Transfer of Advances in Sciences into Dental Education

Angiogenesis in Oral Cancer

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Abstract: Head and neck squamous cell carcinoma (HNSCC) is an aggressive malignancy that develops after years of chronic exposure to alcohol and tobacco products. Exposure to these agents results in alterations of genes that are important in the regulation of various cellular functions. This loss of regulation allows the tumor cells to survive and grow in an unchecked manner by allowing the cells to perform functions that contribute to the growth of the tumor. Some of these important changes include the acquisition of immortality and the ability to invade tissue and/or metastasize to other sights, as well as acquiring the ability to induce angiogenesis. Angiogenesis, the growth of new blood vessels from pre-existing ones, is a complex phenomenon that is absolutely required for the continued growth and survival of solid neoplasms. Without new blood vessels to provide nutrients and remove waste, tumors would be unable to grow larger than 2-3 mm in diameter. Therefore, one could envision its potential role in both the treatment and prevention of malignancies such as HNSCC. The concept of chemoprevention is extremely important in HNSCC since patients often develop multiple independent lesions throughout the mucosa of the upper aerodigestive tract. Therefore, the comprehensive treatment of this disease must address not only the initial primary neoplasm, but also prevent the progression of the premalignant lesions lurking throughout the rest of the mucosal surfaces. This review will outline the basic changes that occur in tumor cells that result in the switch to angiogenic phenotype. In addition, it will discuss the present status of using antiangiogenic agents in the treatment of cancer. Finally, this paper will present a rationale for the use of multiple antiangiogenic agents as a means of developing new chemotherapeutic and chemopreventive protocols that may result in reduced patient toxicity while maintaining similar clinical efficacies.

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Head and neck squamous cell carcinoma (HNSCC) is an aggressive epithelial malignancy that is the sixth most common neoplasm in the world today. At current rates, approximately 50,000 cases in the United States and 500,000 cases worldwide will be diagnosed in the year 2001. Despite advances in surgery, radiation, and chemotherapy, the long-term survival for patients with oral cancer has remained at less than 50 percent for the last forty years. Several factors contribute to this poor outcome. First of all, oral cancer is often diagnosed in an advanced stage. The five-year survival rate of early stage oral cancer is approximately 80 percent, while the survival drops to 19 percent for late stage disease. Second, the development of multiple primary tumors has a major impact on survival. The rate of second primary tumor development in these patients has been reported to be 3-7 percent per year, higher than for any other malignancy. As a result, an individual who is fortunate enough to live five years after the initial primary tumor has up to a 35 percent chance of developing at least one new primary tumor within that time period. The occurrence of new primary tumors can be particularly devastating for individuals whose initial lesions are small. Among patients with early stage disease, second primary tumors are the most common cause of treatment failure and death. The observation of frequent second primary tumors in oral cancer led Slaughter to propose the concept of “field cancerization.” This theory, which is supported by both epidemiologic and molecular studies, suggests that multiple individual primary tumors develop independently in the upper aerodigestive tract as a result of years of chronic exposure of the mucosa to carcinogens.

The term “cancer” describes a heterogeneous group of more than 200 different types of malignant tumors. Like many neoplasms, oral cancer typically
takes many years to be observed, because the development of tumors is a multistep process in which there is a sequential activation of oncogenes and inactivation of tumor suppressor genes in the same clone of cells. The loss of regulation of these important genes is precisely the molecular basis for changes that occur in tumor cells that allow them to survive and grow unchecked. The specific steps involved in the development of human colon carcinoma are the most extensively studied and involve the alteration of a number of these different oncogenes and tumor suppressor genes.9 HNSCC is also believed to arise via a multistep process that involves the activation of oncogenes as well as the inactivation of tumor suppressor genes.10-13 However, the specific pattern of progression and the genetic alterations that are important in human HNSCC have not yet been delineated.

These genetic changes generate concomitant phenotypic changes in the tumor cells that allow them to continue to survive and expand until they become a large and clinically detectable tumor mass. Some of these important phenotypic changes include the development of cell immortality and the ability to invade tissue and metastasize, as well as acquiring the ability to induce angiogenesis.

**Angiogenesis**

Angiogenesis, the growth of new blood vessels from pre-existing ones, is one of the essential phenotypes of tumor formation and is also important in a number of normal physiologic processes including growth and development,14 wound healing,15 and reproduction.16-18 An inadequate amount of angiogenesis contributes to ulcer formation19 and excessive angiogenesis contributes to the pathology of a number of conditions including arthritis, psoriasis, and solid tumors. In a series of now classic experiments, Folkman and colleagues demonstrated that solid tumors cannot grow any larger than 2-3 mm in diameter without being able to induce their own blood supply.20

Whether or not angiogenesis occurs in a particular tissue depends on the balance between the relative amounts of molecules that induce and molecules that inhibit angiogenesis.21 In normal tissues, blood vessels are quiescent, and cells usually secrete low levels of inducers and high levels of inhibitors. As normal cells progress towards malignancy, they must develop the ability to induce angiogenesis. In order to achieve this switch, tumor cells usually increase the amount of inducers and decrease the amount of inhibitors they secrete. There is considerable interest in determining how cells, progressing from normal to tumorigenic, switch from being antiangiogenic to angiogenic. In some animal models, a distinct switch to the angiogenic phenotype is seen.22 In other cases, the cells developing into tumors sequentially become more angiogenic in a stepwise fashion.23-25 The exact mechanisms explaining how this occurs in most neoplasms has for the most part remained elusive. However, one can easily envision that both the activation of oncogenes and the inactivation of tumor suppressor genes play an important role.26-30

**Angiogenesis and HNSCC**

The expression of the angiogenic phenotype in the tumor microenvironment is an extremely complex process involving the interaction of many different cell types. Like all solid tumors, HNSCCs must develop direct and indirect ways to induce the production of new blood vessels in order to continue to expand and metastasize.

A variety of molecules capable of inducing angiogenesis are directly produced by keratinocytes and by HNSCC.31 In a number of studies, Interleukin-8 (IL-8) was a major angiogenic factor.32-36 In addition, in the closely related bronchogenic carcinomas, IL-8 was the primary mediator of angiogenesis found in fresh tumor homogenates.37 Vascular endothelial growth factor (VEGF) is a multifunctional cytokine whose biological activity is primarily associated with endothelial cells. Increased levels of VEGF protein expression are seen in many different neoplasms including HNSCC.35,36,38-41

The interplay between tumor cells and the various constituents of the surrounding stroma are also thought to be critically important in various aspects of tumor biology including the indirect induction of angiogenesis.42 Macrophages, part of the tumor stroma, are members of the mononuclear phagocyte system of inflammatory cells. A very heterogeneous group, they perform a wide variety of functions depending upon the physiologic or pathophysiologic condition to which they are recruited. The presence of macrophages in the tumor stroma is a poor prognostic indicator in a number of neoplasms including
melanomas, gliomas, endometrial cancer, and breast cancer. It is thought that peripheral blood monocytes (PBM) are recruited to the tumor microenvironment as a result of the secretion of various chemotactic cytokines such as Colony Stimulating Factor (CSF-1), Granulocyte macrophage colony stimulating factor (GM-CSF), VEGF, Monocyte Chemotactic Protein (MCP-1), and Transforming Growth Factor Beta (TGFβ-1). Upon their recruitment and activation to sites of neoplasms as well as inflammation, macrophages have been found to secrete a number of angiogenic factors including basic Fibroblast Growth Factor (bFGF), VEGF, Tumor Necrosis Factor-alpha (TNF-α), and IL-8.

In HNSCC, tumor cells attract monocytes and activate them to secrete angiogenic factors. In addition, macrophages produce cytokines that act in a paracrine fashion on the tumor cells, which stimulates them to produce increased levels of IL-8 and VEGF. Taken together, these observations help to underscore the complex interactions between HNSCC and one particular type of stromal cell present in the tumor microenvironment. Other paracrine and autocrine interactions in HNSCC have also been identified and it is likely that additional paracrine interactions involving tumor cells and other stromal cells such as endothelial cells, fibroblasts, and lymphocytes are also occurring. Additional investigation is required to determine the role that each of these cell types may have in the indirect induction of angiogenesis in HNSCC.

There is much less information available as to the identity of endogenous inhibitors of angiogenesis secreted by the oral mucosa. Normal keratinocytes cultured from humans and from hamster buccal pouches secrete high levels of inhibitory activity. This inhibitory activity from normal cells is capable of suppressing angiogenesis induced by media from tumor cells. However, the identities of these inhibitory factors are unknown. These keratinocytes, in order to give rise to oral squamous cell carcinomas, must lose this inhibitory activity, or dramatically increase their secretion of inducers of angiogenesis, if they are to become progressively growing vascular tumors. A similar loss of inhibitors during tumor progression is observed in fibroblasts from Li Fraumeni patients. The fibrosarcomas become angiogenic as a result of a decrease of thrombospondin, a naturally occurring inhibitor of angiogenesis.

The loss of inhibitory activity in the fibroblasts is due to loss of the p53 tumor suppressor gene. A similar suppressor-based mechanism may be operating in human SCC, as keratinocytes in experimental models of oral carcinogenesis also become angiogenic as the result of genetic loss.

**Antiangiogenesis Therapy in Cancer**

An adequate vascular response is essential for the initial development as well as the continued growth of solid tumors. Experimental evidence has demonstrated that tumor growth can be stunted by a variety of agents that have the common ability to inhibit angiogenesis. In addition, experiments have shown that some inhibitors are effective at blocking the growth of HNSCC. Therefore, antiangiogenic therapy is an attractive modality for treating and perhaps preventing the development of malignant neoplasms.

There is an increasing list of agents with antiangiogenic activity that are being tested in clinical trials at this time (Table 1). The National Cancer Institute’s website lists all antiangiogenic agents that are presently under investigation (http://cancertrials.nci.nih.gov/news/angio/table.html). In general, there are at least five possible mechanisms by which antiangiogenic agents can inhibit the tumor-induced blood vessel growth. The first category includes agents that prevent or decrease the secretion of angiogenic factors by tumor cells themselves. Interferons (IFN) is an excellent example of this type of antiangiogenic agent. IFNs can decrease the amount of bFGF that is secreted by renal cell carcinoma tumor cells and can down regulate the production of IL-8 in fibroblasts. The second category includes compounds that increase the secretion of antiangiogenic factors by the tumor cells themselves. Interferons (IFN) is an excellent example of this type of antiangiogenic agent. IFNs can decrease the amount of bFGF that is secreted by renal cell carcinoma tumor cells and can down regulate the production of IL-8 in fibroblasts. The second category includes compounds that increase the secretion of antiangiogenic factors by the tumor cells and/or normal cells surrounding the neoplasm. For example, retinoids can stimulate the production of an unidentified inhibitor of angiogenesis, while (Interleukin-12) IL-12 stimulates the production of IFN-gamma-inducible protein-10 (IP-10), a C-X-C chemokine family member with potent antiangiogenic activity.

The third group includes agents that prevent tumors cells from activating and stimulating other cells such as macrophages or endothelial cells to secrete angiogenic factors. In addition, certain agents that affect macrophage function, and not yet tested...
as antiangiogenic agents, may affect the ability of macrophages to participate in tumor angiogenesis.76,77

The fourth group includes agents that neutralize the biologic activity of angiogenic factors secreted by the tumor cells and/or surrounding normal cells. These compounds make up perhaps the largest category of agents and include: SU5416, SU6668, EMD121974, anti-VEGF, Marimastat, COL-3, Neovastat, and BMS-275291. Finally, the fifth group of antiangiogenic agents consists of drugs that cause endothelial cells to become refractory to inducers of angiogenesis secreted by both the tumor and the surrounding normal cells. This type of antiangiogenic agent would include endostatin, Squalamine, ABT-510, thalidomide, and TNP-470.

There is a great deal of interest in developing additional chemotherapeutic clinical trials that are based upon antiangiogenic therapies.78-81 A wide array of additional antiangiogenic agents can be expected to enter clinical trials in the near future. However, one of the major challenges for designing such therapies is that there are numerous direct and indirect mechanisms by which tumors can induce new blood vessel growth, including eliciting the help of various stromal cells. Therefore, it is reasonable to assume that successful antiangiogenic protocols must address each of the possible mechanisms by which tumors can induce new blood vessel growth. Recent evidence suggests that combinational therapies, using multiple antiangiogenic agents or other treatment modalities in conjunction with inhibitors of angiogenesis, provide improved biologic responses.82-84

Table 1. Agents with antiangiogenic activity currently under investigation in clinical trials

<table>
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<tr>
<th>Agent</th>
<th>Inhibitor</th>
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<tr>
<td>Marimastat</td>
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<tr>
<td>COL-3</td>
<td>Interferon-α</td>
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<tr>
<td>Neovastat</td>
<td>Anti-VEGF</td>
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<td>BMS-275291</td>
<td>EMD121974</td>
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<td>Thalidomide</td>
<td>CAI</td>
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<td>Squalamine</td>
<td>Interleukin-12</td>
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<td>Endostatin</td>
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<td>SU5416</td>
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Chemoprevention and Oral Cancer

In addition to their potential uses as chemotherapeutic agents, inhibitors of angiogenesis may also hold great promise in the realm of chemoprevention. Methods of cancer prevention in patients with recognizable premalignant lesions fall into two general categories. The first is avoidance of further exposure to known carcinogenic agents, notably tobacco and alcohol. This strategy, while clearly important, is not sufficient, as many of the critical genetic alterations leading to transformation may have already occurred in “early” premalignant lesions. The second is chemoprevention, or pharmacologic intervention to reverse or inhibit carcinogenic progression. Chemoprevention can also be considered in two categories: primary chemoprevention directed at individuals with de novo premalignant lesions, and secondary chemoprevention directed at cancer patients following potentially curative therapy to prevent recurrent disease. Chemopreventive agents are being tested for their efficacy in a number of human malignancies including head and neck, lung, uterine cervix, breast, and colon cancer. The rationale for testing chemopreventive agents in humans is the result of both epidemiological studies and laboratory experiments using animal models of carcinogenesis. Epidemiological studies have suggested that certain dietary factors may act as anticarcinogenic agents, and animal models of carcinogenesis have demonstrated that perhaps as many as 500 agents may have some efficacy as chemopreventive agents.

The use of chemopreventive agents in halting the development of HNSCC has been extensively studied in animal models of oral carcinogenesis and in a number of clinical trials. Shklar and colleagues initially demonstrated that various retinoids inhibit the development of carcinomas in the hamster buccal pouch and rat tongue models. They also demonstrated that retinoic acid could cause the regression of precancerous lesions. The mechanism(s) of antitumor action of retinoids appears to be multiple. In some tumor systems the antitumor effects of retinoids correlate with their ability to modulate growth by influencing proliferation, differentiation, programmed cell death and/or angiogenesis.

These encouraging experimental findings resulted in a number of randomized, placebo-controlled studies. An initial clinical trial demonstrated that 13-cis retinoic acid caused the regression of oral cavity leukoplakias, some of which progress to frank carcinomas. Subsequently, 13-cis retinoic acid was found to be effective at blocking the development of new primary tumors of the upper aerodigestive tract in individuals who had a previous SCC. However, initial promising response rates reported with these compounds have not been consistently reproduced. In addition, the toxic side effects from the drug made it an unlikely candidate for long-term usage. As such, forms of targeted chemoprevention that address specific genetic alterations or required tumor phenotypes must be investigated. For example, the Onyx015 chemoprevention protocol has shown early promise in destroying p53 mutant cells. Alternatively, targeting angiogenesis in a chemopreventive fashion may be a therapeutic utility. Experimentally, the expression of the angiogenic phenotype is observed early in the transformation process long before the development of frank neoplasia. Therefore, the use of antiangiogenic agents may be an attractive modality of chemoprevention.

The long-term goals of chemoprevention must be twofold. First of all, we must develop a treatment that can be easily taken by individuals who have had a previous malignancy as well as by individuals who are at high risk for their initial HNSCC. However, the toxic side effects experienced by individuals using the drug(s) must be extremely low in order to achieve widespread acceptance of a chemopreventive regimen that will be used on a long-term basis. This would be particularly important in the case of high-risk patients who have yet to have their first malignancy. Secondly, the treatment must be capable of halting the growth of premalignant lesions before they can be observed clinically. Recent discoveries suggest that one possible way may be to treat patients with a cocktail of antiangiogenic agents that will halt each of the mechanisms by which tumors can directly and indirectly induce blood vessel growth. If such a protocol could be developed, it should provide us with the ability to prevent the development of HNSCC and perhaps other malignancies as well.

Conclusions

There is still a great deal to learn about the biological and molecular mechanisms responsible for
the development of HNSCC. The completion of the Human Genome Project will provide us with new reagents to explore global genetic and phenotypic changes in a more complete fashion. The identification of gene expression profiles required for different cancer-related phenotypes, such as angiogenesis, will enable us to develop targeted therapies with reduced toxicities.

It is important for all dental educators to keep abreast of the field of oral cancer and convey this importance to their students. Dentists are in a unique position to detect both malignant and pre-malignant lesions in the oral cavity. Often, we see our patients on a regular basis. This frequency provides us with the opportunity to observe mucosal changes that may occur over time. Our ability to make these clinical observations and perform diagnostic procedures will position the field of dentistry as the ultimate gatekeepers for new diagnostic and therapeutic measures. In the near future, the battle against oral cancer will include patient education, advanced diagnostics, and chemoprevention.

REFERENCES