric transfers of canstatin with radiotherapy was superior to radiotherapy alone in groups of mice.

In 2004, the SFDA authorized our own recombinant human endostatin (Endostar), which is the first approved endostatin antitumor drug in the world. Clinical trials show that combining Endostar and chemotherapy have better effects than chemotherapy alone.\(^\text{39}\).

### Extracellular matrix targeted molecules

The extracellular matrix is a network structure that is composed of macromolecules, such as collagen and non-collagenous glycoprotein. Degradation and remodeling of the extracellular matrix is essential for tumor cell growth, extension, invasion, and metastasis. Matrix metalloproteinases (MMPs) is a type of zinc-dependent endopeptidase, mediating extracellular matrix degradation and tissue remodeling. Studies show that overexpression of MMP2 and MMP9 are closely related to tumor metastasis and angiogenesis, and have become a new direction for targeted therapy for cancer. AE-941 is a multifunctional drug, not only an inhibitor of both MMP2 and MMP9, but also an inhibitor of the binding of the endothelial growth factor and its receptor, increasing endothelial cell apoptosis. Phase III clinical trials have been run for patients with kidney cancer, but problems in the antitumor effectiveness and toxicity still exist, which need further study. Col3.α, a dual inhibitor, which can inhibit MMP2, MMP9, and the endothelial growth factor, is still in phase II clinical trials. Currently no approved Matrix metalloproteinase inhibitor drugs are available.

With continued development of modern biotechnology and cancer research, more and more tumor cell target molecules have been discovered and cancer therapy has made great progress. At present, targeted therapy can be divided into two types: drugs, antibodies, inhibitors, and other active agents that play direct antitumor roles and target molecules that are just guide agents, with no antitumor activity, that guide the effective ingredients to target tumor cells and play a indirect role in cancer therapy. The former type is the main method of targeted therapy used in clinic and the latter is being a research hot point, more and more researchers are paying attention to it.

Targeted therapy has received great results, but as a therapy, it is still problematic. First, targeted therapy has better effects in only a few types of cancers, such as B-cell lymphoma, breast cancer, and lung cancer, with no exciting results found for other cancers. Second, researchers have not obtained desired therapeutic effects for targeted therapy. Except for Gleevec, the clinical efficiency of the majority of the approved molecular-targeting drugs is just 20%–30%. Lastly, one of the goals of targeted therapy is either lower toxicity or no toxic side effects; yet, the experimental data show that many targeted drugs still have various toxicities. Because of these problems, most targeted drugs are only used as second- or third-line treatment or in combination therapy. Although these problems relate to the complexity of tumor initiation and development, the current status of targeted therapy also directly relates to the problems. (1) Most of the targets are the usual functional sites, deficient of tumor
specificity, and many targets exist in normal tissue as well. For example, signal transduction pathways are selected as targets for cancer therapy because of their abnormal expression in many tumor cells, but they also play regulatory roles in normal cells. Large dosage, toxicity, and other problems in targeted therapy are inevitable. (2) Biologic functions of selected targeted molecules are not understood totally. At present, many functions of targets are unknown, have not been discovered, and the side effects of drugs occur inevitably. If compensatory pathways or the same functional pathways exist, the effect of targeted therapy will be seriously affected. Due to these problems, we need more defined functional tumor-cell specific molecules as targets, changing the status of non-tumor specific target molecules, both increasing the efficiency of targeted therapy and decreasing dosage and toxicity.

To achieve these goals, new biotechnological methods and basic research support are needed. Moreover, we can change the strategy of targeted therapy, using agents to guide the targeted treatment and taking advantage of pharmaceutical agents linked to drugs to complete treatment. This will lead to a new method in targeted treatment. In this field, a group has induced melittin to tumor cells by a short peptide RGD and achieved a good effect.35 Finally, multitargeted therapy strategies can be applied. Different from the mechanism of single-drug multitargets, it means a combination of many functional targets for multitargeted treatment, to increase specificity and pertinence of drugs, and as much as possible to improve the efficiency of targeted therapy under the premise of the lowest toxicity.

Although there are some clinical problems, as a novel therapy, targeted therapy still supplies a new idea for cancer therapy. It is believed that with thorough cancer research and developed biotechnology, the initiation and development of cancer will be definite. More and more tumor-specific target molecules will be identified and targeted therapy will bring more changes in cancer treatment.

References


