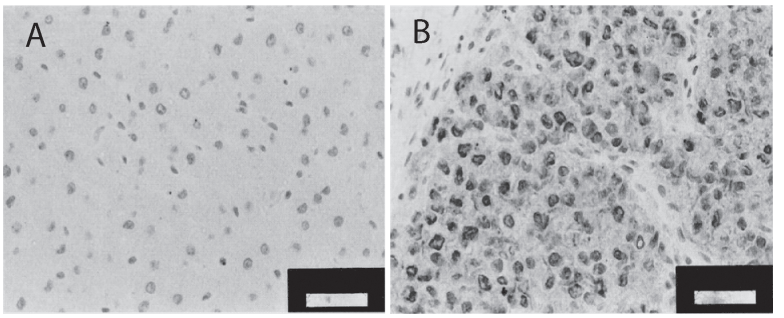
25

REGENERATIVE ENGINEERING FOR CANCER



Cell nucleus density and size in control rat liver and hepatoma: (A) control rat liver; (B) hepatoma. Scale bar: 50 μm. (Reprinted with permission from la Cour JM et al: Up-regulation of ALG-2 in hepatomas and lung cancer tissue, *Am J Pathol* 163:81–9, copyright 2003.) See color insert.

CLASSIFICATION OF CANCERS [25.1]

Cancer is a disorder characterized by the formation of immortal cells that undergo uncontrolled excessive cell proliferation. Cancer cells are aberrantly differentiated cells due to gene mutation and do not possess physiological functions. These cells can aggressively invade neighboring tissues and organs, spread to remote tissues and organs through the lymphatic and vascular systems (a process known as *metastasis*), form colonies in invaded tissues and organs, and expand in the expense of normal cells. As a result, normal cells die because of deprivation of necessary nutrients, leading to the malfunction

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and death of involved tissues and organs. It should be noted that cancer is a malignant type of tumor. There are also tumors that grow slowly, do not invade neighboring tissues, do not metastasize, and do not significantly influence the functions of host tissues and organs. This type of tumor is defined as benign tumors and will not be covered in this book.

Based on the origin of cancer formation, cancers are classified into several types: carcinoma, sarcoma, leukemia, and neural tumors. *Carcinoma* is a type of cancer that arises from epithelial cells, including gland cells, and can be found in a variety of organs, such as the nasal and oral cavities, gastrointestinal tract, liver, pancreas, lung, breast, prostate gland, overy, uterus, bladder, and skin. Carcinoma is the most common type of cancer, accounting for about 80% of total cancers. The high incidence of carcinoma is possibly related to the high turnover or renewal rate of epithelial cells. These cells are developed for the protection of tissues and organs, and are often subject to various types of harmful environmental factors. Thus, mature epithelial cells are still able to differentiate and proliferate for the repair and replacement of injured and lost cells. Such a feature naturally increases the rate of cancer formation. Various terms have been used to describe carcinoma, depending on the type of cells, tissues, and organs where cancers arise. For instance, *hepatoma* is a term for liver carcinoma, *melanoma* is used to describe carcinoma derived from the epidermal melanocytes, which contains the pigment melanin, and *adenocarcinoma* is cancer from gland epithelial cells.

Sarcoma is a type of cancer that arises from connective and muscular tissues. This type of cancer is found in soft connective tissues, bones, cartilages, skeletal muscles, and blood vessels. Since mature connective tissue cells and muscular cells are well-differentiated cells and undergo a low rate of proliferation, the incidence of sarcoma (about 1%) is much lower than that of carcinoma. *Leukemia* is a type of cancer derived from hematopoietic or blood cells, primarily involving leukocytes. Leukemia derived from lymphocytes is called *lymphoma*. This type of cancer accounts for about 9% of the total cancers. Another major type of cancer is *neural cancers*, which are found in the nervous system. This type of cancer increases the volume of the brain within the limited skull space and induces the compression of normal brain tissue, resulting in various symptoms of neural disorders depending on the region involved. Typical nervous cancers include gliomas and retinoblastomas.

PATHOGENESIS OF CANCERS [25.1]

While the pathogenesis of cancers remains a research topic, increasing investigations have suggested that cancers are possibly a result of gene mutation or changes in DNA structure. Cancer may be originated from a single cell that undergoes cancerigenic gene mutation. It is important to note that, although various types of gene mutation may occur under physiological conditions, not all gene mutations lead to carcinogenesis. However, it remains poorly understood what types of gene mutation are carcinogenic.

Gene mutation may occur spontaneously during cell division as a natural process, which is responsible for evolutionary alternations in living organisms. In addition, gene mutation can be induced by environmental factors, including radiation, viral infection, exposure to carcinogens, and therapeutic gene transfer. Radiation (e.g., X rays and ultraviolet light) often causes DNA damage and chromosome disruption. Gene mutation may

be introduced to the genome when damaged genes and chromosomes are repaired. Viruses are able to integrate their genome into the host genome, a process often inducing gene mutation. Chemical carcinogens, such as formaldehyde and peanut mold-toxin (aflatoxin), can cause changes in DNA sequences. Gene transfer is thought a therapeutic method that is used to correct mutant genes. However, the insertion of foreign genes into the genome may induce gene mutation. Cancers may be induced when patients are frequently exposed to these factors.

While many types of gene mutation can contribute to carcinogenesis, there are two types that play a major role: the activation of growth stimulatory genes, which are also known as protooncogenes, and the suppression of growth inhibitory genes, which are also called tumor suppressor genes. *Protooncogenes* are normal genes that encode mitogenic proteins and can be converted to carcinogenic oncogenes or cancer-inducing genes by mutation. Examples of proto-oncogenes include c-fos (activator protein-1 gene), c-jun (another activator protein-1 gene), c-raf (protein serine/threonine kinase gene), c-myc (gene-regulatory protein gene), c-sis (platelet-derived growth factor gene), and c-src (Src protein tyrosine kinase gene). These genes play critical roles in the regulation of physiological development and morphogenesis. These genes can be transformed into oncogenes in response to the stimulation of environmental factors. A typical cause of protooncogene transformation is retrovirus infection. Retroviruses can convert their RNA genome into DNA and insert the converted DNA into selected protooncogenes in the host genome. The protooncogenes can be structurally altered or can be subject to the control of viral gene promoters, leading to the formation of carcinogenic oncogenes that stimulate cell differentiation and proliferation.

Another type of gene mutation is the alteration or loss of tumor suppressor genes. These genes play critical roles in the inhibition of carcinogenesis. The mutation or loss of such genes contributes to the initiation and development of cancers. A typical tumor suppressor gene is the retinoblastoma tumor suppressor gene. This gene is expressed in almost all cell types and encodes a protein that controls the progression of the cell division cycle. Cancers in several organs, including the lung and breast, are associated with reduced expression of the retinoblastoma tumor suppressor gene. The mutation and loss of this gene contributes to carcinogenesis. Another tumor suppressor gene is the p53 gene (see page 236 for the characteristics of p53). Patients with mutation or loss of this gene are susceptible to carcinogenesis. Some viruses, such as papillomaviruses, exert an inhibitory effect on the tumor suppressor genes.

PATHOLOGICAL CHARACTERISTICS OF CANCERS [25.1]

In pathological examinations, cancerous changes can be found at the cellular and tissue levels. Pathological changes at the cellular level include an increase in cell proliferation (assessed by BrdU assay), cell density, and the size of cell nuclei. It is important to note that these changes should be always determined on the basis of a comparison with normal cells. Pathological changes at the tissue level include the formation of tumors without a clearly defined boundary, tumor invasion of neighboring tissues, an increase in angiogenesis, and disruption or destruction of normal tissues where cancer cells invade. The appearance of cancer cells at multiple locations suggests metastasis. Cancers are often

associated with rapid functional deterioration of involved organs. For instance, stomach cancer, when involving a large area, exhibits reduced capability of food digestion and rapid loss of body weight. Lung cancer is associated with difficulties in respiration. The ultimate consequence of cancer is the death of the involved organ. During the late stage, metastasis occurs in almost all cancer patients, often resulting in rapid failure of involved organ systems.

TREATMENT OF CANCERS

Conventional Treatment [25.1]

There are three major conventional approaches for the treatment of cancer: surgical removal, chemotherapy, and radiotherapy. *Surgical removal of cancers* is the most effective treatment when cancers are limited to a local area and are not spread to the surrounding tissues and lymph nodes. Early diagnosis of cancer is critical to the success of surgical treatment. Once cancers spread to neighboring tissues or metastasize to different organs, surgery is no longer effective. Chemotherapy and/or radiotherapy can be used for spread or metastasized cancers. *Chemotherapy* is an approach by which chemical agents are used to suppress the proliferation of cells, including cancer and normal cells. Since normal cells undergo a lower rate of division than cancer cells, chemical agents primarily affect cancer cells. A typical chemotherapeutic agent is 5-fluorouracil (5-FU), a uracil derivative that interrupts DNA synthesis and therefore suppresses cell division when incorporated into the genome of dividing cells. *Radiotherapy* is an approach used to treat cancers by exposing patients to radiation. While radiation destroys cancer cells and is effective for cancer treatment, it also induces normal cell injury and death.

Molecular Engineering Therapies

A number of molecular engineering strategies have been established and used for the treatment of cancers. These include the up-regulation of tumor suppressor genes, correction of mutant tumor suppressor genes, enhancement of anti-cancer immune responses, activation of tumor suppressor drugs, introduction of oncolytic viruses, and inhibition of growth-promoting genes by using antisense and siRNA oligonucleotides. A large number of investigations have been carried out to test these strategies in experimental models. Some of the strategies have been applied to preliminary clinical trials. Selected strategies are discussed as follows.

Overexpression of Tumor Suppressor Genes and Correction of Mutant Tumor Suppressor Genes [25.2]. As discussed on page 265, several proteins, including $p16^{iNK4}$, $p15^{iNK4B}$, $p18^{INK4C}$, $p19^{INK4D}$, p21, p27, and p57, are known to exert an inhibitory effect on cell division. These proteins may suppress the activity of cyclin D/CDK 4/CDK6, an important protein complex that regulates the progression of the cell division cycle from the G1 to S phase. Another protein, p53, suppresses cell division by activating p27. The overexpression of these proteins enhances the inhibitory effect on cell division and suppresses tumor cell growth. In particular, the p53 gene has been tested extensively. A large fraction of cancer patients exhibit mutant p53 gene, a potential factor contributing to the

initiation and development of cancers. Experimental investigations in animal models of cancers have shown that the transfer of the wildtype p53 gene into cancer tissues results in the suppression of cancer cell proliferation and reduction in cancer progression. Preliminary studies in human trials have demonstrated promising results for the therapeutic effect of the wildtype p53 gene. Other growth-inhibitory protein genes, as described above, can also serve as candidate genes for cancer therapy.

Enhancement of Anticancer Immune Responses [25.3]. Cancer cells express tumor antigens that can be recognized by the immune system under physiological conditions. The immune system is capable of destroying recognized cancer cells, as cancer cells are considered as foreign invaders. Such recognition and destruction activities are regulated by several signaling processes. When cancer cells form due to gene mutations, antigens expressed in the cancer cells can be recognized by antigen-presenting cells, which present the antigens to the T-helper cells. The T-helper cells produce and release cytokines, such as interleukin (IL)2, which activate cancer-specific T lymphocytes. The activated T lymphocytes can produce and release killer cytokines to suppress cancer growth and progression.

However, when the function of the immune system is suppressed, the immune system can no longer detect and destroy cancer cells, allowing cancer cells to proliferate and spread. Indeed, the suppression of the immune system may contribute to the initiation and development of cancers. Furthermore, cancer cells can produce and release cytokines that suppress the activity of host immune system and help cancer cells to escape from the immune surveillance and attack. Thus, a major approach for the treatment of cancers is to enhance the tumor-recognizing and suppressing functions of the host immune system. While it is difficult to achieve such a goal, several hypothetical strategies have been proposed and tested. These include the overexpression of T-lymphocyte activating factors, T-cell costimulating factors, and application of tumor-antigen vaccines.

The activity of the T lymphocytes can be stimulated by introducing several factors, such as human leukocyte antigen (HLA), major histocompatibility complex (MHC), cytokines (e.g., IL2 and interferon-γ) to target cells. The level of human leukocyte antigen is reduced in a large fraction of cancer patients. A molecule analogous to human leukocyte antigen is the major histocompatibility complex. The overexpression of the major histocompatibility complex gene in animal models of cancers induces activation of cytotoxic T cells and boosts anti-cancer responses. In human studies, the transfer of HLA-expressing vectors into melanoma cells induces the activation of cytotoxic T-cells and a reduction in tumor growth.

Several cytokines, including IL2, IL4, IL12α (see Table 25.1 for specifics on IL12α and CEA), granulocyte macrophage-colony stimulating factor (GM-CSF), and interferon-γ (see page 631 and the following table in this section for the characteristics of these factors), are known to promote the activation of T lymphocytes. The overexpression of these cytokines by gene transfer is a potential approach for the treatment of cancers. In particular, the IL2 gene has been tested in experimental models for anti-cancer effects. These studies have demonstrated that the overexpression of the IL2 gene activates cytotoxic T cells and reduces tumor growth in murine cancer models. Investigations in human trials have also shown promising results. Direct administration of cytokine proteins is another approach. However, cytokines undergo rapid degradation and the general administration of cytokines often induces toxic responses. Local gene transfer is an approach that can be used to overcome these problems.

TABLE 25.1. Characteristics of Carcinoembryonic Antigen and IL12α*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Carcinoembryonic antigen	CEA, carcinoembryonic antigen-related antigen-related cell adhesion molecule 5 (CEACAM5), meconium antigen 100, CD66e antigen	702	77	Leukocyte, intestine, epithelial cells	Promoting cell survival and proliferation and enhancing cancer cell growth
IL12 α	IL12A, cytotoxic lymphocyte maturation factor, natural killer cell stimulatory factor chain (NKSF1), T-cell-stimulating factor	219	25	Dendritic cells	Inducing the expression of interferon (IFN)γ and promoting the differentiation of Th1 and Th2 cells

*Based on bibliography 25.3.

TABLE 25.2. Characteristics of B7.1*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
B7.1	B7-1 antigen, B lymphocyte activation antigen B7-1, activation B7-1 antigen, CD28 antigen ligand 1 (CD28LG1), CD80 antigen	288	33	Liver, dendritic cells, mast cells, brain, blood vessel	Stimulating activation of T cells

*Based on bibliography 25.3.

Since the repression of the anticancer immune responses is considered a factor that contributes to tumorigenesis, the boosting of immune activities is a potential approach for the treatment of cancers. An effective approach for boosting immune activities is to deliver cancer-associated antigens to the host systems. Such antigens include viral analogues of tumor antigens, mutated oncogene proteins, and carcinoembryonic antigens. The genes of these proteins can be used to construct plasmids, known as recombinant vaccines, which can be used for gene transfer and for producing cancer-associated antigens. The host immune system is able to recognize these antigens and generate specific antibodies, which contribute to the anticancer activities. A typical tumor-associated antigen is the carcinoembryonic antigen, a glycoprotein receptor that is upregulated in certain types of cancer, such as colon cancer. The gene of the carcino-embryonic antigen can be inserted into a viral vector, such as the *Canary pox* virus, forming a recombinant DNA molecule. The overexpression of such a recombinant gene in a transgenic mouse model results in the production of antibodies against the carcinoembryonic antigen, in association with the enhancement of T-cell activities against tumor cells that express the carcinoembryonic antigen. Clinical investigations in patients with colorectal cancer have shown promising results, including the generation of antibodies against the carcinoembryonic antigen and activation of the cytotoxic T cells.

The activation of cytotoxic T cells can be induced by exposure to cancer cell antigens. The activity of the cytotoxic T cells can be boosted by costimulating factors such as the B7.1 protein (see Table 25.2). In certain types of cancer, the expression of costimulating factors is repressed, a potential factor that reduces the anticancer immune responses. Thus, a strategy for enhancing the activity of the cytotoxic T cells is to deliver cancer cell antigens and costimulating factors together. A recombinant gene can be constructed with the carcinoembryonic antigen gene and the costimulating factor B7.1 gene. Such a gene construct has been applied to human patients with adenocarcinoma in a preliminary clinical trail. This study has demonstrated that the overexpression of the recombinant gene is associated with activation of T cells specific to the carcinoembryonic antigen. The application of the costimulating factor gene enhances the anticancer immune reactions.

Activation of Tumor-Suppressing Prodrugs [25.4]. Chemotherapy is an effective approach for the inhibition of cancer metastasis. However, most chemotherapeutic agents destroy not only cancer cells, but also normal cells, significantly repressing the activity of the immune system. It is desired to introduce chemotherapeutic agents that are effective only in targeted cancer cells without influencing the normal cells. Synthetic deoxynucleosides may serve as such chemotherapeutic agents. A typical agent is ganciclovir, an analogue of deoxyguanosine. Ganciclovir can be phosphorylated by the herpes simplex virus tyrosine kinase to form ganciclovir triphosphate, a deoxynucleotide that can be incorporated into DNA during DNA synthesis in place of dGTP. The incorporation of the ganciclovir deoxynucleotide induces the termination of DNA synthesis, thus arresting cell division in the S phase. Ganciclovir without phosphorylation is not effective. Based on such a feature, ganciclovir can be administrated through blood injection, while herpes simplex virus tyrosine kinase gene can be delivered to local target cancer cells. With the expression of the viral tyrosine kinase gene in the local cancer cells, ganciclovir can only be phosphorylated in the cancer cells, thus repressing DNA synthesis in cancers but not in normal cells. Experimental investigations have demonstrated that in animal models of

colon cancer, general ganciclovir administration with local delivery of the herpes simplex viral tyrosine kinase gene results in a significant reduction in tumor growth. Clinical investigations with a similar approach have also shown promising results for the treatment of human colorectal adenocarcinoma.

Another tumor-suppressing prodrug is 5-fluorocytosine, a cytosine derivative. This agent is relatively inactive in its natural form. A cytosine-specific enzyme called cytosine deaminase, which is found in bacteria and fungi, can convert 5-fluorocytosine to 5-fluorouracil, a potent anti-cancer agent that blocks the methylation reaction of deoxyuridylic acid to thymidylic acid and thus interferes with DNA synthesis. A blood injection of 5-fluorocytosine, together with a delivery of a bacterial cytosine deaminase gene into local target cancers can induce local conversion of 5-fluorocytosine to 5-fluorouracil, resulting in the repression of cancer growth with a minimal influence on normal cell function. Such an approach has been shown to effectively inhibit cancer proliferation in animal models as well as in human patients with colorectal cancer.

Application of Oncolytic Viruses [25.5]. Several types of viruses, including adenovirus and herpes simplex virus, can be genetically modified to establish the capability of lysing cancer cells, but not normal cells. A mutant adenovirus has been created by removing the E1B segment of the viral genome, resulting in the deficiency of the E1B-55 kDa viral protein. When delivered to cancer cells, the mutant adenoviruses can replicate in cancer cells lacking p53 and then lyse these cells. Clinical investigations have shown that the delivery of mutant adenoviruses into metastatic tumors results in cancer cell death. However, controversial results have been reported regarding the target specificity of the mutant adenoviruses. Further investigations are necessary to confirm the preliminary discoveries.

Herpes simplex virus is another type of virus that can be modified to establish cancer cell-specific lysing activity. The removal of selective genes, including the tyrosine kinase gene or the ribonucleotide reductase, from the herpes simplex virus results in mutant viruses that can replicate in dividing cells. Since cancer cells undergo a much higher rate of division compared to normal cells, the transfer of the mutant herpes simplex viruses into cancer cells results in the lysis of these cells. Experimental studies have demonstrated selective oncolytic activity of herpes simplex virus in animal colon cancer models.

Application of Antisense Oligonucleotides and siRNA [25.6]. The initiation and development of cancers involve the upregulation of oncogenic genes, such as *abl* (protein tyrosine kinase), *raf* (protein serine/threonine kinase), *ras* (GTP-binding protein), *sis* (platelet-derived growth factor B chain), and *src* (Src protein tyrosine kinase). The expression of other genes that encode mitogenic proteins also contributes to carcinogenesis. The suppression of carcinogenic gene expression is an effective approach for the treatment of cancers. A typical approach for such a purpose is the administration of antisense oligonucleotides specific to a target mRNA molecule. Antisense oligonucleotides are short fragments of nucleotides with a length of 20–40 base pairs and can hybridize to complementary mRNA in the cytoplasm, blocking the translation of proteins. These fragments can be directly delivered to target cells for therapeutic purposes. An alternative method is to transfer a gene construct that encodes a fragment of antisense deoxynucleotides,

which can be expressed and exerts antisense effects. Furthermore, the transfection of target cancer cells with siRNAs specific to proliferative mRNAs can effectively suppress the proliferation of cancer cells (Fig. 25.1).

Application of Combined Therapeutic Approaches [25.7]. The treatment of cancers is a challenging task. Often, the application of a single approach as described above may not be effective, especially in the presence of cancer metastasis. The combination of anticancer therapeutic methods may provide an alternative means for the treatment of cancers. Various combinations have been tested in experimental and clinical investigations. Exam-

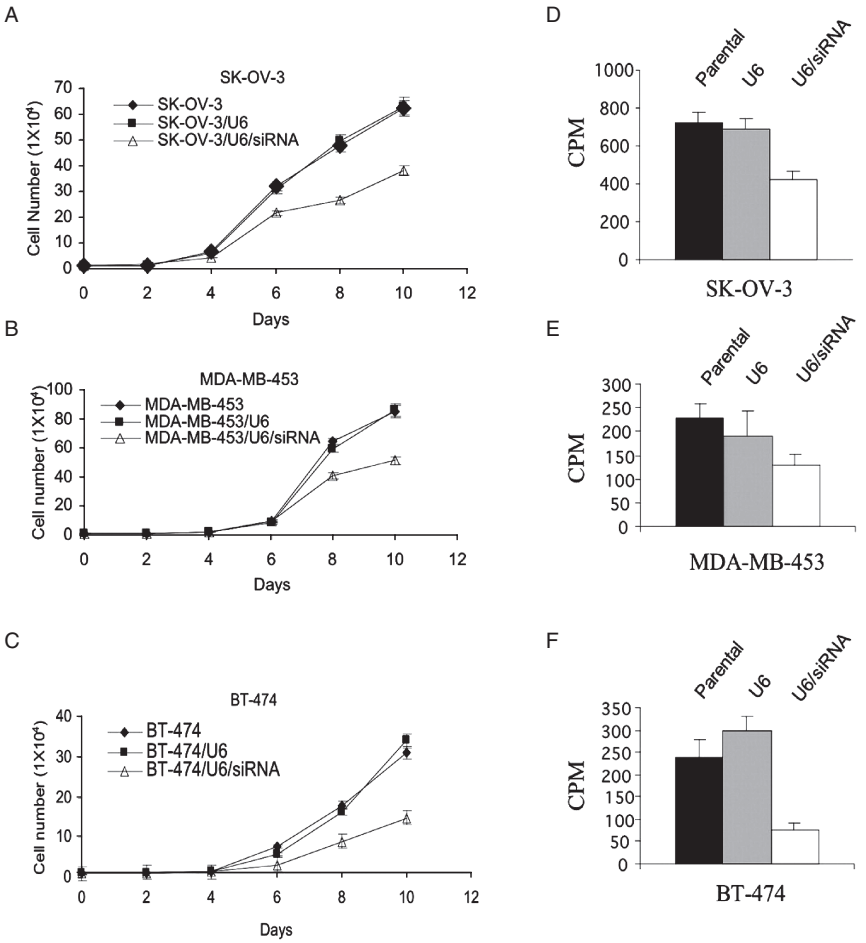

Figure 25.1. Cell proliferation, measured by cell counts (A–C) and [^{3}H]thymidine uptake (D–F) of SK-OV-3, MDA-MB-453, and BT-474 cells with and without transfection with Her-2/neu siRNA. Note that the *Her-2/neu* gene is an oncogene that is overexpressed in ~30% of breast and ovarian cancer cases. The expression of this gene often indicates a poor prognosis. CPM: counts per minute. (Reprinted with permission from Yang G et al: Inhibition of breast and ovarian tumor growth through multiple signaling pathways by using retrovirus-mediated small interfering RNA against Her-2/neu gene expression, *J Biol Chem* 279:4339–45, copyright 2004.)

ples include the combination of oncolytic viruses and anticancer prodrugs, the combination of radiotherapy with oncolytic viruses, and the combination of tumor-suppressing genes and immune response activators. These combination approaches have been shown to be more effective compared to single approaches.

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