Acta Pharmacol Sin 2007 Sep; 28 (9): 1494–1498

Blackwell Publishing

Invited review

From cell signaling to cancer therapy

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Key words

cell signaling; cancer therapy; chemotherapy

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Received 2007-06-14
Accepted 2007-07-25

doi: 10.1111/j.1745-7254.2007.00697.x

Abstract

Cancer has been seriously threatening the health and life of humans for a long period. Despite the intensive effort put into revealing the underlying mechanisms of cancer, the detailled machinery of carcinogenesis is still far from fully understood. Numerous studies have illustrated that cell signaling is extensively involved in tumor initiation, promotion and progression. Therefore, targeting the key molecules in the oncogenic signaling pathway might be one of the most promising ways to conquer cancer. Some targeted drugs, such as imatinib mesylate (Gleevec), herceptin, gefitinib (Iressa), sorafenib (Nexavar) and sunitinib (Sutent), which evolve from monotarget drug into multitarget ones, have been developed with encouraging effects.

Current status of cancer knowledge

Cancer has been one of the most common causes of human death for a long time. Epidemiological data demonstrated that there have been nearly 7 million deaths as a result of cancer per year in the past several decades, and it is estimated that there will be 16 million new cancer cases and 10 million cancer deaths per year by the year of 2020 [World Health Organization].

Despite the efforts of scientists throughout the world for several decades, the mechanism of tumor development has not yet been completely clarified. Scientists in different research fields have given distinct answers on carcinogenesis respectively. As to etiology, cancer develops with the accumulation of multi-gene mutations, which are the result of the interaction between genetic host factors and external agents. Carcinogenic agents can be categorized as physical ones such as ultraviolet (UV) and ionizing radiation; chemical ones such as asbestos and smoking; biological ones such as certain viral and bacterial infections; as well as mycotoxins containing food such as aflatoxins causing liver cancer[1–5]. Research of cancer stem cells, which has been regarded as the origin of cancer, has claimed that the current methods of chemotherapy are targeting the wrong objects[6]. Scientists on system biology contend that traditional biologists have intensively studied the individual components of a living organism, and more efforts should be put into the investigation on how these components interact with each other and form a complex system[7]. Deregulated cell growth is the defining feature of tumors compared with normal tissue. Molecular biologists believe that deregulated cell growth occurs as a result of perturbed signal transduction defined as all cellular signals that modulate cellular behavior or function.

Though great success has been achieved, the war against cancer is complex and hard, and we are still far from winning it. At present, more and more scientists are beginning to explore the mechanism of carcinogenesis by studying the oncogenic signal transduction.

Cells receive an external “signal input” from their environment that controls proliferation, differentiation, migration or death. Such signals come from specific soluble signaling molecules, from matrix molecules or through direct contacts with other cells, and are mediated to the receiving cell through specific receptors. Activation of such receptors initiates a number of intracellular signaling pathways. The signal processing elements within the cell make a response that will be a pattern of reactions manifest as a metabolic, morphologic or electric “signal output”. There has been a notable interest during the past 25 years in the elucidation of these pathways, since their perturbation is linked to the development of serious diseases, including cancer.

Essential signaling pathways involved in tumor development

Cells are organic microsystems with functional compart-

ments interconnected by complex signal pathways[8]. Signal transduction has been proven to not only occur through linear pathways between individual receptors and specific cellular responses, but rather, there is extensive cross-talk between different pathways, and the cell thus contains a network of interacting signaling components. Among those, numerous signaling pathways are accompanied by the activation of kinase cascade, which regulates the processes essential to tumor cell development including cell growth, differentiation, migration and apoptosis etc. Besides p53, phosphatase and tensin homolog deleted on chromosome ten (PTEN) and Rb proteins, which play critical roles in tumor development and have been extensively studied already, cell signaling researchers are also working hard to identify novel targets that act as an Achilles' heel in the signaling pathway to develop new drugs to inhibit cancer development and metastasis. Some key pathways associated with cancer development are briefly summarized as below.

Mitogen activated protein kinase pathways

Mitogen activated protein kinases (MAPKs) are a family of kinases of different lineages that are thought to be important in tumor growth and metastasis[9]. There are three major subfamilies of MAPK: the extracellular-signal-regulated kinases (ERK); the c-jun N-terminal kinase or stress-activated protein kinases (JNK or SAPK); and p38 MAPK. All of these have been proved to play essential roles in the regulation of intracellular metabolism, gene expression and integral actions in many aspects including cell growth, differentiation, apoptosis and cellular responses to external stresses. Lots of evidence has indicated that overexpression and activation of MAPKs are extremely important in the development of cancer[10]. Xu *et al*[11] demonstrated that blockage of the MAPK pathway resulted in the decrease of cyclooxygenase-2 (COX-2) expression and the inhibition of angiogenesis in malignant glioma cells indicating the promising prospect of p38 as a valuable target in brain tumor therapy. Hsiao *et al*[12] reported flavanone and 2'-OH flavanone can inhibit metastasis of lung cancer cells via suppression of the MAPK pathway and perturb the invasion and metastasis of lung cancer cells, thereby constituting an adjuvant treatment for metastasis control.

IKK/NF-κB signaling cascades

IκB kinase/NF-κB (IKK/NF-κB) signaling pathways play critical roles in a variety of physiological and pathological processes[13]. NF-κB is a critical nuclear transcriptional factor that is activated in response to cellular stresses and regulates the expression of genes involved in cell proliferation and cell death[14]. When regulated NF-κB activation is disrupted and cells undergo apoptosis. That is, constitutively elevated or dysregulated NF-κB activation leads to cell death in response to stress. Cross talk between NF-κB and c-Jun N-terminal kinases (JNKs) has been involved in the cell life and death decision under various stresses. Functional suppression of JNK activation by NF-κB has been proposed as a key cellular survival mechanism and contributes to cancer cells escaping from apoptosis. Huang *et al*[15] lately provided a novel scenario of the proapoptotic role of IkappaB kinase beta (IKKbeta)-NF-κB, which can act as the activator of the JNK pathway through the induction of GADD45alpha for triggering mitogen-activated protein kinase kinase 4 (MKK4)/JNK activation. There has been much effort recently to probe the long-recognized relationship between the pathological processes of infection, inflammation and cancer. For example epidemiological studies have shown that 15% of human deaths from cancer are associated with chronic viral or bacterial infections. Hence, inhibition of IKK-driven NF-κB activation offers a strategy for the treatment of different malignancies and can convert inflammation-induced tumor growth to inflammation-induced tumor regression[16].

Wnt signaling pathway

The wnt signal transduction cascade, one of the most powerful pathways in a cell, was originally described in the embryogenesis of vertebrates and non-vertebrates[17]. It is apparent that wnt signaling causes cancer and that tumor promotion by this pathway can proceed through a number of different genetic defects[18]. Additional mechanisms by which defects in the regulation of wnt signaling contribute to tumor progression probably remain undiscovered. The manifestation of cancer by aberrant wnt signaling most likely results from inappropriate gene activation mediated by stabilized β-catenin. Target genes need not contain T cell factor/lymphoid enhancer factor (TCF/LEF) binding sites in their promoters, though, as new potential modes of gene activation by β-catenin are becoming apparent. Several target genes of β-catenin signaling have now been identified and some of their functions are consistent with the control of tumor cell growth, differentiation and survival[19]. It has been demonstrated that 80% of colorectal cancers alone reveal activation of this pathway by either inactivation of the tumor-suppressor gene *adenomatous polyposis coli* or mutation of the proto-oncogene β-catenin. Activation of Wnt/β-catenin signaling has been found to be important for both initiation and progression of cancers of different tissues. Therefore, targeted inhibition of Wnt/β-catenin signaling is a rational and promising new approach for the therapy of cancers of various origins[20].

Cytokine related signaling

Cytokines and their signaling effectors act as key determinants of carcinoma cell behavior. Take transforming growth factor beta (TGF-β) as an example. The autocrine and paracrine effects of TGF-β on tumor cells and the tumor micro-environment exert both positive and negative influences on cancer development[21]. The TGF-β receptor includes Type I and Type II subunits, which are serine-threonine kinases and signal through the Smad family of proteins. Binding of TGF-β to its cell surface receptor leads to the phosphorylation of the Type I receptor by Type II. The Type I receptor is then able to phosphorylate and activate the Smad2 protein. Smad2, in combination with Smad4, is translocated to the nucleus where the activated Smad complex recruits other transcription factors (TF) and together activate the expression of target genes that mediate the biological effects of TGF-β. Some of the activated target genes stimulate tumorigenesis. Accordingly, the TGF-β signaling pathway has been considered as a promoter of tumor development and a potential target of cancer therapy in numerous tumor diseases[22].

Essential role of ROS

It is well accepted that reactive oxygen species (ROS) play a critical role in tumor metastasis. As a signaling messenger, ROS are able to oxidize the critical target molecules such as protein kinase C (PKC) and protein tyrosine phosphates (PTPs), which are involved in tumor cell invasion. MAPK and p21 activated protein kinase (PAK) have been proposed to be regulated by ROS as well[23]. There are several transcriptional factors such as the activator protein 1 (AP-1), Ets, Smad and Snail regulating a lot of genes relevant to metastasis. AP-1 and Smad can be activated by PKC activator and TGF-β1, respectively, in a ROS-dependent manner. In addition, transcription factor Est-1 can be upregulated by ROS via an antioxidant response element in the promoter. The ROS-regulated genes associate with epithelial-mesenchymal transition (EMT) and metastasis include E-cahedrin, integrin and matrix metalloproteinase (MMP). Comprehensive understanding of the ROS-triggered signaling transduction, transcriptional activation and regulation of gene expressions will help with devising a strategy for chemotherapeutic interventions in cancer therapy[24].

Development of targeted drugs

Many years of intensive research into the underlying molecular causes of human cancer have aimed to identify molecular targets of therapeutic importance with the hope that this would enable the development of selective drugs to treat malignancy. There are some successful drugs targeted at the key molecules in the signaling pathway during the last several years, especially for kinase inhibitors.

DNA targeted drug design was once expected to generate novel therapeutics. Given that they are effective drugs in clinical use and have produced significant increases in the survival of cancer patients when used in combination with drugs that have different mechanisms of action, researchers later realized that most pathways can be affected by these drugs at multiple points and these drugs are extremely toxic as well[25]. In the past 10 years, identification of the key protein molecules in cancer signaling as potential therapeutic targets has led to the emergence of a new era of target-directed therapies. Some small-molecule protein kinase inhibitors have shown clinical effects in cancer patients that were discussed by Speake, Holloway and Costello[26]. Similarly, molecules are being isolated or designed to inhibit the activity of other signaling pathways known to be deregulated in cancer, such as MAPK and PI3K/Akt cascade etc.[27,28].

In 1998, the monoclonal antibody trastuzumab (Herceptin) directed against the receptor tyrosine kinase (RTK) HER2 was approved for the treatment of breast cancer by the Food and Drug Administration (FDA) as a milestone of a next-generation anti-cancer therapeutic. Binding of trastuzumab to the extracellular domain of RTK induces the internalization of the receptor, resulting in the down-regulation of HER 2. Then, inhibition of cell-cycle progression and antibody-dependent cellular cytotoxicity can be detected because of immune responses[29]. Moreover, trastuzumab is able to block cleavage of HER2, which would generate a membrane-bound truncated receptor that is constitutively active[30]. Clinical trials showed the positive response in previous treated and untreated patients with metastatic breast cancer[31,32]. Prolonged survival of patients treated with chemotherapy and trastuzumab has been observed on patients overexpressing HER2 compared with chemotherapy alone[33].

STI-571 (Imatinib or Gleevec) targets the ABL (Abelson leukaemia viral oncogene) gene product, a constitutively active tyrosine kinase that drives the proliferation of immature myeloid cells[34,35]. STI-571 thus inhibits tyrosine phosphorylation of the downstream proteins involved in ABL signal transduction and consequently inhibiting BCR-ABL positive cells[36]. Preclinical and clinical studies have verified the remarkable effect of imatinib in the treatment of chronic myeloid leukemia (CML)[37,38]. It was approved by the FDA in 2001 for the treatment of CML at all stages after failure of interferon-α (INF-α) therapy. Furthermore, Imatinib also shows activity against the tyrosine kinases of c-kit and platelet-derived growth factor receptor (PDGFR)[39], so, this drug is also being evaluated in clinical trials for patients with tumors displaying aberrant activation of these signaling pathways[40].

Beyond the success of Gleevec and Herceptin, the development for targeted cancer therapeutics has extended to epidermal growth factor receptor (EGFR) with Iressa, which is another excellent example. Iressa is a small-molecule EGFR inhibitor used in the clinical treatment of patients with non-small-cell lung cancer (NSCLC). Two pivotal phase II studies showed positive results for patients with previous treated advanced[41,42]. However, two phase III trials revealed no additional increase of survival rate compared with routine chemotherapy in NSCLC[43,44]. On the whole, it is somewhat controversial to access the benefits of Iressa, although it was approved in Japan in 2002 and in US in 2003.

Although targeted drugs have achieved great success in the initial stage, drug-resistance of these hormones or antibodies is still inevitable so far. For example, Imatinib resistance in the clinical setting has been attributed to the point mutations of the tyrosine kinase[45]. A potential approach to combating such mutants is to treat patients with a combination of agents that interact differently with their targets at the molecular level. Development of multi-target drugs is one of the ideal choices to solve the problem through a simplified process.

Sorafenib is a kind of novel multi-target anti-cancer drug. It not only inhibits tumor progress by blocking RAF/ MAP kinase kinase (MEK)/ extracellular signal-regulated kinase (ERK) signaling pathways directly, but also prevent angiogenesis in tumor via the suppression of vascular endothelial growth factor (VEGF) and PDGF, thus it indirectly inhibits tumor progress as well[46]. Based on the results of a randomized phase III clinical trial, sorafenib was approved by the FDA to treat advanced renal cell carcinoma in 2005.

Another small molecular drug approved by the FDA for treating gastrointestinal stromal tumor (GIST) and advanced renal cell carcinoma is sunitinib (Sutent, SU11248)[47]. Through the inhibition of VEGF-R2, R3, R1, DGFR-β, KIT, LT-3, RET and their signaling pathways, sunitinib is able to exert anti-cancer effects and works well in renal cell carcinoma, GIST, neuroendocrine tumor, sarcoma, thyroid carcinoma, melanoma, breast cancer, colorectal cancer and non-small-cell lung cancer.

Challenges and prospect

Clinical development of these successful drugs has revealed the importance of target selection. In considering future molecular targets, it is necessary to distinguish between general versus specific ones. General targets essential for cell proliferation and survival, such as those affecting the cell cycle, apoptosis or angiogenesis, are broadly applicable in cancer, while molecular-specific targets represent a unique abnormality of the tumor. The success of imatinib elucidates the importance to identify an appropriate therapeutic target, preferably an early pathogenetic molecule, so as to treat patients at an early stage of disease. Beside specificity, potency, efficacy and biopharma-ceuticals are also key properties required by molecularly-targeted drug development.

Since many tumors accumulate numerous genetic alterations and mutations that can arise in response to drug treatment even in the case of clonal diseases, combinations of new target-specific drugs have notably enhanced antitumor efficacy[48]. Nevertheless, the use of 'cocktails' of target directed drugs that aim at different hallmark characteristics of the tumor are likely to be the future of cancer signaling therapy to achieve maximal therapeutic benefits, underlining the necessity for a comprehensive characterization of oncogenic pathways[49].

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