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# The prognostic role of VEGF in head and neck squamous cell carcinoma

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SCHOOL OF MEDICINE

Thesis

**THE PROGNOSTIC ROLE OF VEGF IN HEAD AND NECK SQUAMOUS CELL  
CARCINOMA**

by

**ROHIT MATHEW**

B.A., Case Western Reserve University, 2012

Submitted in partial fulfillment of the  
requirements for the degree of  
Master of Science

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Approved by

First Reader

---

Isabel Dominguez, Ph.D.  
Assistant Professor of Medicine

Second Reader

---

Amritha Suresh, Ph.D.  
Principal Scientist, Mazumdar Shaw Center for Translational Research

Third Reader

---

Satvika Tadiparthi, M.D.  
Resident Physician

## **DEDICATION**

I would like to dedicate this work to Arvind and Ann

## **ACKNOWLEDGMENTS**

I would like to take this opportunity to thank the people who have provided me the resources, motivation and guidance throughout my life which have afforded me the latitude to pursue a rather muddled path.

# **THE PROGNOSTIC ROLE OF VEGF IN HEAD AND NECK SQUAMOUS CELL**

## **CARCINOMA**

**ROHIT MATHEW**

### **ABSTRACT**

Emerging from potentially malignant disorders that in most cases will never become cancerous, head and neck squamous cell carcinoma (HNSCC) is a cancer that is extremely difficult to diagnose early. This late stage diagnosis has allowed limited improvements in overall survival (OS) as patients are prone to local recurrence, secondary primary tumors, and distant metastasis. As a result, it has become vitally important to assess the prognostic value of biological marker screening to provide an avenue for early diagnosis and identification of local recurrence or residual secondary tumor sites. Many characteristic markers such as EGFR, p16, p53 and VEGF that are constitutively mutated in HNSCC have been identified. However, the dysregulation of VEGF marks a landmark mutation that accelerates the disease's progression and spread. An angiogenic protein normally expressed in response to hypoxic conditions, VEGF allows the creation of new vasculature to remove catabolites and bestows resistance to normal cellular apoptotic signals; pathways often employed by chemotherapeutics. Therefore, early identification of VEGF poses a unique opportunity to employ aggressive therapeutic regimens in combination with precision surgical resection to eliminate the cancer before neovasculture invasion has occurred and the tumor has expanded significantly. For this reason, this Review will examine the current literature available on VEGF's role in HNSCC, its value as a prognostic marker.

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## LIST OF ABBREVIATIONS

BU	Boston University
bFGF	basic Fibroblast Growth Factor
CCRT	Concurrent Chemoradiotherapy
CDDP	Cisplatin
CRT	Chemoradiotherapy
CSCs	Cancer Stem Cells
EGFR	Epidermal Growth Factor Receptor
FA	Fanconi Anemia
HER	Human Epidermal Growth Factor Receptor
HNSCC	Head and Neck Squamous Cell Carcinoma
HUVEC	Human Umbilical Vein Endothelial Cell
IC	Induction Chemotherapy
LOH	Loss of Heterozygosity
MSCTR	Mazumdar Shaw Center for Translational Research
OS	Overall Survival
PI3K	Phosphoinositide 3-Kinase
PKB	Protein Kinase B
PMDs	Potentially Malignant Disorders
QOL	Quality of Life
R/M	Recurrent and/or Metastatic
RT	Radiotherapy

TKR.....Tyrosine Kinase Receptor

VBL.....Vinblastine

## INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in world and collectively refers to the aggressive malignant neoplasms that manifest themselves in the epithelial lining of the oral cavity, oropharynx, larynx or hypopharynx (Abou-Elhamd & Habib, 2008; Kamangar, Dores, & Anderson, 2006). Patients are often diagnosed at advanced stages of the disease with a discernible primary site and a palpable mass. Depending on the location of the tumor, symptoms may include dysphagia, hoarseness, odynophagia, and sore throat (Haddad & Shin, 2008). Typically, most patients range from 60-70 years in age however, a small portion of patients have presented under 45 years of age. Though other cancers also occur in these anatomical regions, they make up a smaller percentage of registered cases, are classified and assessed differently, and therefore are not considered here.

HNSCC is a particularly notorious malignancy. Historically, patients have been treated using disfiguring surgical resection followed by radiotherapy. Such treatment has frequently been accompanied by loss of organ function and decreased quality of life (QOL). In the case of recurrent or metastatic (R/M) HSNCC additional platinum based regimens are employed with some limited response. Unfortunately, little gain in overall survival (OS) has been observed, and platinum based treatments are associated with significant added toxicity, which dramatically reduce patient tolerance and QOL (Sacco & Cohen, 2015).

Recent improvements in technology and research have yielded a better understanding of HNSCC and its biological characteristics, which has ushered in new options for treatment and therapy. However, despite these advances the OS remains at 50% over five years; a rather daunting figure (Forastiere, Koch, Trotti, & Sidransky, 2001; Leemans, Braakhuis, & Brakenhoff, 2011).

With seemingly little improvement in OS made, it is extremely important to understand the factors that contribute to this poor prognosis and what drives them.

Difficulty in diagnosing HNSCC during its emergent stages is primarily responsible for high mortality rates. Approximately two thirds of patients present at advanced stages. At this point the disease has already affected the regional lymph nodes, making recurrences common, and reducing chances of recovery (Argiris, Karamouzis, Raben, & Ferris, 2008). Presenting at such advanced stages requires far more aggressive treatments and limits positive outcomes. Therefore, as with all lifestyle cancers, the most effective treatment in the absence of outright abstinence is early-detection and screening for patient populations with known risk factors.

In addition, once diagnosed the aggressive therapies associated with late stage identification incur significant financial costs; this coupled with the more effective larger doses leading to toxicity and patient intolerance, regimens are often discontinued prematurely. Furthermore, aggressive chemotherapeutics have made little headway in OS, often yielding little to no delay in disease progression and substantially decreasing QOL.

An additional consideration that contributes significantly to poor OS is recurrence. Recurrence is a consistent concern when treating late stage cancer and is especially prevalent among HNSCC cases. One of the modes of recurrence development is the result of tumor cells entering the vasculature implanting in other regions either within the same organ or beyond (in which case, it is termed metastasis). Recently, studies have identified a small sub-population of cells, cancer stem cells (CSCs), that may contribute significantly to recurrence by acting as tumor-progenitor cells (Prince et al., 2007). These cells seem to play a significant role in tumor recurrence and metastasis, which in turn drive high morbidity and mortality.

Finally, HNSCC commonly develops resistance. Thus, once diagnosed patients still run the risk of their cancer developing resistance to the therapeutics being used to treat it, lowering their efficacy. To combat this, practitioners often advocate for aggressive treatments that reduce the likelihood of resistance developing, however, increased toxicity causes patients to prematurely discontinue the cycle. Resistance, significantly reduces treatment options. In recent years, doctors have started to employ combinatorial therapies that mount a multi-front attack, however, resistance can still occur.

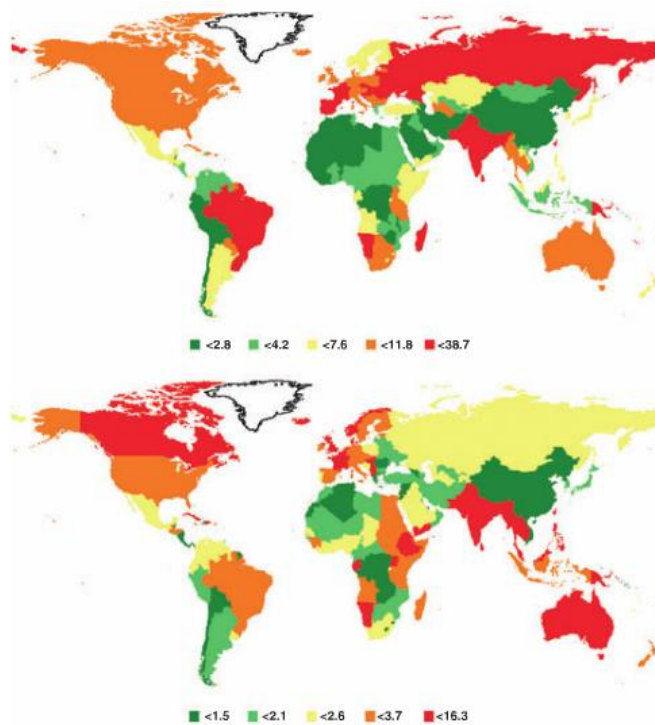
The current standard of care, typically poor prognosis, and significant decrease in QOL, make examination and screening for changes to biological markers like VEGF of particular importance as it marks a profound shift in the lethality of the disease. Determining if VEGF is overexpressed by *in vivo* patient tumors can

provide invaluable foundational knowledge allowing doctors and patients to select the best course of treatment. Based on this knowledge, patients can decide to salvage QOL when associated resistance is present, or pursue curative treatment in its absence.

### **Epidemiology**

In 2008 HNSCC amassed ~500,000 new cases and was responsible for ~273,000 deaths. However, it is unlikely these numbers are representative of HNSCCs global distribution. HNSCC exhibits remarkable geographical variation; two thirds of cases documented occur in developing nations (Ferlay et al., 2010).

**Figure 1: Estimated age-standardized oral and pharyngeal cancer incidence rates per 100,000 population.** The top map shows data for males while the bottom map shows data for females. These maps highlight the marked geographic variation associated with HNSCC. (Johnson & Jayasekara, 2011)



Incidence is influenced by a number of exogenous factors, endogenous susceptibility, and access to quality care, while mortality is influenced largely by staging of cancer at presentation and individual response to therapy (Kamangar et al., 2006).

It is important to consider inherent biases when analyzing the epidemiological data associated with HNSCC. Information pertaining to a cancer that primarily affects those in underdeveloped nations is often poorly documented or not available at all. Additionally, information gathering related to cancer incidence is performed by cancer registries, which in turn introduces bias as only the patients that are able to make the journey to hospitals which have registries are



documented. For example, in regions of rural India where access to hospital-based cancer registries is scarce, difficulty following up, and lack of death certification requirement, suggests the incidence might be significantly higher than reported (Johnson & Jayasekara, 2011). HNSCC's disposition towards underdeveloped nations and high costs associated with therapeutic regimens warrants the investigation of explorative diagnostics that afford informed treatment decisions.

### **Risk Factors**

Factors that contribute to the rise of HNSCC are well known and have been documented extensively. HNSCC is predominantly a lifestyle cancer; largely self-induced, with external factors such as tobacco, betel quid, alcohol, poor fruit and vegetable consumption, although other contributing factors such as HPV are well documented. The consumption of alcohol in parallel with smoking has a synergistic effect, increasing the risk of cancer exponentially (Elango, Gangadharan, Sumithra, & Kuriakose, 2006; Farshadpour, Hordijk, & Koole, 2008; Petti, 2009; Sturgis & Cinciripini, 2007; Vineis, Alavanja, & Buffler, 2004). In addition, recent studies have shown that inherent endogenous susceptibilities and genetic disorders such as Fanconi Anemia (FA) also contribute to the incidence of HNSCC. (Cloos et al., 1996; Kutler et al., 2003; Suárez, Rodrigo, Ferlito, & Cabanillas, 2006).

## **Molecular Progression and Staging**

Cancer arises as an aggregation of genomic aberrations (D. Hanahan & Weinberg, 2000). With this in mind significant effort has been devoted to understanding the alterations that take place as a cell undergoes malignant transformation. Advancements in molecular analysis techniques have allowed the identification of markers that function in a number of cellular functions such as growth, proliferation, communication, and death. This knowledge has provided immense insight into how the dysregulation or activation of these molecules play a role in cancer biology.

Cancer is inherently the result of a series of mutations conferring either a gain-of-function activation of an oncogene or a loss-of-function knockout of a tumor suppression gene. In order for malignant transformation to occur a cell must have acquired enough of the following: rate-limiting mutations, self-stimulation of growth, resistance to inhibitory signaling, resistance to programmed cell death, indefinite regeneration, stimulation of vascular growth, and local tissue invasion. These traits, originally described by Weinberg and Hanahan, have since been revised to include the presence of abnormal metabolic pathways and immune system evasion (D. Hanahan & Weinberg, 2000; Douglas Hanahan & Weinberg, 2011).

Like many other cancers borne of lifestyle choices, HNSCC is usually age dependent; stochastically acquiring mutations, until it has accumulated sufficient rate-limiting mutations to cause malignancy. The apical squamous epithelial cells of

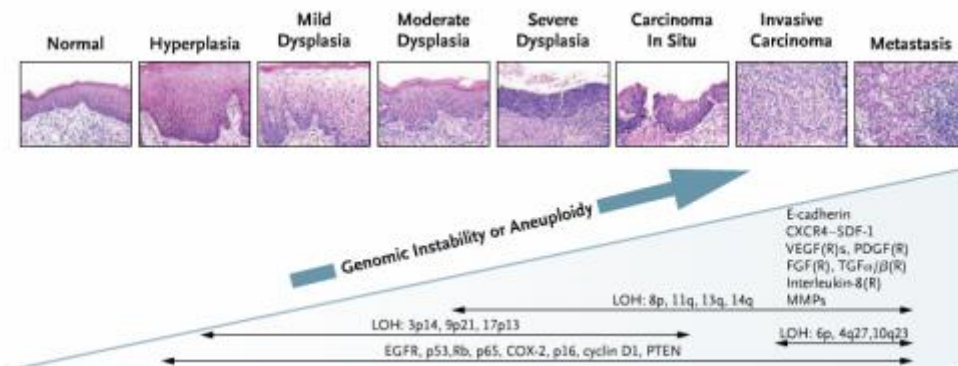
the aero-digestive mucosal lining affected in HNSCC are particularly susceptible to these mutations due to their exposure to environmental carcinogens and their highly replicative nature necessitated by this contact.

### *Potentially Malignant Disorders*

HNSCC progresses in two discernable stages, appearance of white or red patches on the interior of the oral cavity and development of a frank carcinoma. There have been several attempts over the years to unify the nomenclature used to clinically define the lesions or irregularities of the oral mucosa. The most recent evaluation proposed the term potentially malignant disorder (PMD) to describe a 'family of morphological alterations amongst which some may have an increased potential for malignant transformation' (Warnakulasuriya, Johnson, & van der Waal, 2007). It is important to note that a potentially malignant disorder conveys a caveat to identification and classification of these abnormal patches as precancerous; many of these will never become cancerous. Thus, accurate identification largely depends on testing of biopsies to accurately diagnose that malignant disorder. PMDs include a number of disorders, however, we will focus on leukoplakia, the PMD that most commonly undergoes transformation to HNSCC. Leukoplakia can be clinically categorized into two types, homogenous and heterogeneous. Homogenous leukoplakia is defined as a white lesion with a flat, thin appearance which may exhibit shallow cracks, and has a smooth, wrinkled or corrugated surface with consistent texture (Axéll, Pindborg, Smith, van der Waal, & an International

Collaborative Group on Oral White, 1996). Heterogeneous leukoplakia has been identified as a white or white and red lesion (erythroplakia) that is irregularly flat, nodular, or verrucous. Heterogeneous leukoplakia is far more aggressive than other type of oral leukoplakia, progressing in three stages inexorably from its nascent stage to squamous cell carcinoma (Batsakis, Suarez, & El-Naggar, 1999). The transition from PMD to frank carcinoma is marked by a number of chromosomal aberrations constitutively present at key histological presentations as shown in Figure 2. Critical mutations such as those to p16, p53, and VEGF confer many of required traits described by Hanahan and Weinberg in 2000. Mutations to VEGF(R) and FGF(R) typically occur in the late stages of the disease, are linked with invasive/metastatic carcinoma, and are prognostic markers associated with resistance to conventional cytotoxic chemotherapeutics.

**Figure 2: Phenotypic histological and molecular multi-step progression from normal to frank head and neck squamous cell carcinoma. (Haddad & Shin, 2008)**



### *p53*

In general, loss of function of tumor suppressor genes is the most common pathway to tumorigenesis. p53, the most commonly mutated tumor suppressor gene in human cancers, is responsible for arresting cellular growth, repairing damaged DNA and cellular death. In HNSCC, studies have shown that p53 is mutated in ~50% of all patient tumors with significantly higher alteration in invasive HNSCC (43%) than in PMDs (16%) suggesting involvement early in malignant transformation (Boyle, Hakim, Koch, van der Riet, & Hruban, 1993).

### *p16*

Another tumor suppressor gene, p16, responsible for slowing the cellular progression from G1 phase to the S phase by inhibiting cyclin dependent kinase is also found to be inactivated in later stages of tumor progression. IHC analysis of in-situ tumors has also demonstrated that p16 is also inactivated with high frequency in head neck squamous cell carcinoma (Reed et al., 1996). Though specific mechanisms for its inactivation still require further investigation.

### *Chromosomal Region 9p21*

Loss of heterozygosity (LOH), epigenetic methylation or outright deletion on chromosomes are key in many tumor types. The INK4<sub>a</sub>-ARF (CDKN2A) locus which encodes two tumor suppressor genes, p14<sup>ARF</sup> and p16<sup>INK4a</sup>, is located on chromosome 9p21. These two distinct genes mark the convergence of the p53 and

Rb tumor suppressive pathways. Studies have found LOH at 9p21-22 in 72% of informative tumors with somatic mutations and methylation of the p16<sup>ARF</sup> promoter region at the INK4a-ARF locus occurring frequently in both malignant and benign tumors (van der Riet et al., 1994; Weber, Wittekind, & Tannapfel, 2003).

### **Treatment and Current Standard of Care**

Most patients (66%) that are diagnosed with HNSCC usually present with locally advanced, stage III or stage IV malignancies that usually require multi-modal therapy consisting of chemotherapy, radiotherapy, and surgical resection. Surgery and radiotherapy (RT) have been the mainstays of therapy for HNSCC, yielding OS rates ranging from 10%-40% (Vokes, Weichselbaum, Lippman, & Hong, 1993). If patients present with early stages of the disease (stage I, stage II) single-modality therapy, either radiation or surgical resection alone, is sufficient and offers better outcomes. Though in these early disease cases the prognosis is good, local and regional disease recurrence takes place in approximately 30% of patients and distant metastasis appears in 25%, therefore many of these patients are still at risk and must be closely monitored (Cooper, Pajak, & Forastiere, 2004; Pillsbury & Clark, 1997).

#### *Surgical Resection*

Invasive surgery is always challenging; this is ever more true when it involves the head and neck owing to the immense anatomical complexity. Preservation of structure and function when considering treatments options should always be of foremost concern; achieving these goals though is a challenge as both the disease and treatment affect major surrounding vessels. Historically, despite the facial mutilation that often accompanied it, surgery has been preferentially favored due to the decreased likelihood for post-operative metastasis. However, in recent years, surgical techniques have vastly improved and with them so too have prognoses. Minimally invasive keyhole surgeries and multi-modal imaging techniques in conjunction with advances in reconstructive surgery have provided better aesthetic, functional, and clinical outcomes across the board (D'Cruz et al., 2015; Sadick, Schoenberg, Hoermann, & Sadick, 2012; Urken, 2003). Employing these novel methods and the wealth of pre-operative information, surgeons are able to avoid performing the mutilating resections that used to be commonplace and patients no longer face the daunting prospect of perpetual disfigurement. Unfortunately, with so much emphasis placed on surgical skill and resources, surgical outcomes vary widely depending greatly on the treatment center's facilities and level of experience.

### *Chemotherapy*

Advances in treatment have not been isolated to surgery alone, chemotherapy, radiation therapy (RT), and targeted molecular therapies have

evolved immensely in parallel. Chemotherapy, the backbone of the locally advanced disease treatment regimen, is now commonly administered concurrently or prior to radiation. Induction chemotherapy (IC), the administration of chemotherapeutic agents (generally cisplatin and fluorouracil) prior to RT, has been studied for many years. Despite OS remaining the same, some evidence suggest that IC offers improved organ preservation, reduced metastasis, and overall benefit to locoregional control and thus remains a common treatment therapy for advanced HNSCC (Harari, 1997; Ock et al., 2016). Typically, tumors shrink drastically in response to chemotherapy; therefore, IC is utilized to reduce tumor size prior to surgery or RT with the intention to reduce surgical invasiveness or increased toxicity. More recently, concurrent chemoradiotherapy (CCRT) has found use as an alternative to surgical resection, however, it can also be performed post-operatively (adjuvant chemoradiation), if a patient has increased likelihood for recurrence. CCRT delivers chemotherapy concomitantly with radiation and has been widely adopted as the primary treatment for a variety of solid tumors. Though schedule optimization and agent interactions are still under investigation its use has yielded promising results. In HNSCC, CCRT has shown to improve overall 5-year survival by approximately 8% in comparison to RT alone and in high-risk post-operative patients significantly improves the rates of local and regional control and disease-free survival despite the associated adverse effects (Brizel et al., 1998; Cooper et al., 2004; Pignon, Bourhis, Domenge, & Designé, 2000; Zhang et al., 2010).



## *Radiation Therapy*

Fractionation RT, the process by which the total radiation dose is divided among several smaller doses administered over a period of days, is employed in the treatment of multiple cancers and has shown to decrease the toxic side effects on the surrounding healthy cells (Ghita et al., 2016). This has improved overall patient tolerance and therapy success. Fractionation schedules vary; a common schedule may divide a dose into 30 units over the course of 5 to 6 weeks. As technology continues to evolve, conventional fractionation continues to be challenged by interval and dose varying strategies such as hypofractionation and hyperfractionation. Hypofractionation, an amplified version of fractionation RT that delivers higher doses of radiation in a shorter duration of time is currently undergoing evaluation due to its convenience and decreased financial burden. While research continues to be done, moderately hypofractionated therapy is likely to become the standard treatment for many cancers (Koulis, Phan, & Olivotto, 2015; Wilkins et al., 2015).

Another advancement in RT, intensity-modulated radiation (IMRT) enables the precise delivery of RT to the tumor by conforming the dose to the three dimensional shape of the tumor while sparing the surrounding healthy cells (Deloch et al., 2016). Modulating the intensity of the radiation beam to target the tumor alone has also improved outcomes significantly specifically in complex anatomical regions such as the head and neck (Al-Mamgani et al., 2015).

### *Targeted Therapies*

Finally, as our understanding of molecular biology and cancers biological mechanisms have grown, molecular targeted therapies have arisen. Monoclonal antibodies (mAb), molecules that bind to an extracellular epitope, have come to the forefront of cancer treatment regimens as a means to specifically inhibit certain problematic signaling pathways. Cetuximab, one of the mAbs approved for use in the treatment of HNSCC, is a chimeric mouse-human model that functions by binding to EGFR and turning off uncontrolled growth signaling. The use for Cetuximab is supported by EGFR overexpression in 80% to 90% HNSCC tumors and there is evidence to suggest that this occurs as a result of gene amplification (Nagatsuka, Ishiwari, Tsujigiwa, Nakano, & Nagai, 2001).

### *Investigation of Genetic Markers*

Ultimately, the current state of HNSCC offers little hope to those it affects. The treatments available are aggressive, painful and at their culmination offer little by way of success. Furthermore, options for early diagnoses are limited as patients only present once the cancer has progressed to the point that it is noticeable. For these reasons, it is critical that we investigate genetic markers such as VEGF that are constitutively mutated throughout the patient population to identify their value as prognostic markers and potential inclusion in diagnostic panels.

## **PUBLISHED STUDIES**

Vascular endothelial growth factor (VEGF) is a protein that binds to a family of tyrosine kinase receptors (TKRs) known as vascular endothelial growth factor receptors (VEGFR) on the cell surface promoting angiogenesis. Numerous studies have found that VEGF is critically expressed during embryogenesis in order to develop the vascular supply. Throughout adult life VEGF is tightly regulated; it is expressed during wound healing, muscle growth, and during reproductive function. Angiogenic proteins are primarily stimulated by hypoxic cellular conditions and function to return oxygen to these tissues by encouraging proliferation, differentiation, and migration of vascular endothelial cells and increasing capillary permeability (Breslin, Pappas, & Cerveira, 2003; Ferrara & Davis-Smyth, 1997).

A number of pathologies advantageously overexpress VEGF. In proliferative retinopathies and age-related macular degeneration, the expression of VEGF is the primary disease-causing agent leading to leakage, bleeding, clot formation and fibrosis. In cancer, however, VEGF plays a supporting role, allowing solid tumors to overcome the growth limitations imposed by their environment's current-state vasculature. Furthermore, the immature basement membranes of the budding vessels are far more pliable providing tumor cells with an opportunity for vasculature invasion and distant metastasis (Mineta et al., 2000; Sauter, Nesbit, Watson, & Klein-Szanto..., 1999). VEGF expression also prevents cellular apoptosis pathways, which has been linked to increased resistance to certain therapeutics

(Gupta, Kshirsagar, Li, & Gui, 1999; Tran, Master, Joanne, & Rak, 2002). As a result, VEGF expression marks a key transition point in the progress of the cancer strongly indicating a poor prognosis due to its association with increased lymph-node spread, subsequently lowered OS, and increased risk of chemoresistance (Mineta et al., 2000).

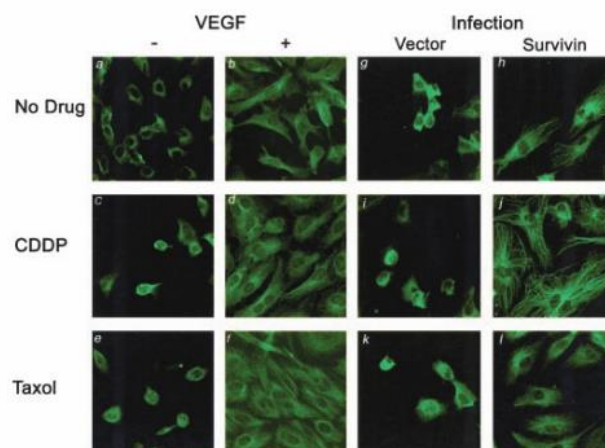
The value of VEGF as prognostic marker has been demonstrated time and again in a number of cancers. However, its value to HNSCC was identified in a meta-analysis study performed in 2005 (Kyzas, Cunha, & Ioannidis, 2005). The study not only identified an association between VEGF overexpression and mortality but also highlighted a more limited association with lymph node metastasis. After a filtering process the investigators ultimately identified 17 studies ( $n = 1,287$  patients) eligible for inclusion in the meta-analyses eight from Japan, eight from Europe, and one from the United States. In each study, VEGF measurements had been taken in patients with HNSCC. The selected studies all had taken measurements at the primary site prior to the administration of chemotherapy or radiation. Of the seventeen studies that were eligible, twelve studies ( $n = 1,002$  patients) contained data on 2-year survival that could be obtained from published or corresponded information. Of the seventeen studies, five studies ( $n = 285$  patients) contained only lymph node status data, while six of the twelve studies that contained 2-year OS ( $n = 437$  patients) contained data on lymph node status as well. Ultimately eleven studies ( $n = 722$  patients) were analyzed for the status of lymph nodes. Across the eligible studies the reported median age of the patients ranged from 58 – 71 years of age. The meta-

analysis identified significant association between prognosis and VEGF overexpression. Mortality was found to be 1.88-fold higher in VEGF positive patients. Moreover, the study also loosely identified an association between lymph node metastasis and VEGF expression, however, the result was not statistically significant.

A study performed in 2001 illustrated the protective role of both basic fibroblast growth factor (bFGF) and VEGF by epigenetically inducing drug resistance in human umbilical vein endothelial cells (HUVECs) (Tran et al., 2002). This study demonstrated that the PI3K and PKB activation are critical for VEGF mediated chemoresistance, by selectively inhibiting/activating each pathway to identify if cells remained resistant. In addition, the induction of anti-apoptotic protein survivin was also examined. As shown in other similar studies survivin levels were upregulated 10 to 20-fold in VEGF stimulated cells and these levels remained the same in the presence of chemotherapy (Taxol and Vinblastine (VBL)). Of note, PI3K activity was required for VEGF-mediated induction of survivin and bFGF also induced a 10 to 20-fold increase in survivin expression. Noticing that survivin protein levels remained high in the presence of disrupting microtubule drugs such as Vinblastine and Taxol, Tran et al., further analyzed the role survivin played on microtubule structure. They found that in the absence of VEGF untreated cells and those treated with either CDDP or Taxol all appeared to have a similar rounded morphology with little microtubule organization (Figure 2; *a, c, e*). In contrast, cells grown in the presence of VEGF (+) display an elongated structure and were spread

out (Figure 2; *b*). The same healthy elongated morphology similar to that of the growing cells were observed for cells in the presence of VEGF and the presence of CDDP and Taxol (Figure 2; *d, f*). Based on this it is clear that VEGF is a mediator of microtubule integrity. To further test survivin's role as the key agent conferring chemoresistance, HUVECs were retrovirally infected with an empty vector or wild-type survivin. The cells retrovirally infected with wild-type surviving display a similar healthy morphology as those treated with VEGF in the presence of CDDP and Taxol (Figure 2; *h, i, j*). Tran et al., concluded that survivin, a rate-limiting regulator of apoptotic threshold, is a primary mediator of VEGF chemoresistance promoting preservation of cellular integrity in the presence of chemotherapeutic drugs (Tran et al., 2002).

**Figure 3: Induction of survivin mediates the integrity of the microtubule network in the presence of VEGF in HUVECs. HUVECs were stained for  $\beta$ -tubulin and grown in the presence of VEGF and with or without drug. (Tran et al., 2002)**



## DISCUSSION

VEGF plays a vital and irreplaceable role in the body; one that HNSCC and many other cancers takes advantage of to proliferate and expand. Based on our current understanding it is clear that the investigation of biological markers, such as VEGF, as indicators of prognosis has significant therapeutic value. While VEGF's role as a prognostic marker in multiple neoplastic cancers has been known for some time, it has not yet found ubiquitous adoption as a tool to inform therapeutic decisions taken by patients and their doctors.

For lifestyle cancers, such as HNSCC, the most successful and obvious curative method is, of course, prevention through abstinence of risk factors. However, in lieu of this it is critical that we employ the recognizable pattern of mutations that lead up to outright carcinoma by adopting early-detection screening. Early-detection screening for known at-risk patients will likely have a profound effect on the OS for HNSCC, which currently remains steady at approximately five years. Close monitoring of patients in the pre-malignant stages of the cancer will allow for far earlier detection, less invasive surgical procedures, less aggressive therapeutic regimens and likely will drive OS up.

VEGF upregulation marks a critical transition point in the progression of HNSCC indicating that research dollars should be allocated to the identification and detection of VEGF and other mutations occurring prior to its dysregulation; most importantly those that occur during the transition from severe dysplasia to

carcinoma in-situ. While current research has identified a preliminary pattern of mutations that lead up to outright carcinoma a clear panel of markers and their correlated staging remains to be created. As a diagnostic tool this offers a promising low-cost solution to inform HNSCC therapy decisions in the third world nations in which it is most prevalent.

Moving forward it is important that care decisions leverage information garnered from early-detection screening and marker panels. As technology and medical innovation usher in a new era of low-cost, efficient, connected, personalized healthcare it is critical that practitioners augment their practices to account for these advances. This new paradigm for personalized treatment curtailed to the specific characteristics of an individual's disease offers patients the ability to take ownership in their own care. Ultimately, we enable patients to dictate the narrative of their own lives, whether it be to pursue curative treatment or to live out the remainder of their days in the comfort of their own homes.



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