REVIEW

STROMATOGENESIS AND ANGIOGENESIS, TWO ESSENTIAL PROCESSES OF TUMOR INVASION IN MALIGNANT EPITHELIAL NEOPLASMS - A BRIEF REVIEW OF PLANOCELLULAR SKIN CARCINOMA

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Abstract

Each neoplasm creates its own unique micro-environment in which the tumor grows and modifies. Although majority of host cells in the neoplasm stroma have obligatory tumor suppression ability, the stroma changes during the malignant process and even promotes growth, invasion and metastasis. The genetic changes that occur during cancer evolution, driven by malignant cells, lead to changes in the stroma of the host. At the same time angiogenesis occurs, and formation of new vascular net from the existing normal capillaries. The stroma in the neoplasm does not only give mechanical support to the tumor, but also provides metabolically rich and active environment where the cancer cells survive and multiply. It also contributes to tumor growth and through the newly formed vascular pathways enables further guidance to the circulation, so that the four features of the cancers are achieved: migration, invasion, angiogenesis and metastasis. The understanding of the molecular events in the process of stromatogenesis and angiogenesis is essential for clinical research.

ПРЕГЛЕД НА ЛИТЕРАТУРА

СТРОМАТОГЕНЕЗА И АНГИОГЕНЕЗА, ДВА БИТНИ ПРОЦЕСИ ВО ТУМОРСКАТА ИНВАЗИЈА КАЈ МАЛИГНИТЕ ЕПИТЕЛНИ НЕОПЛАЗМИ - КРАТОК ОСВРТ НА ПЛАНОЦЕЛУЛАРЕН КАРЦИНОМ НА КОЖАТА

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Клучни зборови: строматогенеза, екстрацелуларен матрикс, металопротеинази, ангиогенеза, сквамозен карцином

***Кореспонденција:** Љубица Ташева, Институт за медицинска хистологија и ембриологија, Медицински факултет, Универзитет Св. Кирил и Методиј"- Скопје, Република Северна Македонија. E-mail: ljubica.tasheva@medf.ukim.edu.mk

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Печатарски права: ©2024. Љубица Ташева, Ирена Костадинова-Петрова, Наташа Стојковска, Елида Митевска, Лена Какашева-Маженковска. Оваа статија е со отворен пристап дистрибуирана под условите на нелокализирана лиценца, која овозможува неограничена употреба, дистрибуција и репродукција на било кој медиум, доколку се цитираа тоориналниот(ите) автор(и) и изворот.

Конкурентски интереси: Авторот изјавува дека нема конкурентски интереси.

Извадок

Секоја неоплазма создава своја единствена микросредина во која туморот расте и се модифицира. Иако повеќето клетки на домаќинот во стромата на неоплазмата имаат задолжителна способност за супресија на туморот, стромата се менува за време на малигниот процес, па дури и почнува да промовира раст, инвазија и метастазирање. Генетските промени кои се случуваат за време на еволуцијата на неоплазмата, поттикнати од малигните клетки, доведуваат до промени во стромата на домаќинот. Исто така, во исто време се јавува ангиогенеза, формирање на нова васкуларна мрежа од постојните нормални капилари. Стромата во неоплазмата не дава само механичка поддршка на туморот, туку обезбедува и метаболички богата и активна средина каде што канцерозните клетки преживуваат и се размножуваат. Придонесува и за раст на туморот, и преку формираните васкуларни патишта овозможува понатамошно водење на циркулацијата, така што се постигнуваат четирите карактеристики на карциномот: миграција, инвазија, ангиогенеза и метастаза. Разбирањето на молекуларните настани во процесот на строматогенезата и ангиогенезата е од суштинско значење за клиничките истражувања.

Introduction

The skin is placed on the body`s surface, between the internal environment of the human organism and the external habitat. It is considered a morphologically complex and multifunctional organ, the largest in the human body.

Sun exposure, i.e. the ultraviolet radiation, polluted environment, use of different chemical substances, alcohol, nicotine, arsenic poisoning, the process of industrialization in the bigger cities, chemical substances such as vinyl chloride, poly-cyclic aromatic hydrocarbons, HPV infection etc., are some of the factors that cause various skin changes[1,2]. 95% of skin cancers present as non-melanoma cancer and squamous cell carcinoma account for 20% of them. Squamous cell carcinoma can cause metastasis and death, and therefore it is in the top five causes of cancer death around the world[3].

Squamous cell carcinoma

Squamous cell carcinoma (SCC) is a malignant tumor of the epidermal epithelium. This type of neoplasm, depending on the location in the body, presents with different symptomatology, origin, prognosis and treatment[4,5].

The main etiological factors in the occurrence of squamous cell carcinoma are: ultraviolet B radiation, previous skin burns, HPV infections, radiological radiation, inflammatory lesions and long-term skin ulcerations and arsenic poisoning.

People who have undergone immunosuppression therapy as a result of organ transplant are more likely to develop squamous cell carcinoma. This type of cancer appears mostly on the uncovered body parts that are directly exposed to sun, such as: forehead, face, ears, scalp, neck, arms and lips (vermillion). SCC has a higher occurrence rate in light-skinned people and in elderly people. The incidence is higher in countries near the equator. This cancer rarely appears in dark-skinned population.5 There were 2,402,221 global incidence cases and 356,054 deaths of SCC in 2019. Data from the Institute of Public Health of the Republic of Macedonia cover two categories of malignant skin neoplasm: malignant melanoma of the skin and other malignant neoplasm of the skin that include squamous cell carcinoma, basal-cell carcinoma and malignant neoplasm of the sweat and sebaceous glands.

Squamous cell carcinoma skin changes can be detected by eye as shallow ulcers, often crusted on top and elevated, with an indurated surrounding, as plaques or nodules[6].

This type of carcinoma is composed of nests, sheets and strands of squamous epithelial cells that invade the epidermis and extend into the dermis with variable depth. The cells have rich eosinophilic cytoplasm, a large vesicular nucleus with notable nucleolus; some of them are hyperchromatic and show numerous mitosis. There is variable central keratinization and horn pearls formation, depending on tumor differentiation. The level of anaplasia in the nests is used to determine the tumor differentiation and the categorization of well differentiated, moderately and poorly differentiated tumors. Squamous cell carcinomas occasionally infiltrate along nerve sheaths, the adventitia of blood vessels, lymphat-

ic system and fascial planes. The presence of perineural lymphocytes is a sign of cancer spreading in the deeper parts.[6] In the periphery of the neoplasm, a variable quantity of inflammatory infiltrate may exist. Rare histological variants of SCC include clear cell, signet-ring, pigment, basaloid and rhabdoid types. The cells of squamous cell carcinoma are positive to the epithelial membrane antigen and cytokeratin.[6,7]

Skin squamous cell carcinoma is a locally aggressive tumor, which can appear in several modalities. It has an aggressive course in patients infected by HPV. If the tumor invasion is severe, poorly differentiated, it is more likely to recur or metastasize. The risk of metastasis in the skin damaged by sun radiation is low and is around 0.5%, while in cancers in areas unexposed to the sun the risk is higher and is between 2-3%. Lesions located on the lips, penis, vulva, perineum, as well as on the so-called Marjolin's ulcer, radiation scar or thermal burns have even higher potential for metastasizing. The depth of tumor invasion is a prognostic factor. Lesions invading less than 2 mm in the skin can rarely metastasize; tumors between 2 and 5 mm invasion depth have a medium risk of around 5% chance to lead to metastases, while tumors invading deeper than 5 mm have a high risk to metastasize, around 20%. Tumors that are larger than 2 cm in diameter have a higher risk of recurrence and metastasis, compared to smaller lesions.8

The aim of this paper was to evaluate the current evidence that science has had confirmed by now, regarding the role of stromatogenesis and angiogenesis in the evolution of epithelial malignancies with reference to squamous cell carcinoma.

Tumor stroma

The histological architecture of the tumor includes parenchyma, specific for each tumor type, which is composed of tumor cells, and stroma, a structural mass built of connective tissue, blood vessels and inflammatory cells, which originates from the host. Without the presence of the stroma, the cancer cells cannot sustain themselves, because it supplies them with nutrients essential for their survival, growth and spreading.[9]

A network of blood vessels, specialized mesenchymal cells, cancer-associated fibroblasts (CAFs)/ myofibroblasts, immune cells, mastocytes, macrophages, leukocytes and adipocytes, all together incorporated in the extracellular matrix (ECM), are the main components of the stroma. (On the other hand) the principal elements of the extracellular matrix are structural proteins (collagen and elastin), specialized proteins (fibrillin, fibronectin and elastin) and proteoglycans. The process of the cancer development leads to genetic changes guided by the malignant cells. These genetic changes alternate the stroma of the host. In the process of tumor development and invasion, the basal membrane is deteriorated and the stroma becomes more dynamic as a result of an increased number of fibroblasts, inflammatory infiltrates and newly composed capillaries. The previously mentioned tissue elements are in direct contact with tumor cells. These changes lead to cancer invasion.[10]

Stromatogenesis represents formation of a new, specific type of tissue located around active tumor cells as a vital part of the invading process. Tumor stromatogenesis is managed by the tumor cells themselves with absolute tolerance and participation of host's local fibroblasts. Tumor stromatogenesis differs from the usual reactive fibrosis that surrounds benign lesions (fibrous capsule) and the formation of avascular connective tissue that forms the scar tissue.

Carcinogenesis is defined as uncontrolled replication of tissue cells with a monoclonal character, implying origin from a single cell mutation. These monoclonal cells are in fact tumor cells that interact with the surrounding tissue including the ECM growth factors and cytokines. The monoclonal cells are associated with the ECM, as well as with the nearby endothelial cells, fibroblasts, macrophages, mastocytes, neutrophils, pericytes and adipocytes. The mitigation of the tumor cells, tumor invasion, metastasis and angiogenesis are reliant on the tumor microsurrounding. The key players in this process are matrix metalloproteinases (MMP), enzymes that break cell adhesion molecules, change the connections between the cells and the connections of the cells with the ECM.[11,12]

The following mechanisms of the malignant cells are believed to lead to breaking the normal tissue barriers and causing invasion:

- Rapid growth and replication of the malignant cells cause mechanic pressure on the surrounding tissue with the least resistance;
- Tumor cells lose the surface molecules of the cadherins and integrins, which leads to weakened tumor cell adhesions;
- Tumor cells become more motile through secretion of mitogen cytokines and increase the number of growth factor receptors;
- The extracellular matrix is degraded by the release of more proteolytic enzymes such as metalloproteases, collagenases, plasminogens, cathepsins, etc.
- The secretion of the vascular endothelial growth factors (VEGF) and thymidine phosphorylase (TP) stimulates angiogenesis, providing survival of the tumor cells and their penetration in the circulation.[11]

The matrix metalloproteinases (MMP) provide proteolytic activity necessary for breaking down the physical barriers in the surrounding tissue, which contributes to tumor expansion and intravasation in the nearby blood vessels. MMPs locally stimulate the production of the so-called invadopodia that support the invasion, localized on the surface of the cells. The invadopodia are found in places where ECM degradation happens.

MMPs are also involved in the tumor cell proliferation by modifying the bioavailability of the growth factors and the functions of the surface cell receptors. The members of the MMP release cell membrane precursors for the insulin-like growth factor (IGF) and the epidermal growth factor (EGF) that support the proliferation. MMP-1, -2, -3, -7, -9, -11 and -19 connect to the IGF connecting pro-

tein and regulate the bioavailability of the growth factor.[13]

Many authors have pointed out to the association between glycose aminoglycans, matrix metalloproteinase and growth factors. Glycosaminoglycans (GAGs) induce the matrix metalloproteinase (MMPs) to release growth factors (GFs) from the cell surface that leads to proliferation of the cancer cells.

MMP are also involved in the tumor angiogenesis. MMP-9 increases the bioavailability of the vascular endothelial growth factor, which is a potent mediator in the tumor vasculature, and human basic fibroblast growth factor (bFBGF), by degradation of the extracellular components such as collagen type IV, VIII and perlecan. MMPs also release tumstatin, endostatin, angiostatin and endorepellin - products of the degradation of collagen type 4 and type 17, as well as plasminogen and perlecan. By releasing the previously mentioned products of degradation, MMPs regulate the balance of angiogenesis. MMP-2, -3, -9, -13, -14 reduce the intracellular interactions and increase the migrational capacity of the epithelial cells. During this process, the epithelial cells lose their polarity and take on mesenchymal phenotype. Breaking the cell adhesions results in cell migration; also the connections between the cells are disturbed because of the elimination of e-cadherins.[14]

As a result of the degradation of EMC and other extracellular molecules, fragments with new bioactivities appear which can suppress angiogenesis. For example, active endostatin appears in the process of ripping collagen type XVIII caused by MMP-3, -7, -9, -13, -20. Also, pathological vascularization and increased tumor growth can be found in mice with MMP-9 deficiency. MMP-2, -9 and -12 degrade plasminogen and this process produces angiostatin which has anti-angiogenic function. It means that the MMP can generate angiogenesis inhibition, as well as angiogenesis stimulation. MMPs also play a role in the process of lymphangiogenesis. Studies have reported that the MMPs expand the bioavailability of the vascular endothelial growth factor, which also supports the lymphangiogenesis and promotes the metastasis spreading in the lymph. This has been confirmed in experiments done in fragments of ductus thoracicus of mice incorporated in collagen, that resulted in formation of lymph capillaries in the lumen. The increased level of MMP-1, -2 and MMP-3 is associated with the lymph invasion and metastasis in the lymph nodes. The inhibition of MMP-2, -9 and -14 decreases the angiogenesis, lymphogenesis and reduces the appearance of metastasis in the lymph nodes.[15,16]

MMP-9 was found in the stroma of the invasion surroundings in the micro-invasive squamous cell carcinoma.[17] The MMP-2 level can serve as a predictive factor in the appearance of metastasis in the oral squamous cell carcinoma, and the high level of MMP-2 and -9 correlates with the level of invasion of the squamous cell carcinoma.[18,19] MMP-1 can be found in neoplastic nests of the SCC and in the stroma fibroblasts which surround the tumor epithelial cells.[20] The increased expression of MMP-9 and -7 detected in the cancer cells is in correlation with deeper tumor invasion.[21]

Most of the epithelial tumors accumulate connective tissue cells and extracellular material in their surroundings. This appearance/phenomenon is called a stromal reaction. The key element of the stromal reaction are myofibroblasts. Myofibroblasts are in fact fibroblasts with acquired capacity for expression of alpha smooth muscle actin, (a-SMA), which are vascular smooth muscle cells. Myofibroblasts are capable of synthesizing collagen and other extracellular components and play a key role in the connective tissue remodeling that occurs during wound healing and development of fibrosis.[22] These cells can remodel the connective tissues, but can also react with the epithelial cells and other connective tissue cells, and control processes such as tumor invasion and angiogenesis. Fibroblasts and myofibroblasts produce collagen and extracellular proteins, thus creating desmoplastic reaction.[23] Desmoplasia is a process in which host cells respond to the inductive stimulus of the tumor cells. The stroma cells produce collagen, ECM proteins and initiate desmoplastic reaction for mediation in the invasion process of the tumor cells. Therefore, a question is raised whether the stroma around the cancer cells acts as a protective mechanism, or it accelerates the tumor activity. The stromal collagen is accumulated by the myofibroblasts during the cancer invasion, which is associated with desmoplasia. Myofibroblasts produce paracrine motility factor and cytokines such as hepatocyte growth factor (HGF) and fibroblast growth factor (FGF) also initiate growth of blood vessels and increase the cancer invasion and the metastasis potential[24,25] (Figure 1).

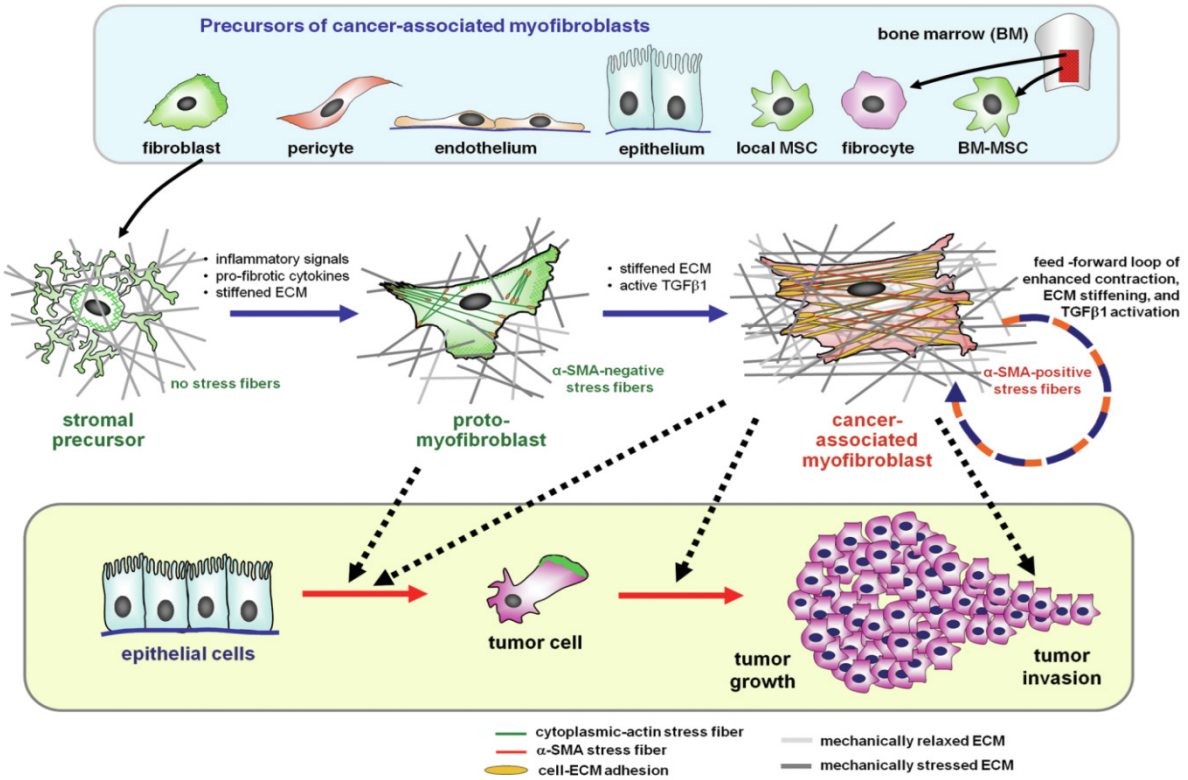


Figure 1 Myofibroblast in the tumor stroma
(See ref. Otranto M. *et al.*[39])

A study that analyzed samples from oral leukoplakia and squamous cancer stained immunohistochemically with a-SMA, confirmed that there were no myofibroblasts in the normal mucosa and in the oral leukoplakia, but were found occasionally in samples of squamous cell carcinoma. Myofibroblastic presence was more frequently found in the more invasive squamous cell carcinoma compared to those with smaller invasion.[26] The analysis of myofibroblasts marked with alpha smooth muscle actin and CD34 in the non-metastatic and metastatic oral squamous cell carcinoma has shown more a-SMA positive fibroblasts in the metastatic group of carcinomas.[27] It has been confirmed that myofibroblasts are strong indicators of the invasion and the proliferation of oral squamous cell carcinoma.[28] Analyzing the myofibroblasts and examining their presence in the stroma in keratoacanthoma and squamous cell carcinoma, it was found that these were significantly more common in the stroma of squamous cell carcinoma.[29]

Angiogenesis

Angiogenesis is the process of formation of new blood vessels from the existing normal capillaries. The higher the angiogenesis activity is, the more aggressive the tumor is, which leads to a poor prognosis for the patients.[30] It is believed that tumors which have more blood vessels than the others are angiogenetic. Tumors also show ability for expression of angiogenesis growth factors such as VEGF, TP, etc.[31]

Stromal and tumor-associated macrophages produce molecules which activate proliferation of endothelial cells, their migration and maturation by forming vascular channels. These growth factors are mainly VEGF. Also, the increased presence of hepatocyte growth factor, the basal fibroblast growth factor and some other interleukins may manifest angiogenesis activities. Weidner *et al.* used immunochemical staining of the endothelial cells in order to differentiate neoplastic lessons into rich angiogenesis and poor angiogenesis tumors. Tumors with increased vascular density have higher chance of metastasizing and have worse postoperative prognosis.[32]

Endothelial cells, which compose the inner lining of the tumor blood vessels, are vital in delivering nutrients and oxygen to the malignant cells. The VEGF expression by endothelial cells amplify vascular permeability. This occurrence assists in the extravasation of fluids and macromolecules, which leads to tumor development and metastasis. The process mentioned above encourages the development of a tumor vasculature, establishing a continuous supply of oxygen and nutrients to support neoplastic proliferation. Research has shown that the TME of SCC often exhibits increased angiogenesis.[33] Thompson *et al.* described the vascular density in breast adenocarcinoma. They found that angiogenesis dominated on the invasion front of the tumor and even more it was found on the border between the normal tissue and the malignant tissue. This finding helped the authors understand that the tumors get the blood supply by the blood-vessel joining, and not by its proliferation.

The vascular density of the hot spots in the tumor periphery was appointed as T1; the area in the middle (between the periphery and the center) was marked as T2 and T3 was named the vascular density area in the center of the tumor. T1 area had the highest vascular density, and it was decreasing going towards the internal part of the tumor, with variations depending on the different types of neoplasm. This occurrence of the vascular regression has also been confirmed by immunochemical staining used for VEGF and kinase insert domain receptor (KDR) complex. VRGF and KDR are present on the surface of the vascular endothelium and VEGF/KDR positive vascular density is higher in the periphery of the tumor and on places where it touches the normal tissue. This finding suggests that angiogenesis activity occurs also in the normal tissue near the invasive tumor front.[34]

Angiogenesis and stromatogenesis are parallel processes in the active tumor cell invasion. The newly formed stroma is edematous and convenient for endothelial cell migration and proliferation of tumor cells. These overlapping processes along with the invasive tumor cells form a well-organized tumor. Angiogenesis and stromatogenesis happen at the same time with the tumor cell invasion and are closely related with tumor metastasis. The conditions in the tumor micro-surrounding, as oxygen level, acidity level and the amount of nutritious products are dominant specifications of the tumor environment. From the above mentioned, a question is raised on how the tumor cells survive in the central parts of the neoplasm when the conditions are less appropriate compared to the peripheral parts of the tumor. The presumption would be that the tumor cells and the young blood vessels are more tolerant to hypoxia by activation of the glycolytic anaerobic pathways.[35] Tumor hypoxia is a microenvironment factor that causes cancer development and resistance to cancer treatment. Approximately 60% of tumors show evident levels of hypoxia or anoxia in tumor tissues. The adaption to hypoxia environment is the base for cancer tissue survival and growth. Abnormal and dysfunctional tumor blood vessels are unable of restoring oxygenation because of the loss in the transportation of oxygen, therefore sustaining hypoxia, which results in promoting cancer progression, metastasis, and resistance to antitumor therapies.[36]

During hypoxia, cancer cells change their metabolism by shifting from oxidative phosphorylation to aerobic glycolysis that results in acidification of the extracellular space. Acidosis is a major link associated with hypoxia which allows selected cells to adapt to acidic conditions. As proliferation of tumor cells occurs, the consumption of energy increases prompting oxygen deprivation. Oxygen deprivation leads to metabolic shift into aerobic glycolysis, known as Warburg effect, by converting glucose into lactate, subsequently fermenting lactic acid in the cytosol. The pH of tumors can decrease to 5.7 in comparison to the pH of healthy tissues which is 7.4.[37]

The tumor potential to withstand lower blood flow in its central parts depends on the ability for production of proteins with anti-apoptotic

features. Proteins are produced and secreted from the tumor and the endothelial cells that determine an important feature of the tumor, the so-called vascular survival ability (VSA). The blood vessels that supply the peripheral part of the tumor are typical blood vessels. But the blood vessels in the internal part of the tumor are irregularly shaped, dilated, tortuous and can have dead ends. These blood vessels are not organized in venules, arterioles and capillaries, but rather have chaotic arrangement. The blood vessels of the vascular network formed in the tumors contain wide splots and this is the cause of bleeding in the surrounding tissues. The perivascular cells often become loosely associated or less abundant. Tumor vessels have cancer cells incorporated in its walls. The blood flow is irregular, with slower and sometimes even oscillating course. The reason for this peculiar occurrence is unknown, but it is believed to be a result of disorder in the appearance and functioning of the angiogenesis factors.

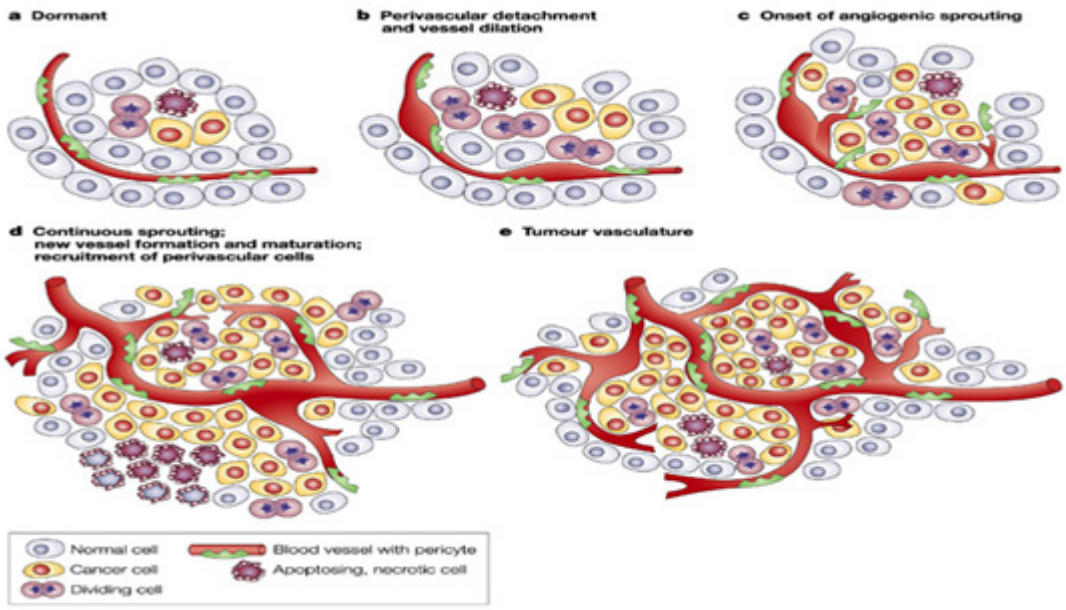


Figure 2 Classical angiogenic switch
(See ref. Bergers G. *et al.*[33])

In oral squamous carcinoma association between the micro-vascular density and positive lymph node metastasis indicates that the angiogenesis plays an essential role in the oral carcinoma.,[38] Angiogenesis is a constitutive part of tumor progression which plays a key role in tumor growth and metastasis. In the 1970s, Professor Folkman stated that tumor growth and metastasis depend on angiogenesis, and targeting its angiogenesis can be used as a therapeutic strategy for tumor treatment.[40]

Conclusion

The stroma in the neoplasm does not only give mechanical support to the tumor, but also provides metabolically rich and active environment where the cancer cells survive and multiply. It also contributes to tumor growth and through the newly formed vascular pathways enables further guidance to the circulation, so that the four features of the cancers are achieved: migration, invasion, angiogenesis and metastasis. The understanding of the molecular events in the process of stromatogenesis and angiogenesis is essential for clinical research. Studying the anti-angiogenesis or blocking of the enzymes included in the complex cell matrix interactions can provide relevant information that would be further included in the treatment protocol of the neoplasm.

Squamous cell carcinoma as a more common type of skin cancer needs further research, taking into consideration that most of the published studies are related to the oral squamous cell carcinoma. Cutaneous squamous cell carcinoma with the mechanisms of its invasion and metastasis, as well as the interactions between the epithelium and the stroma still remains unclear and can be considered as an interesting field of research, not only because of the frequent occurrence of this tumor, but also because of the access to materials for research. The elaboration of the abovementioned mechanisms has not only academic value, but could be considered as a basis for determination of prognosis and treatment modality.

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