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Cellular Growth, Development, and Defensive Response

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1. Normal and Abnormal Cell Growth

1.1. Cell Cycle

The cells of living organisms experience one of three fates: (1) live and reproduce, (2) live without reproducing, or (3) death. A cell reproduces by carrying out a series of processes in which it duplicates its contents and divides itself into two daughter cells. The cycle of duplication and division is known as the cell cycle, which involves four major events: cell growth, DNA replication, chromosome segregation into two identical sets, and cell division. The entire process can be divided into two fundamental parts: interphase (including G_1 , S, G_2 phases) and mitosis (M phase) (1–2), as illustrated in Fig. 1.

Interphase starts with a gap phase 1, or G_1 phase, during which the cell grows and prepares for the initiation of DNA replication. After the cell enters S phase, DNA synthesis starts and the whole chromosomes are replicated. In order to prepare for cell division, the cell enters the second gap period, or G_2 phase, and continues to grow and synthesize proteins such as mitosis-promoting factors for the next phase. The culmination of cell cycle is the triggering of the mitosis phase, during which the chromosome segregation and cell division take place. Interphase can be further subdivided into five phases: prophase, prometaphase, metaphase, anaphase, and telophase. During mitosis, chromosomes are condensed and nuclear envelope is broken down. The mitotic spindle is then formed and the chromosomes move to the opposite poles followed by cell division.

Typically, interphase lasts much longer time than the mitosis phase. For example, interphase lasts about 23 h, whereas mitosis lasts only about 1 h for human cell, with a total cycle time of 24 h. Although S phase and M phase are two major phases in a cell cycle, the two gap phases, G_1 and G_2 , monitor the internal and external environment to ensure that conditions are suitable and preparations are complete for the S and M phases, respectively. The length of the G_1 phase is the most variable among all the phases, and it is greatly dependent on external conditions and extracellular signals. If extracellular conditions are unfavorable, cells delay progress through G_1 and may even enter a quiescent state known as G_0 , in which they can remain metabolically active but no longer proliferate, unless called on to do so by appropriate extracellular signals. If extracellular conditions are favorable and signals for growing and dividing are present, cells in early G_1 or G_0 progress through a commitment point near the end of G_1 known

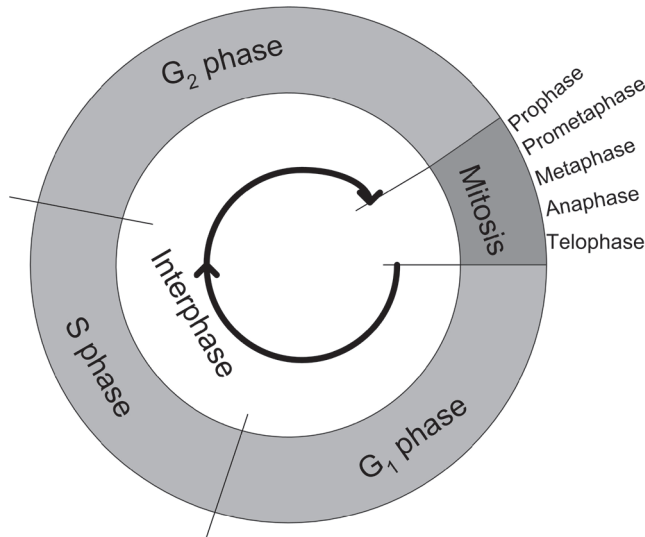


Fig 1. A typical diagram of cell cycle.

as the restriction point. After passing this point, cells are committed to DNA replication, even if the extracellular signals that stimulate cell growth and division are removed.

1.2. Intracellular and Extracellular Control of Cell Cycle

Normally, the cell cycle is a rather complicated and precisely coordinated process. In eukaryotes, it is mainly controlled by enzymes known as cyclin-dependent kinases (Cdks) that serve as the control system to coordinate the major transitions in a cell cycle. Coordination of the timing and order of these processes is achieved by a regulatory system that represents the checkpoints in the major transitions in the cell cycle. Two key checkpoints are at the G₁/S and G₂/M phase transitions. G₁ and G₂ cyclins and their associated catalytic subunits, Cdks, are responsible for controlling the transition. In most cells, the control system responds to various signals from both inside and outside the cell. Intracellularly, the control system monitors progression to ensure that the transitions are properly timed and ordered. For example, it is critically important that the cell does not begin mitosis until replication of the genome has been completed. Extracellularly, the control system analyzes its environmental conditions and regulates the progression according to the extracellular signals. Cdk activities oscillate as the cell progresses through the cell cycle. These oscillations lead directly to cyclical changes in the phosphorylation of key components of the cell-cycle process, resulting in the initiation of different cell-cycle events. For example, an increase in Cdk activity at the beginning of mitosis leads to protein phosphorylation, which controls spindle assembly, nuclear envelope structure, and chromosome condensation. The Cdk activities are in turn controlled by a combination of small inhibitory proteins known as cyclin dependent-kinase inhibitors (CKIs). It should be noted that a variety of other mechanisms also contribute to the control of Cdk activity, e.g., some Cdks exemplified by Cdk7 are able to control the activities of other Cdks, which make the control system a complex Cdk regulatory network (3).

Extracellular signals contribute to the cell-cycle control by helping the cells determine whether there is need and whether it is ready to divide to ensure that the organism

and its organs achieve and maintain an appropriate size. They are classified into three types based on the effects of these regulators on the progression in a cell cycle: cell division, cell growth, and cell survival (1,2). By removing the restriction point that restricts the cell-cycle progression in G_1 phase, mitogens can stimulate the cell-division rate. On the other hand, growth factor can promote cell mass increase by stimulating the biosynthesis while inhibiting the biodegradation of macromolecules. Survival factors can inhibit the process of apoptosis and lead to an increase in cell numbers.

1.3. Abnormal Growth of Cell

Because the cell cycle is tightly regulated by intracellular and extracellular signals, it normally runs smoothly. However, cell mutation is a routine process in human body. Although most cell mutations are corrected efficiently by the human body, some can cause permanent disruption. Mutations, especially mutations of key regulatory genes, may cause much more rapid cell division than that of normal cells and lead to a growing mutant clone. If the mutant cells continue to grow vigorously and out of control, cancer can develop. The cancer cells do not obey the normal cell division restraints and they can invade the territories of neighboring cells. They can even transfer to other organs that are far away from them through body circulation. Cancers can be classified into two major categories based on their original cell or tissue type: carcinomas and sarcomas. The former refers to cancers arising from epithelial cells, and the latter refers to those arising from connective tissue or muscle cells. Many cancers do not fit into either of these two categories, e.g., various leukemias derive from hemopoietic cells, and others derive from the cells of nervous system.

The development of cancer can be viewed as a multistep process involving mutations and selection for cells with progressively increasing capacity of proliferation, survival, invasion, and metastasis. It starts with tumor initiation, which is the result of genetic alteration leading to abnormal proliferation of a single cell. The tumor progression turns an initially mild disorder of cell behavior gradually into a full-blown cancer. Clonal selection, a process similar to natural selection that a new clone of tumor cells has evolved on the basis of its parent by gaining increased growth rate, survival, invasion, and metastasis ability, makes the tumors become more rapidly growing and increasingly malignant.

A cell must acquire a whole range of aberrant properties as it evolves in order to turn an abnormal cell into cancer. Different cancers may require different combinations of properties. One of the common characteristics of cancer cells is that they disregard the external and internal signals that normally keep cell proliferation under tight control. A primary distinction between cancer cells and normal cells is that normal cells display density-dependent inhibition of cell proliferation. Normal cells proliferate to reach certain density and then cease proliferating, whereas cancer cells are insensitive to these signals. Cancer cells are also less stringently regulated, and insensitive to cell-contact inhibition, which makes them tend to be invasive and able to metastasize. In order to develop cancer, cancer cells tend to have longer life span as compared to their normal cell counterparts. Because cancer cells are usually blocked at an early stage of differentiation, they fail to differentiate normally, which is related to abnormal proliferation. Another important property of cancer cells is that they are genetically unstable, which makes it possible for further genetic alterations required for neoplasia and malignancy to occur.

1.4. Cell Cycle and Cancer Drug Targeting

Components of the cell-cycle machinery are frequently altered in cancer cells. Deregulated Cdk activity, combined with aberrant checkpoint control (at the G₁/S and G₂/M boundaries), leads to undesirable cell proliferation. The Cdks offer multiple mechanisms for intervention in the transformed state. In S phase, Cdk2/cyclin A is important for phosphorylation and inactivation of the E2F/DP1 transcription factor. Inhibition of Cdk2/cyclin A results in elevated E2F concentrations, which leads to S-phase arrest and apoptosis.

Among various regulatory proteins in a cell cycle, Cdks are essential for driving the cell through each stage of a cell cycle. Thus it is not surprising that Cdks are important targets for therapeutic intervention in various proliferative diseases, including cancer. By modulating Cdks, it is possible to block cell-cycle progression, induce apoptosis, promote differentiation, inhibit angiogenesis, and modulate transcription. For example, flavonoid can cause cell-cycle arrest at G₁ or G₂ and can inhibit the activation and the activity of several Cdks (4). Because flavonoid affects Cdk activity directly, it is called a direct modulator. Other direct modulators include staurosporines, paullones, indirubins, roscovitine, olomoucine, and purvalanol (3). On the other hand, indirect modulators, such as proteasome inhibitors, can block G₁ or G₂ transition. They have offered a promising new approach to treating cancers. The 26S proteasome regulates the turnover of proteins involved in cell-cycle control and apoptosis. It is relevant to human cancer because many intracellular proteins regulated by the ubiquitin-mediated proteasome degradative pathway govern the cell cycle, tumor growth, and survival. Lactacystin is one such inhibitor that was found to arrest umbilical vein cells at the G₁ phase of the cell cycle and to induce the nuclear accumulation of p53 in these cells (5).

The variety of mechanisms controlling the major transitions in the cell cycle provide many possibilities for drug targeting along the cell cycle, such as drugs arresting at G₀/G₁, drugs arresting at G₁/S, and drugs arresting at G₂/M. For example, the anticarcinogenic potential of grape-seed polyphenols were found to be involved in the modulation of mitogenic signaling, induction of G₁ arrest, and apoptotic cell death (6). Okadaic acid was found to arrest plasmacytoma cells at both G₂/M and S phases and induces vimentin expression in these cells (7), thereby inducing apoptosis and causing differentiation of tumor cells. Although okadaic acid has not been tested in the clinic, it merits further development for the treatment of human proliferative diseases like cancers from the modulatory effects in the cell cycle.

2. Genetic Process and Control

2.1. DNA–RNA–Protein Process Overview

The growth and development of all cells and living organisms are dependent on the faithful transmission of genetic information from parent to offspring. Thus accurate replication of DNA is essential for all cells and organisms. DNA replication undergoes a process called DNA templating, in which the nucleotide sequence of a DNA strand is copied by complementary base-pairing (A with T, and G with C) into a complementary DNA sequence. The replication is done through a semi-conservative process, i.e., the resulting DNA contains a newly created strand and a strand from the parent DNA. DNA repair will follow to correct the mistakes in DNA replication through one of the two major DNA repair pathways: (1) direct reversal of chemical reaction responsible for the mistake, and (2) removal of the damaged bases followed by their replacement

with newly synthesized DNA. The replication and repair mechanisms ensure that the daughter DNAs carry exactly the same genetic information as their parent DNA.

When a cell needs a particular protein, the nucleotide sequence of the appropriate portion in DNA molecule is first transcribed into mRNA. Once mRNA is produced, it can be used to synthesize the corresponding protein. Each group of three consecutive nucleotides in RNA is called a codon, and each codon specifies either an amino acid or a stop codon in the translation process. The translation of the nucleotide sequence of an mRNA molecule into protein takes place in the cytoplasm on a large ribonucleoprotein assembly called a ribosome, a complex catalytic machine made of the ribosomal proteins and the ribosomal RNAs (rRNAs). This process is generally divided into three stages: initiation, elongation, and termination. To initiate translation, a small ribosomal subunit binds to the mRNA molecule at a start codon (AUG) that is recognized by a unique initiator tRNA molecule. Elongation phase of protein synthesis follows, and each aminoacyl-tRNAs bearing a specific amino acid binds sequentially to the appropriate codon in mRNA by forming complementary base pairs with the tRNA anticodon. Each amino acid is added to the C-terminal end of the growing polypeptide. In the direction of 5'-to-3', the mRNA molecule progresses through the ribosome codon by codon until stop codon is reached. After the release factor binds to ribosome, translation is terminated and complete polypeptide is released. The flow of genetic information from DNA to RNA to protein is illustrated in Fig. 2.

2.2. The Control of Gene Expression and Drug Targeting

Different cell types in a multicellular organism differ dramatically in both their structures and functions, resulting from the different sets of RNA and protein molecules synthesized and accumulated.

The control of gene expression is exerted at multiple levels by changes of DNA content or position and changes in gene activity. The four main levels of gene activity control are transcriptional control, posttranscriptional control, translational control, and posttranslational control. Gene expression is a very complicated process, and many regulatory factors, including proteins and nucleic acids, are involved in this process. For example, it was recently discovered that many small RNAs play an important role in gene-expression control by affecting mRNA degradation (8). In theory, all regulatory factors in this process can serve as the therapeutic targets for targeted drug development. These regulated steps and the corresponding drug targeting strategies are discussed in the following sections.

2.2.1. Transcriptional Control

In eukaryotic cells, transcription is a complex process in which many proteins work together to transcribe DNAs into mRNAs. It is the most regulated step in the genetic path from DNA to proteins. The DNA sequence to which RNA polymerase binds to initiate transcription of gene is called the promoter. Multiple proteins are required by the RNA polymerase to act as initiation factors. Some of these proteins bind to promoter sequences and direct the polymerase to the transcription start site. Transcription is initiated by eukaryotic RNA polymerase II, and it can be further stimulated by activators and inhibited by repressors. Transcription can also be regulated by some extracellular signals such as hormones.

In recent years, researchers have found that gene expression can be altered by the change of the chromatin shape, although the reason of such change is still mysterious.

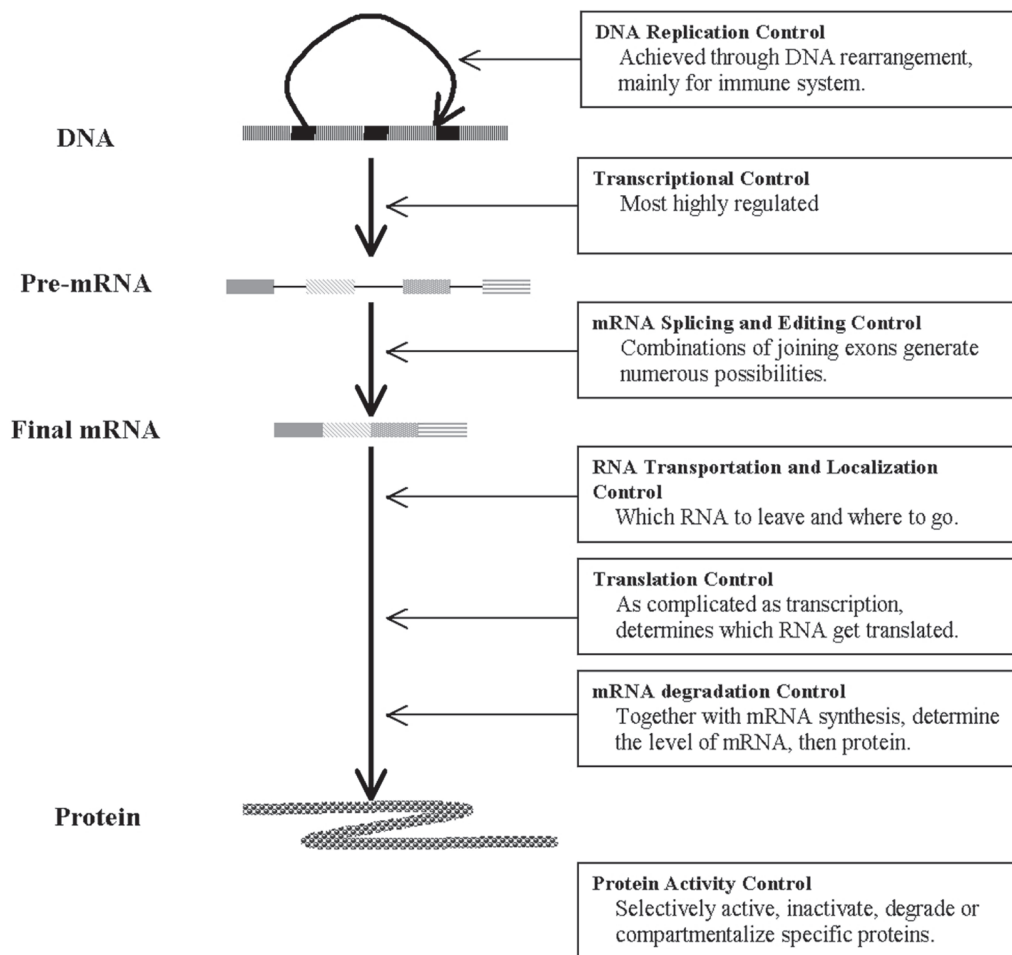


Fig 2. Diagram of genetic information flow (DNA–RNA–protein).

For example, in many chromosomes, loops or puffs of exposed DNA are sites of heavy transcription. Genes can be amplified when there is a heavy demand for a protein coded by a gene or set of genes, as the cell can make multiple copies of the genes so that more mRNAs can be simultaneously made.

The complicated control network of transcription provides numerous possibilities for drug targeting. Triple helix-forming oligonucleotides, for example, can be used to inhibit transcription initiation. The inhibition of binding of transcription activating factors by triplex formation can modulate the level of transcription of the target gene. This provides rationale for the development of new tools for cellular biology and of new therapeutic approaches to control gene expression at the transcriptional level (9).

2.2.2. Posttranscriptional Control

In eukaryotes, transcription of protein-coding genes yields pre-mRNAs, which are spliced and edited into functional mature mRNAs that are used to guide protein synthesis. In eukaryotic cells, nascent pre-mRNAs are associated with a complex set of hnRNP

proteins before transcription is completed. One function of these RNA-binding proteins is to help the formation of the structures recognized by RNA-processing factors.

In multicellular organisms, most pre-mRNAs are spliced to remove noncoding introns. Small nuclear ribonucleoprotein particles (snRNPs; U₁, U₂, U₄, U₅, and U₆) play a key role in this process. The snRNPs associate with splice sites to form a spliceosome (composed of many proteins and snRNPs), in which the splicing reactions occur. In this process, U₁ of snRNP is first bound to yield 5' splice site of pre-mRNA. The recognition of 5' splice sites is based on base pairing between the 5' splice site consensus sequence and a complementary sequence at the 5' end of U₁ snRNP. After U₂ snRNP binds to the branch point by a similar mechanism, a complex consisting of U₄/U₆ and U₅ snRNPs is then incorporated into the spliceosome.

Because most pre-mRNAs contain multiple introns, different mRNAs can be produced from the same gene by different combinations of 5' and 3' splice sites. The possibility of joining exons in varied combinations provides exponential ways of getting mature RNA from the same pre-mRNA. This process, called alternative splicing, increases the diversity of proteins that can be expressed from a single transcription unit. Alternative splicing plays an important role in the gene-expression process, and it is estimated that at least 35% of all human genes are alternatively spliced (10).

Although the exact mechanism of splicing is unknown, it has been shown that several proteins contribute to the selection of a splice site and can affect the use of an alternative splice site in a pre-mRNA molecule. Sequence-specific RNA-binding proteins, for example, have been shown to bind near specific splice sites to either inhibit or activate splicing to the nearby site. Because transcriptional activators consist of DNA-binding domain and activation domain, which are generally encoded in separate exons, alternative splicing enables the production of many activators and repressors from the same gene. It has been shown that alternative splicing can be modified by using antisense oligonucleotides, and this provides a very promising potential chemotherapeutic target for cancer and other proliferative diseases (10).

2.2.3. Control of mRNA Degradation

In mammalian cells, the levels of both rRNA and tRNA are relatively stable, whereas the stability of different mRNAs may vary greatly. These unstable mRNAs often code for regulatory proteins whose levels need to be changed rapidly to respond to various signals. For example, the hormone lymphokine produced by lymphocytes, which coordinate cell-cell interactions between the cells involved in the immune response of mammals, are synthesized and secreted in short bursts. Correspondingly, the mRNAs encoding these proteins must be synthesized and degraded in a very short period of time, and the switches in this controlling machinery must be turned on and off promptly.

The degradation of most eukaryotic mRNAs is completed by a gradual shortening of their poly-A tails. It has been demonstrated recently that some small RNAs contribute to mRNA degradation in RNA interference (RNAi). The exact mechanism about this process is still unknown, but scientists believe that enzyme complex called RISC uses the sequence information in small RNAs (miRNAs and siRNAs), which are combined to identify and degrade mRNAs with complementary sequence (8).

The degradation of mRNAs can also be affected by extracellular signals. For example, mRNA encoding for transferrin receptors is regulated by the iron level within the cell. In the presence of adequate amounts of iron, transferrin receptor mRNA is degraded rapidly as a result of specific nuclease cleavage at a sequence near its 3' end.

Otherwise, the mRNA is stabilized, resulting in increased synthesis of transferrin receptor and more iron uptake by the cell. This regulation is mediated by a protein that binds to specific sequences (called the iron response element [IRE] near the 3' end of transferrin receptor mRNA, inhibiting the mRNA from cleavage.

2.2.4. Translational Control

Translation completes the flow of genetic information within the cell from DNA to protein. It is a complicated procedure wherein the information encoded in RNAs is translated into proteins. The translation process generally is divided into three stages: initiation, elongation, and termination. Ribosomes, which are composed of proteins and rRNAs, are the sites of protein synthesis for mammalian cells. During translation, the rRNA plays the dominant role in determining the overall structure of the ribosome, forming the binding sites for the tRNAs, matching the tRNAs to codons in the mRNA, and providing the peptidyl transferase enzyme to link amino acids together during translation.

Translational control can be achieved by binding repressor proteins, which block translation to specific mRNA sequences. It has been shown that malignant transformation in cancer could be caused by the increased translation of a subset of mRNAs encoding important proteins for cell growth and proliferation, which usually possess regulatory sequences that render their translation more sensitive to changes in the activity of translation initiation factors. By repressing the translation of the mRNA, that is required for cancer development, cancer cell growth and proliferation could be effectively inhibited. It has been reported that translational control in cancer cells could be an excellent target for anticancer drug development (11).

2.2.5. Posttranslational Control

One important function of proteins is to serve as enzymes, and the regulation of enzyme activity plays an important role in governing cellular behavior. The catalytic activity of enzymes can be altered by changing their conformations. This can be achieved by binding small molecules, such as amino acids or nucleotides, to regulate enzyme activity. Protein activity can also be regulated by the interactions between the polypeptide chains that form proteins, and many activators and repressors function through protein-protein interactions. Proteins are targets of most traditional drugs, which use small molecules to inhibit functions of particular proteins (12).

Just as with other molecules in the cells, the levels of proteins are determined by the difference between the rates of synthesis and the rates of degradation. Different rates of protein degradation affect the behavior of the cells, serving as an important factor for cell regulation. For example, regulatory molecules such as transcription factors usually have shorter half-lives to allow their levels to change quickly in response to various signals from both the inside and the outside cells. The ubiquitin/proteasome-dependent protein degradation pathway plays an essential role in both cell proliferation and cell death in human cancer cells (13). The knowledge that proteasome function is required for tumor cell survival has prompted the design, synthesis, and evaluation of various pharmacological proteasome inhibitors. Both in vitro and in vivo experimental results have demonstrated the potential use of proteasome inhibitors as novel anticancer drugs.

2.2.6. Expression of Receptor Protein and Drug Targeting

It is known that many cancer cells overexpress certain cell-surface receptors, including transferrin receptor (14), folate receptor (15), and low-density lipoprotein (LDL)

receptor (16,17), to meet the increased cell proliferation and growth requirements. One strategy of developing targeted drug delivery for cancer therapy is to take advantage of these overexpressed cell-surface receptors. By incorporating the corresponding ligands for the cancer cell-surface receptors onto drug or drug carriers, an anticancer drug can be specifically delivered to the cancer site.

Tumor growth and development is related closely to tumor neovascularization, the growth of new blood vessels. The endothelial cells play a very important role in this process, and therefore, they are attractive targets for cancer therapy. More importantly, angiogenic endothelial cells overexpress certain proteins on the cell surface, which can be recognized only by a certain peptide sequences (18,19). Thus, targeting angiogenic endothelial cells may become a very promising strategy in the treatment of cancers.

3. Cellular Defensive Response Systems

3.1. Human Immune System

Our body, like other living organisms, is in an environment that encounters many foreign invaders. Most of the foreign invaders that confront the human immune system are microscopic pathogens, including fungi, parasites, bacteria, and viruses. All foreign microbes in the human body display special markers, and it is these special markers that can be recognized as harmful and identified for destruction by the immune system. In order to survive in an environment with various types of pathogens, the body must have well-developed mechanisms in place to resist the infection by pathogens. These defenses can be categorized into two types: innate immune responses and adaptive immune responses (20).

Innate immunity refers to antigen-nonspecific defense mechanisms and is the immunity with which one is born. It serves as the first line of defense for the body and is switched on immediately after an infection begins; it does not depend on the host's prior exposure to the pathogen. Adaptive immunity, however, operates later in an infection, is highly specific to the pathogen that has induced it, and is much more powerful. However, the adaptive immune responses are slow to develop on first exposure to a new pathogen. It may take a week or so before the responses are effective. The key difference between the two immune responses is that innate immune responses are not specific to a particular pathogen, whereas adaptive immune responses are.

The function of immune system is to remove or destroy invading pathogens and any toxic molecules they may produce. It is a crucial task for a healthy immune system to be able to determine what is foreign and what is its own. This applies to both innate and adaptive immune systems, although it is more important to the adaptive immune system, which is both more powerful and more destructive. Occasionally, the system fails to make this distinction and reacts destructively against the host's own molecules. Such autoimmune diseases can be fatal. On the other hand, tolerance can be problematic in some other cases like cancer. To the immune system, the difference between a cancer and a normal cell is so small that the immune system largely tolerates cancer cells rather than attacking them. In order to use the immune system to attack cancer cells, stimulations must be made to the immune system strongly enough to overcome this tolerance. Although the relation between the cancer and the immune system is still not clear, it is believed by most scientists that the cancer cells either have generated tolerance in the immune system or have developed ways of resisting immune recognition. In terms of cancer treatment, we need to identify ways to break the tolerance or circum-

vent resistance mechanisms (21). Cancer vaccine is such a solution and will be discussed in later section.

3.2. Innate Immune System

Innate immunity consists primarily of a chemical response system, including complement, endocytosis, and phagocytosis. Macrophages, for example, detect and engulf extracellular molecules and materials, clearing the system of both debris and pathogens. The innate immune responses in vertebrates are also required to activate adaptive immune responses.

Innate immune responses rely on the body's ability to recognize conserved features of pathogens that are not considered as self, because the pathogen surface contains various classes of common pathogen-associated immunostimulants, such as many types of molecules on microbial surfaces and the double-stranded RNA of some viruses (1,2). These molecules can be recognized by some dedicated receptors in the host, which are collectively called pattern-recognition receptors. The cell-surface receptors have two functions: (1) to initiate the phagocytosis of the pathogen, and (2) to stimulate innate immune responses.

Phagocytic cells display a variety of cell-surface receptors that enable them to recognize and engulf pathogens. These include pattern recognition receptors such as toll-like receptors (TLRs). There are two kinds of phagocytic cells: macrophages and neutrophils. Macrophages are usually long-lived cells residing in tissues throughout the body. They patrol the tissues of the body and are among the first cells to encounter invading microbes. Neutrophils, on the other hand, are short-lived cells that are abundant in blood but are not present in healthy tissues. They are rapidly recruited and dispatched to sites of infection by both activated macrophages and molecules released by the microbes themselves. Phagocytic cells trigger inflammatory responses to help fight infection and begin to activate the adaptive immune system. The adaptive immune system plays a major role after this point.

In recent years, macrophage-mediated gene delivery has been studied for cancer treatment by arming macrophages with the ability to express a therapeutic gene. In experiment, a hypoxia-regulated adenoviral vector was used to transduce human macrophages with either a reporter or a therapeutic gene encoding human cytochrome P4502B6. Infiltration of transduced macrophages into a tumor spheroid results in the induction of gene expression. They have significant tumor cell-killing ability in the presence of cyclophosphamide via activation by P4502B6. It has also been shown that this can be further targeted to tumors through hypoxia-regulated gene expression (22).

It has been found that macrophages play very important roles in human immunodeficiency virus (HIV) dissemination to the whole immune systems. When they are infected by HIV, they stay alive, allowing HIV to live and replicate for a long time. Because macrophages express scavenger receptor proteins such as modified LDL receptor in the cell surface (23), they have been considered to be very important therapeutic target for HIV infection. During the development of disease atherosclerosis, lipid-loaded macrophages appear in the blood vessel wall. Because of the scavenger receptors in these cells, they can serve as ideal therapeutic targets for this disease.

3.3. Adaptive Immune System

The adaptive immune system is a more advanced and powerful system than the innate immune system. The adaptive immune system is called on by the innate immune

system to respond to pathogens. Unlike innate immune responses, the adaptive responses are highly specific to the particular pathogen that induced them, and the protection is also long-lasting. There are two broad classes of such responses: antibody responses and cell-mediated immune responses, which are carried out by different classes of lymphocytes, called B cells and T cells, respectively.

In the process of immune response, the pattern-recognition receptors present on the surface of various types of host cells activate intracellular signaling pathways in response to the binding of pathogen-associated immunostimulants. This leads to the production of extracellular signal molecules that promote inflammation and help activate adaptive immune responses if needed.

3.3.1. B Cells and Antibody-Mediated Drug Targeting

B cells respond to antigens by secreting antibodies. In antibody responses, the antibodies circulate in the bloodstream and permeate to other body fluids, where they bind specifically to the foreign antigen that stimulated their production. Binding of antibody inactivates viruses and microbial toxins by blocking their ability to bind to the receptors on host cells, thus blocking viruses from entering cells. Antibody binding also marks invading pathogens for destruction by making it easier for phagocytic cells of the innate immune system to ingest them.

There are two phases in the development of B cells: (1) the antigen-independent phase and (2) the antigen-dependent phase. The first phase involves the generation of diversity and the acquisition of rearranged heavy (H)- and light (L)-chain genes for synthesizing IgM antibody as a surface receptor protein. In the antigen-dependent phase, a B cell uses one of its receptors to bind to its matching antigen, which the B-cell engulfs and processes. The B cell then displays a piece of the antigen, bound to a class II major histocompatibility complex (MHC) protein on the cell surface. The whole complex binds to an activated helper T (Th) cell. This binding process stimulates the transformation of the B cell into an antibody-secreting plasma cell (Fig. 3).

Each B-cell clone makes antibody molecules with a unique antigen-binding site. Initially, during the development of B cell in the bone marrow, the antibody molecules are inserted into the plasma membrane, where they serve as receptors for antigen. In peripheral lymphoid organs, antigen binding to these receptors, together with costimulatory signals provided by Th cells, activates the B cells to proliferate and differentiate into either memory cells or antibody-secreting effector cells. The effector cells secrete antibodies with the same unique antigen-binding site as the membrane-bound antibodies. There are five classes of antibodies in mammals, namely IgM, IgD, IgG, IgA, IgE, each of which mediates a characteristic biological response following antigen binding.

Because antibodies are the product of immune response of B-cells to immunogens, antibodies can be used either as therapeutics or ligands for drug targeting. Because proteins like Her2 are abundant on surface of cancer cells, antibodies against such proteins can be armed with a toxin, or made to carry an enzyme that cleaves a harmless “prodrug” into a toxic molecule. The second case is especially useful because one molecule of enzyme can then generate a large number of toxic molecules. One virtue of this strategy is that the toxic drug generated enzymatically can then diffuse to neighboring tumor cells, increasing the odds that they can be killed, even if the antibody did not bind to them directly (1,2).

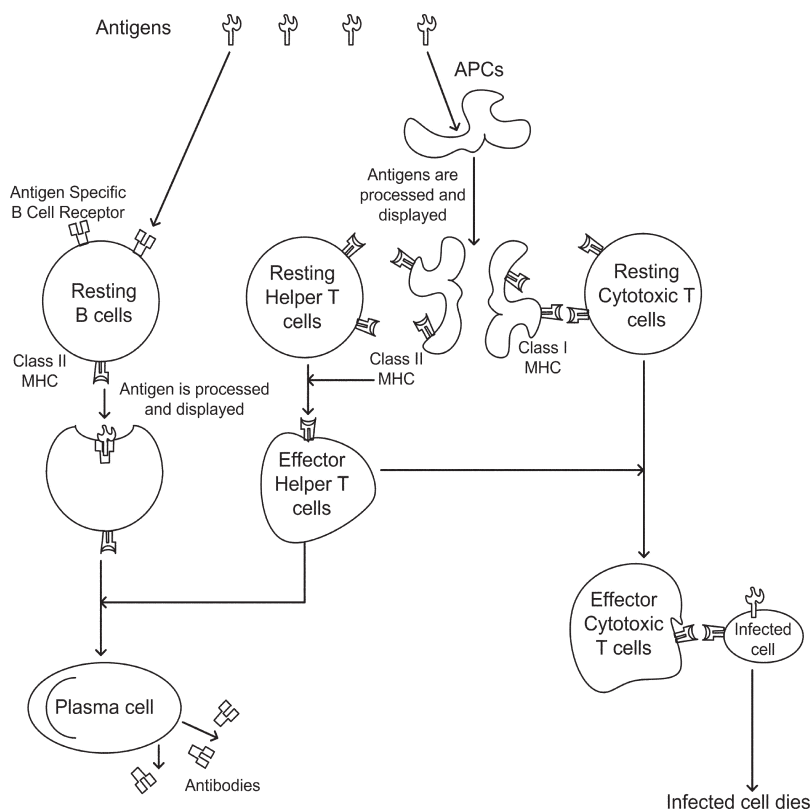


Fig 3. Activation of B cell and T cell.

3.3.2. T Cells in the Adaptive Immune System

The diverse responses of T cells are collectively called cell-mediated immune reactions. Most adaptive immune responses, including antibody responses, require Th cells for their initiation. Most importantly, unlike B cells, T cells can help eliminate pathogens that reside inside host cells. Activated T cells react directly against a foreign antigen that is presented to them on the surface of a host cell. For example, killer T cells possibly kill a virus-infected host cell, thereby eliminating the infected cell before the virus has a chance to replicate. T cells are also responsible for orchestrating, regulating, and coordinating the overall immune response. For example, Th cells alert B cells to start making antibodies (Fig. 3).

Like antibody responses, T-cell responses are exquisitely antigen-specific. But T-cell responses differ from B-cell responses in at least two crucial ways. First, they differ in their mechanism of antigen recognition. T cells depend on antigen-presenting cells (APCs) in peripheral lymphoid organs that present antigen to them. Protein antigens are partly degraded inside the APC, and are then carried to the surface of the presenting cell on special molecules called MHC proteins, which bind the fragments and present them to T cells. The second difference is the mechanism that T cells degrade the antigen. Once activated, effector T cells act only at short range, either within a secondary lymphoid organ or at the site of infection. They interact directly with other

cells in the body, during which they either kill or signal in some way. Activated B cells, by contrast, secrete antibodies that can act far away.

Based on their functions in the immune system, T cells can be categorized into two classes: cytotoxic T cells and Th cells. The former kill infected cells directly by inducing them to undergo apoptosis, whereas the latter help activate B cells to make antibody responses and macrophages to destroy microorganisms. The Th cells also stimulate the activation of cytotoxic T cells. They are dependent on the unique cell-surface molecules, MHC, to help them recognize antigen fragments. They have receptors on their cell surface similar to antibodies, which recognize fragments of foreign proteins that are displayed on the surface of host cells in association with MHC proteins. Both are activated in secondary lymphoid organs by APCs, which express peptide-MHC complexes, costimulatory proteins, and various cell-cell adhesion molecules on their cell surface. Two kinds of MHC proteins, MHC-I and MHC-II, play crucial roles in presenting the antigens molecules to the cytotoxic T cells and Th cells, respectively. MHC-I are expressed in almost all vertebrate cells, whereas MHC-II are normally expressed in those cells interacting with Th cells, such as dendritic cells (DCs), macrophages, and B lymphocytes (12).

MHC proteins have a single peptide-binding groove, which can bind a large and characteristic set of small peptide fragments derived from proteins. After they have formed inside the target cell, the peptide-MHC complexes are transported to the cell surface. Complexes containing a peptide derived from a foreign protein are recognized by T-cell receptors, which interact with both the peptide and the walls of the peptide-binding groove. The MHC molecules appear to be the keys to understanding and manipulating T cells, including both T-4 and T-8 cells. Based on the knowledge concerning the presentation of peptides by MHC molecules, a reliable method for predicting peptides that can bind to MHC, thus inducing T-cell immunity, can be of great value in modulating immune responses.

T cells play very important roles in human immune systems. However, they are subject to attack by foreign invaders, e.g., T-4 cells are the primary target of the HIV-1 virus. Because T-4 cells are responsible for the effective immune activity of macrophages, B lymphocytes, and other T lymphocytes, when they are depleted because of the HIV infection, the patient develops acquired immune deficiency syndrome (AIDS). The intensive study of the HIV replication has provided insight into the fundamental cellular mechanisms. One very important example is the discovery of protein TAT, an 86 amino acid transcriptional activator protein. TAT can be synthesized in one HIV-infected cell and transited into a neighboring cell without the requirement for the receptor-mediated event. This phenomenon has provided a great advantage to use TAT or fragment of TAT for efficient gene and drug delivery (24,25).

3.3.3. Diversity and Specificity of the Adaptive Immune System

The most remarkable feature of the adaptive immune system is that it can respond to millions of different foreign antigens in a highly specific manner. There are two important issues related to adaptive immune system: (1) the generation of diversity, and (2) the specificity of the antibody and MHC molecules.

The immune system has the capacity to recognize and respond to a large amount of antigens. This extreme diversity can be generated in at least three possible ways: (1) multiple genes in the germ-line DNA, (2) variable recombination during the differen-

tiation of germ-line cells into B cells, and (3) mutation during the differentiation of germ-line cells into B cells. The diversity of MHC molecules is generated in a similar process.

Once the diversified antigen-binding receptors are generated, how does the immune system pick the appropriate ones to respond to a specific antigen? This can be explained by the clonal-selection theory. The adaptive immune system is composed of millions of lymphocyte clones, with the cells in each clone sharing a unique cell-surface receptor that enables them to bind a particular antigen. With the binding of antigen on the cell-surface receptors, the lymphocytes proliferate and differentiate into functional lymphocytes. Lymphocytes have three possible fates: (1) some lymphocytes proliferate and differentiate into memory cells, which are able to respond faster and more efficiently the next time the same pathogen invades to prevent the body from the same illness in the future; (2) lymphocytes that would react against self molecules are induced to alter their receptors, induced to kill themselves, inactivated, or suppressed, ensuring self-tolerance; and (3) not to react at all, and subsequent death. In this process, both B and T cells circulate continuously between the blood and lymph until they encounter their specific foreign antigen in a peripheral lymphoid organ. After that, they proliferate and differentiate into effector cells or memory cells (26).

3.4. Artificially Acquired Immunity

Based on the key properties of the immune system, we can defend our body using artificially acquired immunity. We can get the immunity artificially either passively or actively. Passive immunity is usually achieved by injecting antibodies into the body. For example, administering tetanus antitoxin to a patient is a way of conferring passive immunity (27). Passive immunization is effective very quickly, but it lasts only a short time. It is mainly used to protect people when they are particularly vulnerable, such as immediately after exposure to a serious disease.

The purpose of vaccines is to stimulate the immune response like antibody formation or T-cell responses without subjecting a person to the risk of actual infection (27). Most traditional vaccines rely on the vaccine's capacity to evoke antibody formation, but more attention is paid to vaccines evoking T-cell responses, especially for HIV vaccines and cancer vaccines. Recent developments in vaccine research have provided many ways to obtain active immunity, including conjugate vaccines, subunit vaccines, recombinant vector vaccines, naked DNA techniques, and vaccine presentation (27). Conjugate vaccines are used to deal with certain bacteria having special outer coats that disguise antigens so that the immature immune systems are unable to recognize these harmful bacteria. Proteins or toxins from a second type of organism that is easier for an immature immune system to recognize are linked to the outer coats of the disease-causing bacteria. This enables an immature immune system to respond and defend against the disease agent. Subunit vaccines can be made by using a fragment of the microbe, because the fragment of the microbe is able to trigger the immune system while having far fewer side effects. Subunit vaccines made from *Streptococcus pneumoniae* have been used to protect against pneumonia. A recombinant subunit vaccine for hepatitis B virus infection made by inserting a tiny portion of the hepatitis B virus' genetic material into common baker's yeast is also in clinical trial.

A vaccine vector is a weakened virus or bacterium into which harmless genetic material from another disease-causing organism can be inserted. The vaccinia virus, for example, can be used to make recombinant vector vaccines because it is relatively

large and has ample room to accept additional genetic fragments. A vaccinia virus with several genes from the HIV is currently being tested as a vaccine against AIDS. In addition, naked DNA technique has also been used to obtain active immunity. This is achieved by incorporating “naked DNA” encoding certain proteins from a disease-causing organism into the body’s own cells. The proteins encoded by the DNA work as antigens to stimulate the immune system. In this way, the DNA will have an effect similar to that of a live, attenuated vaccine, thus produces antigens for years. The exclusion of genes critical to the disease-causing organism’s survival also assures that the vaccines are safe and do not actually cause disease.

Vaccine presentation deals with presenting the vaccine to the immune system, which is closely related to drug delivery. Microspheres—tiny spheres containing bits of antigenic material—show promise in that they can release small doses of vaccine over extended periods of time as they gradually dissolve in the body. This makes two or more doses of vaccine in one administration possible.

3.4.1. HIV Vaccine

The focus of HIV vaccine research has progressed from HIV surface antigens and the role of antibodies in early stages to the importance of cytotoxic T cells. Subunit vaccines for HIV based on viral-surface proteins such as gp120 have the advantage of being safe and simple to prepare. It remains to be confirmed whether these vaccines can elicit antibodies capable of neutralizing primary HIV isolates. A vaccine candidate based on gp120 from two different HIV clades recently entered Phase III testing in the United States. Vectored vaccines employ non-HIV viruses (e.g., avian pox viruses) engineered to carry genes encoding one or more HIV epitopes. A related vector comparison study is ongoing and is evaluating three potential products to determine which vector produces the most robust immune response. By combining a canarypox-vectored product with a subunit vaccine, a hybrid vaccine has been studied to determine if a robust cellular and humoral response to HIV can be elicited. In addition, attenuated live or whole-killed HIV vaccines have shown promise in nonhuman primates.

3.4.2. Cancer Vaccines

Cancer vaccines work primarily to prevent cancer from recurring by alerting the body for certain characteristics of cancer cells and killing the remaining cancer cells. Although there are some promising results reported, the research in this area is still in a very early stage. It has been shown that the most effective anti-tumor immune responses are achieved by stimulating T-cells, which can recognize and kill tumor cells directly.

Cancer vaccine can be produced from cancer cells, parts of cells, or pure antigens (27). Tumor-cell vaccines use killed cancer cells removed during surgery. The antigens on the killed tumor-cell surfaces can stimulate a specific immune-system response. As a result, cancer cells carrying these antigens are recognized and attacked. To increase the effectiveness of the vaccine, killed tumor cells may be further enhanced with non-specific adjuvants. The advantages of using whole tumor cells is that they may expose the immune system to a large number of important cancer antigens, some of which may have not yet been identified. There are two basic kinds of tumor-cell vaccines: autologous and allogeneic. The former takes advantage of the tumor cells from the patient itself, whereas the latter utilizes cells from someone else (28).

Dendritic cells are the most important APCs. When exposed to a foreign molecule, DCs can take up the foreign substance and display it, and stimulate the immune

responses. This process is rather specific, as each patient's own DCs must be used as the foundation of the vaccine. It was shown recently at the Duke Comprehensive Cancer Center that RNA can be used instead of protein to make dendritic vaccines, which have dramatically expanded the scope of cancer vaccines, thus making the technology broadly applicable (29).

By creating antigens that are easier for the immune system to recognize, antigen vaccines stimulate the immune system using individual antigens, rather than the whole tumor cells that contain many thousands of antigens. Although antigen vaccines may be specific for a certain type of cancer, they are not patient-specific like cell vaccines. Although a large amount of work needs to be accomplished before making significant progress, cancer vaccine holds promise in the battle with cancers.

4. Concluding Remarks

As the basic structural and functional unit of all living organisms, the cell is very complicated machinery with highly ordered structure and precisely controlled biological processes. Any deviation of the cell structure or function from the normal state can cause disease in the human body. In order to correct the cellular malfunction, drugs must be delivered efficiently to the cells to produce a pharmacological effect. Understanding the cell cycle, growth control, and cellular defensive response becomes essential for the design and development of therapeutics for targeted cellular drug delivery. The rapid development in human genomics, cell biology, and molecular biology will lead to the complete understanding of various cellular processes, and more and more targeted drug delivery systems will be developed in the near future.

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