1-1- Vasculogenesis

The cardiovascular network is the first system that develops in the embryonic gastrula stage. The first organization of endothelial cells that leads to the creation of blood vessels is called tissue vascular expansion or vasculogenesis, and before that there is no other vascular system[1] (1).

1-2- Angiogenesis

1-2-1- Definition of angiogenesis

The growth of new capillaries from existing blood vessels is called angiogenesis, which includes a series of cell events that lead to new vascularization.

Angiogenesis can be seen in physiological conditions such as wound healing, embryo formation and development, menstrual cycle, etc., as well as pathological conditions such as cancer and numerous ischemic and inflammatory diseases[1] (1). The fact that tumor growth is dependent on new blood vessels leads to the investigation of the chemical factors that determine angiogenesis so that we can find new possibilities for the diagnosis and treatment of these diseases[2] (2).

Angiogenesis is regulated by both activating and inhibitory molecules. In the normal state, the inhibitors are dominant and prevent the growth. There must be a need for new blood vessels so that the number of angiogenic activators increases and inhibitors decrease. This causes the growth and division of vascular endothelial cells and finally new blood vessels are formed. The ability of cancer to spread to other organs of the body makes it a life-threatening factor. Tumor cells can penetrate blood and lymph vessels and circulate through the intravascular flow and then multiply in another area, which is called metastasis. For tumor metastasis, the growth of the vascular network is important. As mentioned, the processes in which new blood and lymphatic vessels are formed are called angiogenesis and lymphatic angiogenesis, respectively. Both of these processes play an essential role in the formation of a new vascular network to provide food, oxygen and immune cells, as well as to dispose of waste materials[3] (3).

1-2-2- Angiogenesis in cancer

The growth of solid tumors is completely dependent on the creation of new vessels and meeting the nutritional needs of the tumor. Special compounds (angiogenic factors) are released into the environment by tumor cells and stimulate different types of normal cells. Among the cells stimulated in this way are the endothelial cells of the adjacent capillaries, during which they break down their base membrane and separate from the neighboring cells and enter the extracellular matrix, towards the mass. Tumors migrate. Along with these changes, cell division also happened in the bud, and with the increase in the migration of endothelial cells, a string of these cells is formed and the inter- and intracellular basement membrane evolves. It is useful to consider that the endothelial cells of the blood vessels reproduce on average only once every 1000 days. The rings of these hollow threads communicate with each other and form the structure of new vessels, which are finally connected to the blood circulation system. In this way, the capillary network is created in the tumor mass and can continue to grow[3] (3).

On the other hand, the growth of tumors causes advanced hypoxia and acidosis. Tumor vessels do not have the normal function of the vascular system and create a positive feedback for continuous tumor proliferation, which leads to continuous hypoxia and angiogenesis[4] (4). Generally, angiogenesis is regulated by two types of activating and inhibitory molecules. However, increasing the activity of angiogenic factors by itself is not enough to cause angiogenesis, and in addition, it is necessary to reduce the activity of angiogenesis inhibitors[3] (3).

In 1971, Falkman published a hypothesis that tumor growth is dependent on angiogenesis and by inhibiting angiogenesis, the stage of treatment can be reached[5] (5). This article also introduced the term anti-angiogenesis with the meaning of inhibiting the sprouting of new vessels from the existing vessels by a tumor. This hypothesis predicts that tumors are able to advance more than 1-32 mm without creating new capillary blood vessels, which concept is now accepted due to confirmation by laboratory study data and clinical observations[6] (6).

Growth arrest of micrometastases may be governed by angiogenesis. As Falkman and colleagues found, when a primary tumor is transplanted into a mouse, metastasis is not observed until new vessels are allowed to form. In the absence of angiogenesis, micrometastases rarely reach a diameter greater than 0.2 mm and the number of proliferating tumor cells is in balance with the number of apoptotic cells. When angiogenesis takes place, the growth of micrometastases becomes faster, in principle, growth arrest is a state of tumors in which inhibition of angiogenesis leads to the balance of tumor cell proliferation and apoptosis[7] (7).

1-2-3- The importance of investigating angiogenesis in cancer

Due to the fact that the increasing resistance of cancers to common treatments has become a troublesome problem, efforts are being made to discover and identify new anticancer agents that increase the sensitivity of cancer cells. The resistance of cancer cells to chemical drugs leads to a decrease in the response level of these cells to the drug and as a result to the failure of treatment measures. Therefore, research and development of more effective drugs or with less side effects is of increasing importance. Folkman was one of the first researchers who proposed the use of inhibiting the formation of tumor vessels for cancer treatment. His suggestion and other researchers have led to the development of clinical research and investigation of more than 20 different drugs that inhibit various stages of vasculitis. Among the potential advantages of this type of treatment that can be mentioned include easy access to intravascular targets, the absence of the problem of tumor cell resistance in comparison with conventional chemotherapy against cancer, and also the widespread use of this type of strategy to treat many types of angiogenesis-related diseases. is. Therefore, the vascular inhibition strategy is important in the sense that cancer cells may show less resistance to treatment with this method, because this issue is directly and more related to the stroma and not to the tumor cells, which are genetically unstable. The design of this idea is based on the fact that the processes used by endothelial cells in vascularization are not the result of genetic changes in the activity of the oncogene suppressor gene of endothelial cells. In other words, the absence of drug resistance to angiogenesis inhibitors in the antiangiogenesis process is most likely due to the fact that endothelial cells are genetically active and do not mutate into drug-resistant forms. Although this type of treatment is very promising, lack of resistance strongly depends on the type of angiostatic treatment used.

Considering what was mentioned and the importance of angiogenesis in research related to the discovery and identification of angiogenic factors and angiogenesis inhibitory factors for the treatment of various diseases including types of tumors that are closely related to angiogenesis and are dependent on it, the methods of angiogenesis inhibition that aim interfere with this important process, they are considered a promising path for the treatment of diseases related to angiogenesis. Based on this, the development and use of different models of angiogenesis for this purpose becomes more important to the point that many researchers around the world benefit from different models of angiogenesis to study this important phenomenon and its influencing factors[8] (8).

1-2-4- Hypoxia in tumors

All eukaryotic organisms require oxygen to provide oxidative phosphorylation (pO2) to produce adenosine triphosphate (ATP). Therefore, a constant supply of nutrients by the vascular system in mammals is essential for development, homeostasis and tissue function. Tissue oxygenation is managed by the balance between the oxygen supply from the vessels and the metabolic demand of the tissues. Hypoxia occurs in conditions where there are changes in the amount of oxygen source and the amount of tissue oxygen demand, which is seen in both healthy developing tissues and human diseases. Abnormal functioning of blood vessels due to vessel blockage or fracture can reduce oxygen delivery, and this is a pathological reason for diabetic retinopathy, peripheral vascular disease, and ischemic heart disease. Contrary to this situation, rapid cell division during embryonic period or tumor growth can increase oxygen demand due to increased metabolism and cause chronic hypoxia[9] (9). Hypoxia occurs in tumors in two ways. The first form is chronic hypoxia as a result of long diffusion distance between tumor vessels. The second form of acute hypoxia is related to the temporary blockage of tumor vessels.

Cells that are chronically hypoxic are placed in an environment where pO2 is less than 10 mmHg for several hours, and such cells may never reach normoxic conditions when hypoxic. Cells become acutely hypoxic when they are kept in an environment where pO2 is less than 10 mmHg for many minutes[10] (10).

Note: It is important to consider that hypoxia in biological systems is relative because the oxygen concentration changes from one tissue to another in a normal physiological state. For example, arterial blood has a pO2 of 14%, myocardium muscle has a pO2 of 10%, and other tissues often have a pO2 of 5%. Also, cartilage, bone marrow and thymus are areas with less than 1% oxygen. These observations show that hypoxia is not only not always a pathological component, but in many cases it is an important component for cellular structures, especially in the case of stem cells and reproduction. However, low oxygen and its molecular consequences play an important role in the pathogenesis of many diseases[9] (9).

1-2-4-1- source of chronic hypoxia

The balance between the amount of delivered and consumed oxygen determines the tissue oxygen concentration. Several factors play a role in chronic hypoxia in tumors, including the longitudinal gradient of pO2, irregular distribution of vessels, low density of vessels, and changes in blood viscosity. In this case, oxygen consumption is more than the amount that reaches the tissue, and among these factors, oxygen consumption has a more dynamic effect on tissue pO2[10] (10).

1-2-4-2- Change in blood viscosity

It has been observed that vascular hypoxia in tumors is responsible for the change in blood viscosity, which causes slow blood flow in tumors. Using the specified viscometer, when pO2 decreases below 10 mmHg and pH below 6.8, the viscosity of the suspension increases by about 40%, and this increase in viscosity is caused by the shrinkage of red blood cells. This increase in viscosity causes a decrease in blood flow, which subsequently aggravates vascular hypoxia.

1-2-4-3- Dispersion of vessels

As mentioned before, chronic hypoxia occurs because the distance between vessels in tumors is too large, but the irregular distribution of the vascular network is also important. The existence of a disorder in the vascular network leads to tumor vessels having a large amount of plasma but not enough red blood cells, which is due to the abnormal angle of branching and the altered flow of red blood cells, and on the other hand, the arteriole feeding the tumor is relatively It has less oxygen than normal arterioles, and the pO2 of tumor arterioles is 60% of normal arterioles. The relative reduction of oxygen in these arterioles is due to the acidic pH of the tissue and the tendency to the right of the hemoglobin saturation diagram of the tissue arteriole, which has acidosis, and because the arteriole passes through the area with high proliferation, the rate of oxygen consumption is also higher[11] (11).

1-2-4-4- acute hypoxia

Acute hypoxia occurs as a result of temporary blockage of blood vessels, which is caused by one of the following three reasons, i.e. vascular collapse, accumulation of leukocytes or accumulation of tumor cells inside the vessels. Although these factors may play a role in vascular occlusion, they are not the only main cause of acute hypoxia. Instability of blood flow in tumors and vascular collapse can lead to temporary hypoxia, and interestingly, many genes involved in angiogenesis are regulated by hypoxia[12] (12).

1-2-4-5- The effect of hypoxia on angiogenesis

In the initial phase of malignant progression, in an avascular area of the body, the tumor remains in a state of arrest in which the rate of cell proliferation is equal to the rate of cell apoptosis. Then the tumor undergoes an event called angiogenesis, which means passing from a state without angiogenesis to a state with active growth of vessels. Angiogenesis is very important for tumor growth and supply of food and oxygen to tumor cells. In fact, preventing angiogenesis has been effective in controlling tumor growth in animal models. In contrast, reduction of intrinsic angiogenesis inhibitors such as thrombospondin-1 and thrombospondin-2 accelerated tumor growth of an in vitro model of spontaneous colorectal cancer as well as chemically induced skin cancer. Therefore, angiogenesis is critical for both tumor production and progression.

Despite active angiogenesis, tumor vessels are highly irregular and leaky and have poor function. This characteristic leads to the creation of hypoxic regions and the stabilization of hypoxia-inducible factors (HIF-α) (transfer factors that respond to changes in the oxygen level of the cellular environment to reduce hypoxia), in tumors and even in vascularized tumors. It increases. In addition, factors associated with malignant transformation can stabilize hypoxia-dependent HIF-α. Hypoxia and HIF activity affect tumor biology, for example, HIF-1α and HIF-2α expression is associated with poor prognosis and metastasis of several cancers such as brain, breast, colon, head and neck, liver, lung, skin, and pancreas. Was. This is partially due to tumor treatment resistance, for example, hypoxic tumors are resistant to radiation therapy because O2 molecules are necessary to produce the toxic effect of ionizing radiation. Also, chemotherapy in hypoxic tumors remains ineffective due to poor drug delivery to the tissue and the expression of ATP-dependent drug-releasing pumps that are regulated by HIF. Hypoxia and activation of HIF pathways in tumor cells are good stimuli for blood vessel growth. In fact, HIF-1α and HIF-2α express proangiogenic genes including Vegf, Ang-1, Ang-2 and Tie-2. And they also regulate genes that are used as biomarkers to determine tumor hypoxia. In fact, HIF-1α directly regulates Vegf expression in Hepa-1 xenograft tumors in vivo[9] (9).

Almost all angiogenic agents promote the growth of new vessels in the same way. They cause new vessels to sprout from other small vessels. As mentioned before, the first step is to degrade the basement membrane of the endothelial cells at the site of budding. Then the new endothelial cells multiply rapidly and are pulled out from the vessel wall in the form of ropes and move towards the origin of the angiogenic factor. The cells in each cord continue to divide and quickly form a tube. Then this tube joins another tube and forms a capillary ring in which blood starts to flow[13] (13).

1-2-5- The role of tumor cells in angiogenesis

Tumor cells are able to contribute to the induction of angiogenesis by several different mechanisms. First, tumors continue to grow by inducing angiogenesis, and increased angiogenesis activity is the first finding in many preneoplastic cells and may be the first result for the cell population to become malignant. Second, tumor cells usually have the ability to produce inhibitors. They lose normal angiogenesis with or without increasing angiogenic factors. There is now evidence that one of the primary changes in cells undergoing neoplastic changes is that they lose the production of internal inhibitors of angiogenesis. Third, tumor cells are able to recruit host defense cells such as macrophages, which are a common cell in neoplastic stromal tissues. When these defense cells are activated, they are able to stop cell growth or destroy neoplastic cells. In vivo and in vitro studies show that activated macrophages have antitumor activity and have various functions in tissue regeneration, inflammation and immunity, but the analysis of the function of tumor-related macrophages suggests that these cells have a great impact with several functions. They affect the progress and growth of the tumor, which includes the creation of vessels, the speed of tumor growth and metastasis, and restructuring of the stroma. The fourth mechanism is that tumor cells divert the host's defense system, which is an obstacle against angiogenesis. A good example for this mechanism is the kapposis sarcoma tumor. One of the normal endothelial changes compared to these tumors is the reduction or lack of activity of angiogenesis inhibitors by these tumor cells, and one of the inhibitors that is reduced in this type of tumor is thrombospondin-1 (TSP-1). Is. Therefore, when there is no inhibitory force created by TSP-1, endothelial cells are capable of angiogenic activity even if they do not produce a high amount of angiogenic factors. Macrophages actively participate in wound angiogenesis and produce a range of factors and soluble mediators. Macrophages are able to stimulate, grow and migrate the endothelial cells of small vessels, and their action during the process of tumor angiogenesis causes both tumor growth and new vessel formation[14, 15] (14, 15).

Macrophages may be involved in the growth of new capillaries by several different mechanisms. The first mechanism is that macrophages produce a large number of growth factors, cytokines, proteolytic enzymes and matrix molecules that stimulate proliferation, migration and differentiation of endothelial cells in vitro and angiogenesis in vivo. do The second mechanism, changes in the extracellular matrix are modulated by macrophages, and they may change the composition of the extracellular matrix through the direct production of extracellular matrix compounds. The third mechanism is the production of substances that inhibit angiogenesis, and one of these angiogenesis inhibitors derived from macrophages is TSP-1, which has received attention in recent years[16] (16).



Figure Vascularization model during breast cancer tumor cell growth in nude mice[17]

In short, the growth and differentiation of capillaries in the tissue environment that contains a high level of soluble angiogenic mediators is controlled by changes in the composition or function of the extracellular matrix. Changes in the extracellular matrix are caused by soluble factors produced by tumor cells or host cells such as macrophages, and it is clear that macrophages have an effect on angiogenesis in pathological and physiological conditions. Macrophages produce both stimulators of angiogenesis and inhibitors of angiogenesis. Therefore, they have the ability to modulate angiogenesis both in a positive and negative direction. In addition, tumor cells are able to change the angiogenic activity of macrophages and use them to increase angiogenic activity.

1-2-6- Metastasis

Cancer spreads through the blood stream by metastasis, which is the ability of cancer cells to penetrate into blood or lymphoma cells, and then invades normal tissues and grows in them. On a larger scale, it is the ability to spread to other tissues or organs that makes cancer a deadly disease. Therefore, it becomes important to understand what factors make it possible for cancer tumor to metastasize. In other words, metastasis is a dynamic process in which the cell is stimulated under the influence of autocrine or paracrine factors and reproduces after leaving the primary environment and moving to distant or nearby places in the body. Successful metastasis requires invasion, angiogenesis and proliferation.

1-2-6-1- Angiogenesis in the pathogenesis of metastasis

The continued growth of primary neoplasm and metastasis depends on sufficient blood supply to that area. The process of forming new vessels, i.e., angiogenesis, allows tumors to grow larger than 1 to 2 cubic millimeters[18] (18). With the exception of benign tumors that have little angiogenesis and their growth rate is slow, malignant tumors have many vessels and their growth is fast. An increase in the vascular system increases the possibility of tumor cells invading by entering the bloodstream and spreading to other organs[19] (19). Studies have shown that the formation of the vascular system in malignant cancer is directly related to the power of metastasis, and every angiogenesis process before metastasis includes successive stages that begin with the local destruction of the basement membrane around the capillaries, followed by the invasion of endothelial cells. to the surrounding stroma in the direction of the angiogenic signal, and endothelial cell migration is accompanied by proliferation in the terminal area where the cell migrates, and endothelial cells begin to form three-dimensional structures to form capillary tubes. Angiogenesis is induced by positive and negative regulatory molecules that are released by both tumor cells and host cells, which include endothelial, epithelial, mesothelial, and leukocytes. And the balance between molecules regulating angiogenesis is important in this process[20] (20).

In stable normal tissues, factors that prevent angiogenesis are dominant, but in rapidly dividing tissues, molecules that stimulate the angiogenesis process are dominant. For example, in cultured fibroblasts, the loss of the TP53 suppressor gene leads to an angiogenic phenotype, the result of which is the reduction of TSP-1 production[21] (21).

1-2-7- Characteristics of tumor blood vessels

The blood vessels of the tumor provide nutrients and oxygen to the tumor, which leads to the development of the tumor. They also act as gatekeepers for tumor metastasis. More than 40 years ago, Dr. Falkman proposed the concept of targeting tumor endothelial cells by angiogenesis inhibitors (anti-angiogenesis therapy). After that, this proposal was considered as an important strategy for cancer treatment and many anti-angiogenic drugs were discovered and tested.

First, it was assumed that tumor endothelial cells are the same for all tumor types and an effective antiangiogenic drug alone can be used to treat different forms of cancer. In addition, in contrast to tumor cells, tumor endothelial cells are genetically stable and therefore have a very low probability of drug resistance. However, recent studies revealed that the regulation of tumor angiogenesis is very complex. It was also assumed at the beginning that anti-angiogenic drugs cannot be as toxic as cytotoxic drugs, but later it was found that they have severe side effects such as fatal hemoptysis and intestinal perforation. The cause of these complications is that common antiangiogenic drugs are inhibitors of vascular endothelial growth factor (VEGFR) receptors, which is very important for the survival of normal endothelial cells. Because the increase of VEGF usually leads to blood leakage and abnormalities in the tumor vessels, preventing it leads to the normalization of the vascularization process. Normalized blood vessels can be effective in delivering anti-cancer drugs and improve blood nutrition, which restores the oxygen level of the tumor tissue, which will be useful for the radiation treatment process. In addition, long-term antiangiogenic therapy sometimes leads to tumor hypoxia. Therefore, it is clear that a specific time window (good timing) is needed for the normalization of tumor vessels, which is not an easy task. Therefore, the development of safer anti-angiogenic drugs for tumors is an important goal in cancer treatment, the success of which depends on having a complete understanding of the biology of tumor endothelial cells.

Tumor cells produce several angiogenic factors that increase the formation of new vessels, such as VEGF, bFGF, angiopoietins, hepatocyte growth factor, chemokines, and platelet growth factor. Although VEGF is the main angiogenesis factor, other growth factors such as angiopoietin and ephrin family play an important role in tumor angiogenesis. Compared to normal blood vessels, tumor blood vessels show a distinct morphology. As a characteristic, tumor vessels are disorganized, while normal vessels show a regular branching pattern of arteries, veins, and capillaries. Tumor blood vessels often have an irregular appearance and due to the density of tumor cells on the immature vessel wall, they have unequal vessel diameters. The pressure of the interstitial fluid in a tumor leads to the collapse of the blood vessel and thus causes a drop in blood flow. Tumor vessels have irregular blood flow or no flow at all, and are also leaky due to weak connections between endothelial cells. These characteristics of tumor vessels partly explain the high vascularity of tumor tissue and why it is usually hypoxic and resistant to radiotherapy. Anti-VEGF treatments have an effect on the normalization of these abnormal vessels.

Normal endothelial cells form a uniform and continuous single layer, while tumoral endothelial cells have an irregular shape and size. Tumor blood vessels allow tumor cells to spread, which is an initial step for metastasis[22] (22).

1-2-8- study of tumor endothelial cells

The apparent abnormalities seen in tumor blood vessels compared to normal blood vessels, raise the question of whether there are phenotypic differences at the molecular and functional levels between tumor and normal endothelial cells. Recent evidence shows that vascular endothelium cells are heterogeneous and originate depending on the organ, and their functional state shows a specific phenotype.

Several studies have been conducted in which normal endothelial cells were used as substitutes for tumoral endothelial cells. Normal endothelial cells such as human umbilical cord vascular endothelial cells (HUVEC) and human skin vascular endothelial cells (HMVEC) were extracted and cultured under hypoxic conditions or in the presence of growth factors in order to adapt to the tumor environment and conditions. They simulate. These strategies are useful in that they produce a relatively pure population of endothelial cells. However, these in vitro endothelial cell models do not fully represent the in vivo tumor conditions because the length of the treatment period may not be sufficient and also several factors apply in the in vivo environment[22] (22).

1-2-9- Angiogenesis investigation methods

1-2-9-1- Methods of investigating angiogenesis in the in vitro environment

Endothelial cells are the primary builders of new blood vessels, and many functions of endothelial cells are required for angiogenesis, including cell matrix degradation, migration, proliferation, and morphogenesis. Various techniques are used to evaluate endothelial cells, which allows us to investigate the effects of angiogenic and antiangiogenic factors.

Migration

Following matrix destruction, endothelial cells migrate into the surrounding tissue in response to angiogenic chemical stimuli. Growth factors can contribute to the mobility of endothelial cells by causing random cell movement (chemokines) or targeted cell migration towards a stimulating factor (chemotaxis). Cell motility is a target of interest for the design of anticancer therapies because cell migration is required for both tumor invasion and angiogenesis. The methods that make it possible to measure endothelial cell motility in response to various external and internal factors are: the scrap wound method, the transfer well method and the agarose method.

In the scrap wound method or scratch wound method, endothelial cells are grown to the point of completely filling the floor and a wound is created by emptying a part of the floor using a head sampler or needle. Cell filling of the scratched area is done first by cell migration and then by proliferation of migrated cells. Because some growth factors stimulate both migration and proliferation processes, only anti-proliferation agents are used in the cell environment to investigate migration. The quantification of this method includes measuring the distance of endothelial cells movement, the area covered by endothelial cells or the amount of time required to completely close the wound area.

One of the advantages of this method is its compatibility with large-scale surveys and the ability to evaluate a well at different times. Disadvantages include: the lack of ease in creating scratches with the same size and border, the difficulty of quantification, changes in the types of wells used and possible changes in the degree of filling of containers. This method is a measure of cell motility, but it is not used to evaluate the effect of a treatment on chemokinesis and chemotaxis. The transfer well method is much easier than the small wound method. In this method, endothelial cells are cultured on one side of the porous membrane and a solution containing the migration enhancement factor is placed on that side of the membrane. For endothelial cells, a pore diameter of 3 micrometers is suitable and the porous membrane is covered with collagen or fibronectin to improve cell adhesion. After 3-18 hours of incubation, the migrated cells are stained and counted. Because the concentrations of angiogenic or antiangiogenic factors are normalized between the upper and lower compartments, the movement of cells may be in response to chemokines rather than guided migration. Therefore, a series of experiments should be performed to distinguish the state of chemokinesis and chemotaxis. To obtain the number of migrated cells, it is counted after removing the wells containing non-migrated cells. The advantages of the transfer well technique are the sensitivity of the method to low amounts of chemical factors and its high reproducibility compared to other migration methods. The lack of advantages of this method include the high price of these membranes and the fact that migration is usually evaluated only for a fraction of the total membrane surface.

A migration method that allows us to measure directed migration is the sub-agarose method. In this method, agarose gels are prepared in tissue culture plates and then we fix the well in the gel so that 2 mm of agarose remains between them. Endothelial cells are cultured in one well and migration factor is added to another plate. Migration is defined as the distance of movement of endothelial cells in the direction of stimulation (chemotaxis) compared to the distance of movement of cells in the opposite direction of the well (chemokines). The sub-agarose method is cheap and allows a clear distinction between chemokines and guided migration, but its sensitivity is lower than the transfer well method, its quantification is more difficult and it is not suitable for large-scale analyzes[23] (23).

reproduction

Proliferation of endothelial cells along with increased survival provide cells for the formation of new blood vessels. The effects of angiogenic and anti-angiogenic factors on proliferation can be evaluated by direct cell counting, quantification of DNA synthesis or evaluation of metabolic activity. Proliferation testing methods can be used to measure angiogenic activity in conditions of low growth factor or low serum or anti-angiogenic activity when the culture medium contains normal amounts of serum or growth factor. The easiest way to evaluate the number of cells after the application of angiogenic or antiangiogenic factors is direct cell counting. Live cells can be stained with trypan blue and counted with a hemocytometer, or trypsinized and counted by a coulter counter. Although direct cell counting is simple and inexpensive, this technique can detect the cause of changes in cell numbers. It doesn't matter if these changes were caused by proliferation, apoptosis or both.

Mitotic cell division can be measured by labeled reagents during DNA synthesis. In the thymidine method, thymidine bound to tritium bound to newly synthesized DNA is measured using a scintillation counter. In the BrdU method, bromodeoxyuridine, a pyrimidine analogue is attached during DNA synthesis and measured by immunohistochemical method. This method gives similar results to the results of the thymidine method. The main advantage of the BrdU method over thymidine is that it does not require radioactivity. The immunohistochemistry required for BrdU is time-consuming and difficult to quantify.

The MTT method evaluates mitochondrial activity by evaluating the number of living cells. Active mitochondria convert the yellow MTT substrate to formazan, which is a purple product. This colorimetric change is quantified using spectrophotometry and is related to the number of cells. The MTT method is suitable for large-scale work, but because some factors affect the MTT reduction process that have nothing to do with the viability of the cells, the results of the MTT method must be confirmed by cell counting[23] (23).

survive

Many angiogenic factors increase the number of cells by increasing the proliferation and survival of endothelial cells. Cell death occurs as a result of toxic stimulation in the form of necrosis, passive response or apoptosis. During apoptosis, external and internal signals activate caspase 3, 6, and 7, which can cause DNA fragmentation, DNA budding, and chromatin condensation as characteristics of programmed cell death. to be Endothelial cell apoptosis can be induced by serum starvation conditions in vitro, therefore, normal cell culture conditions are used to investigate the apoptotic effects of anti-angiogenic agents. Two common techniques to investigate endothelial cell apoptosis are TUNEL and annexin V.

The TUNEL method measures DNA fragmentation. In this method, deoxynucleotide transferase binds to the 3'-OH region of fragmented DNA and then binds to a fluorescent dye. Labeled cells can be identified by microscopy or flow cytometry, and the number of apoptotic cells is calculated relative to the total number of cells. Because TUNEL labels both necrotic and apoptotic cells, cells should be examined visually to confirm apoptotic cell death.

During premature apoptosis, the cell membrane component phosphatidylserine is transferred from the inner surface to the outer surface. annexin V is a protein that binds to phosphatidylserine; Therefore, annexin V attached to fluorescent is used to detect apoptotic cells. Like the TUNEL method, annexin V labels both necrotic and apoptotic cells. These processes are differentiated by staining cells with PI prepidium iodide because PI labels necrotic cells[23] (23).

Morphogenesis in angiogenesis

Angiogenesis requires the accumulation of endothelial cells inside vascular tubes. The stage of angiogenic tube formation in the in vitro environment can be modeled by the culture of endothelial cells with extracellular matrix components. Morphogenesis methods use substrates such as matrigel or type I collagen and endothelial cells cultured on, inside or between these substrates.

The most common applied method for investigating endothelial cell morphogenesis is the culture of human umbilical cord endothelial cells (HUVEC) on Matrigel, which is an extracellular matrix obtained from Engelbreth-Holm-Swarm rat sarcoma cells.

HUVEC cultured on matrigel with low density form a network of branched structures within 12-24 hours. Quantification is done by taking a photo of the wells and measuring the length or area of capillary-like structures (CLS) per surface unit. Among the advantages of this method, it can be mentioned that the test time is short, the settings are easy and it can be done in 96 plates. It is important to consider that Matrigel is the tumor endothelial cell matrix and contains a lot of endothelial growth factor. In fact, CLS formation for HUVEC within normal matrigel with VEGF angiogenic factor will be below baseline.

Another method of producing endothelial cell tubes in vitro is the collagen sandwich method. In this method, a layer of collagen gel is poured on the bottom of the plate and becomes solid. Endothelial cells are cultured on the gel until it is less than full. This method improves the modeling of the in vivo environment for cells[23] (23).

Evaluation of sprouting angiogenesis using endothelial cell culture:

 The morphogenesis methods mentioned above explain the formation of CLS of cultured endothelial cells to an extent less than the filling limit on or inside the extracellular matrix components. Angiogenesis in vivo involves the sprouting of endothelium filled into pre-existing vessels. Several in vitro methods have been developed to model the formation of vascular sprouts from filled endothelial cell monolayers.

Cells grown on collagen or fibrin gels can form encapsulated tubes by penetrating the matrix in response to factors such as PMA, bFGF and VEGF. Germination is evaluated by taking a slice from the gels or taking pictures of them. Invasive buds are more similar to capillaries than CLS produced by cells cultured on the extracellular matrix due to having a lumen or capsule. Analysis and quantification of buds when the vessels grow in a three-dimensional environment is difficult, which can be facilitated by cutting.

In another technique, to model sprouted angiogenesis, endothelial cells are grown to saturation on microbeads. Then these microbeads are immersed in fibrin gel. Endothelial sprouts can be seen in calf pulmonary artery endothelial cell culture that is accompanied by angiogenic factors, about 3 days after culture, and factors that induce sprouting are distinguished from other factors that only induce migration. . Endothelial cells isolated from the adrenal cortex can also be used to evaluate angiogenic factors. In this method, the growth of the veins can be seen with microscopic images, and quantification can be done by measuring the length of the vein or the number of veins per willow. Because endothelial cells are covered on all surfaces of the microbead, therefore we will have vessels in all directions[23] (23). The microbead method is also used to detect angiogenic activity with HUVEC cells, but the use of a fibroblast supporting cell layer can help HUVEC sprouting[24] (24). Also, this type of cell needs more growth factor than other types of endothelial to germinate. Endothelial cell differentiation or in other words the formation of tube or capsule in the in vitro environment can be investigated in both 2D and 3D conditions. Endothelial cells cultured on plates containing matrix proteins such as matrigel, collagen or fibrin can be differentiated[25] (25).

1-2-9-2- Methods of investigating angiogenesis in the in vivo environment

Angiogenesis can be quantified with the help of invasive and non-invasive methods. With the removed tissues, techniques include measuring morphological parameters by light or electron microscope of vessels and histological examination of tissue sections stained with endothelial antibodies or markers such as colloidal carbon, India ink, marked blood cells. They are used with radioactive and tracers with high molecular weight. The main limitation of histological techniques is that they are invasive and static. While angiogenesis is a dynamic process and involves time. Non-invasive quantification techniques have also been developed, such as dynamic MR scan, functional CT and PET scan, optical scan and ultrasound[26] (26). Vascular imaging provides the possibility of obtaining the number and distance of blood vessels, as well as measuring blood flow and vessel permeability and analyzing cellular and molecular abnormalities in blood vessels. Also, these methods enable repeated and continuous measurement of the angiogenic response. Of course, the spatial resolution of these images will be lower than microscopic methods. The measurement of microvessel density (MVD) in histological sections is still considered as the gold standard for quantitative evaluation of vascular response[27, 28] (27, 28).

Most imaging methods have morphological and qualitative information. Of course, quantitative methods such as functional and purposeful methods have recently emerged[29] (29). Also, there are different types of models to investigate the effect of external factors on angiogenesis in the in vivo environment, two types of which will be mentioned below.

Various imaging techniques

Functional imaging techniques

 ultrasound

Ultrasound, using a combination of gray scale ultrasound modes and power and color Doppler, can show the power and flow dynamics of larger vessels in tumors. These vessels are generally larger than the diameter of angiogenic capillaries (less than 500 µm). Therefore, Doppler ultrasound most likely shows flow and flow resistance in the main vessels or in the case of mature vessels serving the tumor. Spectral waveform analysis that measures blood velocity, direction, and downstream resistance will be successful in differentiating between benign and malignant injuries. For example, the resistive index (RI) can reflect the resistance of diastolic vessels in the tumor bed. In peripheral tumor vessels, low vascular resistance is related to the lack of vascular motor control and venous artery branching, because in central vessels, high vascular resistance is related to high intratumoral pressures. Therefore, the heterogeneous nature of tumor vascularization may result in the detection of both low and high RI values in the same wound; In addition, the RI values of malignant and benign lesions may overlap significantly. RI sizes correlated with the density of microvessels in breast and uterine cancers have been reported, but this relationship is indirect because the size of microvessels is significantly below the spatial resolution of ultrasound. By using microbubbles and increasing the contrast of ultrasound images, small vessels can be identified better, but still, due to the poor spatial resolution and limited field of view and short time window for imaging, microbubble ultrasound is used in very few parts of the world[29]. (29).

Perfusion CT

Perfusion CT or dynamic contrast-enhanced CT (DCE-CT) is the acquisition of serial images of the same volume over time after injection of iodinated contrast material. Because of the linear relationship between tissue attenuation and iodine concentration, DCE-CT is able to analyze blood flow and blood volume within tumors. This technique was first used to evaluate patients with acute stroke, but later it was reported that it is effective for revealing tumor angiogenesis and evaluating the response to anti-angiogenic therapy. The tissue enhancement curve can be mathematically fitted on compartmental or deconvolution models, and quantitative parameters such as blood volume, blood flow, mean transit time, and capillary permeability level can be obtained. Ma et al evaluated the relationship between perfusion CT results with tumor angiogenesis and vascular endothelial growth factor (VEGF) expression in patients with malignant and benign pulmonary nodules. They investigated several parameters related to the glands such as peak height enhancement and ratio of wound to aorta peak height enhancement and calculated parameters such as blood flow, blood volume and capillary permeability level were obtained on the perfusion plate. They correlated CT perfusion parameters with tumor angiogenesis, MVD and VEGF expression. Despite the advantages of this method, perfusion CT has radiation exposure that increases general radiation. This reduces the allowed number of clinical trials of this method; However, the use of low-dose protocols can lead to the predominant use of this technique[29] (29).

Dynamic contrast-enhanced MRI

Dynamic contrast-enhanced MRI (DCE-MRI) is performed using TI-weighted 3D MRI sequences before, during, and after intravenous injection of low molecular weight gadolinium chelate. On DCE-MRI, tumors show a sharp increase following a decrease, which is faster than normal background tissue. However, there are significant changes in this pattern between tumor types and significant heterogeneity within a single tumor. Although these features can be detected in larger wounds, they are difficult to see in smaller wounds.

MRI images can be evaluated using different analytical techniques. With descriptive analyses, the shape of the intensity versus time curve can be described by the on time relative to the injection time, the slope of the initial enhancement, the time to reach the peak, the slope of the washout curve and the area under the curve. Descriptive analysis is relatively easy to perform, but it depends on the exact conditions of the scan, injection time, patient's condition and a series of other variables, and therefore it is difficult to repeat. In addition, the data are somewhat descriptive and have no inherent physiological meaning. Another analytical method quantifies gadolinium concentration using T1 and fitting a gadolinium concentration curve to a kinetic model. In MRI, there is no linear relationship between gadolinium concentration and tissue signal intensity. However, the T1 map can cause errors if the scanners are not fully calibrated. A pharmacokinetic model is used to fit the enhancement curves, which is able to calculate the vascular permeability constants. Kinetic parameters are usually higher in the tumor than in the surrounding healthy tissues, but they can significantly decrease after anti-angiogenic treatment. The central drawback of the DCE-MRI method is its poor adherence to standard nomenclature and pharmacokinetic models and data acquisition methods. Therefore, it is difficult to compare studies with different initial conditions.

DCE-MRI is used as a biological marker to monitor the response to chemotherapy, anti-angiogenic treatment, radiotherapy and ambulatory therapy of different types of cancer such as breast, prostate, colon and gynecological malignancies. Thukral et al. compared three methods of DCE-MRI analysis (exploratory, sugar percentage, and overall kinetic models) to determine the parameter or combination of parameters that are associated with microvascular changes during treatment. They reported that Ktrans, Kep and the integrated area under the gadolinium concentration curve were closely related to the physiological response to treatment.

DCE-MRI is commonly performed in the clinic with low-molecular-weight gadolinium chelates, which rapidly cross the endothelium of blood vessels. Because inflammatory tissues and neoplastic tissues have leaky vessels, enhancement is non-specific; However, neoplastic tissue has larger vascular pores than inflammatory tissue. Therefore, macromolecule MR contrast agents (such as very small paramagnetic iron oxide, gadolinium albumin, gadolinium liposome, gadolinium dendrimer, and gadolinium viral particles) are more selective for tumor-induced angiogenesis than low molecular weight contrast agents. Macromolecular contrast agents generally produce lower permeability constants and lower effective vascular fractions than low-weight molecules. So they will strengthen less, which can be compensated by better release of macromolecules. However, due to the lack of sufficient experience, these materials are only used in animal experiments[29] (29).

Functional PET

PET is performed as both a functional imaging technique and a targeted imaging technique. For functional PET, 15O-labeled radiotracers such as H215O and C15O have been used to quantify tumor angiogenesis. For example, when H215O is injected as a bolus, it can be used to measure blood flow using the Patlak method. C15O immediately binds to RBC and allows us to evaluate blood volume. 11C and 18F-FDG are also used as radiotracers[29] (29).

Targeted imaging techniques

Molecules expressed on new angiogenic vessels: targets

Considering the new molecular treatments that are directed at specific targets, it should be possible to perform molecular imaging in the same related targets. This is useful for predicting drug response. A targeted approach relies on the binding of labeled molecules to markers expressed on the endothelium of tumor vessels. Examples of molecules that can be targeted for imaging include: VEGF and its receptors (VEGF receptor types 1, 2, and 3), integrins, and matrix metalloproteinases (MMPs).

VEGF/VEGF receptor axis is one of the most extensive signaling pathways related to the study of angiogenesis. VEGF receptor 1 is useful for physiological and structural angiogenesis, while VEGF receptor 2 is important for the mitogenic, angiogenic and permeability enhancing effects of VEGF[29] (29).

Targeted imaging with ultrasound

Ultrasound with targeted microbubbles has been reported to be effective for both in vivo molecular imaging and VEGF receptor 2 and integrin quantification. Microbubbles with target ligand expression on their outer surface are made. Expression of VEGF receptor 2 in human breast cancer and murine angiosarcoma tumor models is demonstrated using ultrasound with targeted microbubbles. For integrins, microbubbles labeled with ανβ3 integrin-targeting peptides are used to non-invasively detect angiogenesis in murine models of glioma. Using ultrasound in the presence of targeted microbubbles on both VEGF receptor 2 and ανβ3, tumor angiogenesis in human cervical tumor is better visualized. Targeted microbubbles have extraordinary sensitivity so that only a few microbubbles are enough for detection[29] (29).

Targeted imaging with MRI

In addition to its excellent quality for imaging tumor angiogenesis, MRI is also used for targeted imaging, especially for revealing the expression of integrinβ3 in tumors. Schmeider et al demonstrated ανβ3 integrin expression in murine melanoma tumor models using targeted paramagnetic nanoparticles at 1.5 tesla. Despite the progress of targeted MRI of angiogenic molecules, imaging of new tumor vessels using MRI is difficult because vessels make up only a small percentage of a tumor. Higher field intensities with newly designed coils and molecular materials with high comfort have improved targeted MRI imaging of angiogenesis[29] (29).

Targeted imaging with PET

PET is recognized as a better technique for targeted imaging of tumor angiogenesis due to its higher sensitivity. Molecules such as 64Cu, 111In, 89Zr and 18F are used for targeted PET imaging of tumor angiogenesis[29] (29).

Optical imaging

Optical imaging is a rapidly developing branch of medical imaging that does not require the use of ionizing radiation and can be obtained with inexpensive and portable equipment. In general, biological tissues show high photon absorption in both the wavelength range of visible light (350-700 nm) and infrared range (900 nm and larger). This is despite the fact that in the near infrared region (700-900 nm) the absorption spectrum for all biomolecules reaches a minimum. Therefore, NIR fluorescence light has a unique advantage for imaging pathophysiological conditions. Optical imaging probes use fluorophores, which must first be stimulated with a photon before producing photons with a longer wavelength[30] (30). Near infrared (NIR) fluorophores have the best tissue penetration. Therefore, they are preferable for intradermal imaging. The main limitation of optical imaging as a whole-body imaging technique is its limited depth of penetration. Therefore, optical imaging is limited for superficial tissues and during endoscopy or surgery. Another limitation of optical imaging is autofluorescence, that is, the light produced by internal molecules and non-specific external molecules in the body. Cy5.5-RGD peptides and Cy5.5-RAFT-cRGD peptides are among the targeted agents considered for optical imaging of angiogenesis. In addition, optical fluorophores can be combined with smart probes and used to image MMPs[29] (29).

Immunohistochemistry

An introduction to immunohistochemistry

Immunohistochemistry (IHC) refers to the process of antigen detection (for example, protein) in the cells of a tissue section, which is done by exploiting the principle of antigen-specific additional antibody in biological tissues.

Immunohistochemical staining is widely used in the diagnosis of abnormal cells such as cancer tumor cells. There are specific molecular markers for specific cellular events such as proliferation or cell death (apoptosis). Immunohistochemistry is also widely used in basic research to understand the distribution and localization of biomarkers and different proteins expressed in different parts of a biological tissue.

 Demonstration of antibody-antigen interaction can be done in several ways. In the most common example, an antibody is conjugated to an enzyme, such as peroxidase, which can catalyze a color reaction. Antibodies can also be labeled with a fluorophore.

Types of antibodies

Antibodies used for identification can be polyclonal or monoclonal. Polyclonal antibodies are made by injecting the desired proteins into the body of animals or a peptide fragment, then a secondary immune response is stimulated that isolates the antibodies in the total serum. Therefore, polyclonal antibodies are a heterogeneous mixture of antibodies that identify several epitopes.

For immunohistochemical identification strategies, antibodies are classified as primary and secondary. Primary antibodies enter against the desired antigen but are not incorporated, while secondary antibodies enter against the immunoglobulins of the primary antibodies. The secondary antibody is usually attached to a linker molecule such as biotin through which it binds to the reporter molecules, or the secondary antibody binds directly to the reporter molecule.

Reporter molecules

The reporter molecules change based on the nature of the detection method. The most common detection methods include chromogenic and fluorescence, which are determined by an enzyme or a fluorophore, respectively. With chromogenic reporters, an enzyme reacts with a substrate and produces an intensely colored product that can be analyzed using a conventional light microscope. The list of enzyme substrates is very wide, and among them, alkaline phosphatase (AP) and horseradish peroxidase (HRP) can be mentioned, these two enzymes are used for protein detection. There is a class of chromogenic, fluorogenic and diminuluminescent substrates for use with these enzymes, such as DAB or BCIP/NBT, which produce brown or purple colors, respectively, wherever they are bound to the enzymes (31).

H & E staining method

Hematoxylin, toluidine blue and methylene blue are examples of alkaline dyes and acid eosin and fuchsia are also examples of acid dyes. Among the dyes in general staining, hematoxylin and eosin (H&E) is used the most. With this staining, hematoxylin turns the cell nucleus and acidic structures such as RNA-rich organelles blue or purple, and eosin turns the cytoplasm red (32).

Angiogenesis study models

There are several models for researching the effective factors in angiogenesis, as well as obtaining more information and understanding the processes involved in angiogenesis, two of which are mentioned below.

Chicken chorioallantoic membrane (CAM) measurement model:

CAM method has been the most widely used in in vivo angiogenesis studies. The preparation of the test substance is done in polymer pellets with slow release that are either absorbed using gelatin sponges or dried on plastic containers with air; These materials are then grafted on the CAM between the cut pieces of the egg shell. Lack of immune and mature system in 7-8 days old chicken embryos allows to study tumor induced angiogenesis. The effects of angiogenesis are measured by counting the number of blood vessels on a certain surface using a stereomicroscope. In another type of CAM method, embryos without shell are cultured in petri dishes of the test female. This method allows us to examine blood vessels in a wider area of CAM. The CAM method is a relatively cheap and simple in vivo method suitable for large-scale studies. However, considering that the CAM itself has its own vascular network, it will be difficult to distinguish new capillaries from existing capillaries. In addition, 7-8-day CAM shows inflammatory effects in front of some stimuli, such as pollen produced from the shell of the hengan that creates a window in it, which can cause problems in identifying new vessels. Therefore, it is better to consider a pause of 3 days between the time of creating the window and the connection of the test substance[31] (33).

Model of formation of new vessels in the cornea

The cornea is an area without vessels, and therefore any vessel that penetrates from an organ in it can be identified as a new vessel. In this method, a pocket is created in the corneal stroma of a laboratory animal (usually rabbits are used in this method, but this method has also been performed with rats and small laboratory mice). The angiogenic response should start after slow release pellet or polymer containing angiogenic agent. The quantification of this method is done by killing the animal and analyzing the images taken from the corneal perfusion with Indian ink. This method is reliable, but compared to the CAM method, it is more expensive and technical, and cannot be used on a large scale. In addition, although the use of mice has improved the possibility of cheapness and availability of the experiment, the reduction of the size of the animal's eye will lead to surgical problems, and the scientists of this method will face a serious ethical problem due to the damage to a major organ[31]. (33).

1-2-9-3- Factors affecting angiogenesis

Intracellular stimulatory and inhibitory factors

As soon as researchers realized that cancer cells can release molecules to activate the angiogenesis process, the challenge began to find and study these stimulating molecules in animal and human tumors. These proteins or smaller molecules are called angiogenic, which means that they are released by tumors as signals for angiogenesis. Among these molecules, two proteins are more important for promoting tumor growth: vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). VEGF and bFGF are produced by most types of cancer cells as well as some normal cells. These factors are first synthesized inside the tumor cells and then secreted into the surrounding tissue. When they encounter endothelial cells, they bind to specific proteins called receptors and sit on the outer surface of the cells. The binding of VEGF or bFGF with the appropriate receptor activates a series of signaling proteins in endothelial cells. The nuclear signal finally activates a group of genes, which in turn provide the products needed for the growth of new endothelial cells. The activation of endothelial cells by VEGF or bFGF goes through a series of steps towards the production of new blood vessels. First, activated endothelial cells produce matrix metalloproteinases (MMPs), a special class of degrading enzymes. Then these enzymes are released from the endothelial cells in the surrounding tissue. MMPs break down the extracellular matrix. The breakdown of this matrix allows cells to migrate. When they migrate into the surrounding tissues, the activated endothelial cells begin to divide. They are soon organized in the form of hollow tubes and gradually become a mature network of blood vessels.

Although most tumors produce angiogenic molecules such as VEGF or bFGF, these molecules must be able to overcome the inhibitors to initiate angiogenesis. Until today, a large number of endogenous factors that inhibit or inhibit angiogenesis have been identified, many of which originate naturally from the extracellular matrix, and some are actually basement membrane proteins. Angiogenesis agents are divided into two main classes, including matrix-derived inhibitors and non-matrix-derived inhibitors. Arrestin, endostatin and thrombospondins can be mentioned among the famous examples of the first class. From the second class, we can mention angiostatin, interferons and various types of interleukins (Figure 3)[32] (34).

Researchers are always looking for angiogenesis inhibitors to prevent the growth of tumor metastasis. For this purpose, almost twelve angiogenesis inhibitors were tested on cancer patients. These inhibitors are divided into several classes based on their mechanism of action. Some directly affect the endothelial cells themselves, while others affect the signaling pathway of angiogenesis or the ability of endothelial cells to They destroy the breaking of the extracellular matrix[26] (26).

A series of angiogenesis inhibitors that have been tested in cancer patients are molecules that directly affect the growth of endothelial cells. Endostatin is among this group that prevents tumor growth in animals. Other drugs that interact with a molecule called integrin can also affect the proliferation of endothelial cells. Another drug is thalidomide, which causes abortion in pregnant women. This drug prevents the formation of new blood vessels by endothelial cells and is therefore useful in the treatment of non-pregnant cancer patients. The second group of angiogenesis inhibitors that have been used in the clinic on humans are molecules that interfere with the stages of the angiogenesis signaling pathway. Among these data, there are anti-VEGF antibodies that prevent the VEGF receptor from binding to the growth factor. Another factor is alpha interferon, which is a naturally produced protein that prevents the production of VEGF and bFGF. Of course, we also have several synthesized drugs that interfere with endothelial cell receptors. The third group of angiogenesis inhibitors act against one of the primary products of endothelial cell growth, such as MMPs and enzymes that facilitate the breakdown of the extracellular matrix. Because the breakdown of the matrix is necessary before the endothelial cells can move into the tissue and multiply in the form of new blood vessels, so these drugs can prevent angiogenesis[33] (35).

Antivascular chemotherapy

Browder in Falkman's laboratory was the first to show the concept of optimizing the conventional chemotherapy plan in order to achieve more stable apoptosis of endothelial cells in the vascular bed of the tumor and stated that even if the tumor cells are resistant to drugs, it may be possible to Controlled tumor growth in mice.

Conventional chemotherapy is prescribed with the maximum tolerance dose and time intervals of 2-3 weeks until bone marrow and intestinal cells can regenerate themselves during this period. But anti-angiogenic chemotherapy is prescribed more continuously and in lower doses. During antiangiogenic chemotherapy, endothelial cell apoptosis and capillary removal increases the death of tumor cells that are around each capillary.

Commonly, several components with angiostatic activity are considered for clinical use, and most of them are in the final stage of development. However, clinical trials have not shown the antitumor effects expected from parabolic studies, and it seems that the clinical applications of antiangiogenic therapy are very complicated. The main problem in the production of antiangiogenic factors is that several angiogenic molecules may be produced by tumors and tumors provide their blood sources in different stages of progression depending on different angiogenic factors. Therefore, blocking an angiogenic molecule will have only a small effect on tumor growth. Today, with the introduction of the targeted treatment method, with the production of targeted anti-angiogenic agents including their use in the combination of various anti-angiogenic inhibitory agents to achieve the goal of blocking tumor angiogenesis, the hope for anti-angiogenic treatment of tumors has increased[13] (13).

Magnetic field as a physical factor affecting angiogenesis

In several studies conducted on the biological effects of the magnetic field, the results indicated the effect of the field with different parameters on angiogenesis as a stimulus or inhibitor[34-38] (36-40). In this section, a brief introduction to the physics of electromagnetic and magnetic fields and the biological effects of these fields along with the mechanisms of action and finally an overview of the studies conducted on the effect of magnetic fields on angiogenesis and tumors are given.

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