The expression of E-cadherin and β-catenin in squamous cell carcinoma of the esophagus

By

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DECLARATION

I, CORNELIUS MUZI NKOSI hereby declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise) and that neither the whole work or any part has been, is being or is to be submitted for another degree at this or any other university or tertiary education institution or examining body

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DEDICATION

First and foremost I would like to thank God for being there with me all the way. I also thank my family Nomthandazo Nkosi my mother, Sizakele ,Khumbuzile and Anele my three lovely sisters and my two wonderful brothers Mbuso and Musa for their constant support and guidance they gave me though my years of study and not forgetting my late dad Mr Dan Nkosi thanking you once more for opening doors to my dreams. I also dedicate this work to a most instrumental person in my life my Fiancee Kebawetse Eulain Mashogoane and Entle Khaya Nkosi my son, with your love and support I will always rise above all odds.

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INDEX OF CONTENTS

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SUMMARY

Background

Esophageal squamous cell carcinoma (SCC) remains a disease of poor prognosis. Early diagnosis is compromised by the delayed onset of symptoms. By the time of surgical intervention metastases and organ infiltration have already occurred this reduces the prognosis significantly and the 5-year survival rate of operative advanced esophageal SCC remains poor. In order to select an appropriate therapeutic regime and guard against both over- and under treatment, reproducible prognostic markers are needed at the time of diagnosis. The study evaluates the phenotypic expression of E-cadherin and β- catenin in SCC of the esophagus.

Methods: The expression patterns of both β-catenin and E-cadherin was determined using immunohistochemistry technique in patients with esophageal SCC with the application of the Broders and Brynes grading systems in assessing clinical outcome**.** Forty four cases were randomly selected, one case was esophagectomy, and 43 were endoscopic biopsies with one case of Broders Grade I, 37 Grade II and 6 Grade III and 9 cases had pattern 2 and 35 had pattern 3 with Brynes Grade.

Results: The reduced expression of E-cadherin and β-catenin was 45.5% and 47.7% respectively. No significant level was observed with E-cadherin (P= 0.20) and for β-catenin (P= 0.18) but the low protein level of both biomarkers was associated with tumor cell differentiation with Broders classification. The reduced expression of E-cadherin on invasive tumor front was 27% and 57% for reduced expression of β-catenin. The level of significance was found to be (P=1.00) for E-cadherin expression and (P=0.02) for β-catenin. E-cadherin and β-catenin showed reduced expression on invading tumor front and β-catenin was associated with tumor cell invasiveness.

Conclusion: The expression of E-cadherin and β-catenin with regard to Broders classification showed no significance on tumor cell differentiation and these expressions do not play a role in guiding nor

predict the behavior or progression of the tumor. However, the assessment β-catenin on the tumor invasive front (Brynes) shows a high correlated with tumor behavior as it is involved in regulation E-cadherin function.

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: INTRODUCTION AND REVIEW OF THE LITERATURE

1.1 Anatomy of the esophagus

The esophagus is a muscular tube approximately 10 inches (25 cm.) long extending from the hypopharynx to the stomach. The esophagus is located posterior to the trachea and the heart, and passes through the mediastinum and the hiatus, an opening in the diaphragm, in its descent from the thoracic- to the abdominal cavities. The esophagus has no serosal layer and the tissue around it is referred to as the adventitia. There are two sub-site descriptions for the esophagus and they are not equal in length. The first is the cervical esophagus, which begins at the lower end of pharynx (level of 6th vertebra or lower border of cricoid cartilage) and extends to the thoracic inlet (suprasternal notch 18 cm from incisors), followed by the thoracic esophagus which is subdivided into: 1) Upper thoracic (from thoracic inlet to level of tracheal bifurcation; 18-23 cm) 2) Mid thoracic (from tracheal bifuraction midway to gastroesophageal junction; 24-32 cm) 3) Lower thoracic (from midway between tracheal bifurcation and gastroesophageal junction to the gastric junction, including abdominal esophagus; 32-40 cm) which is considered part of lower thoracic esophagus.[1]

The mucosa of the esophagus is lined by a squamous epithelium. Categorization of the epithelium is based on the number of layers and on the morphology of the most superficial cells. Squamous epithelium usually consists of 5-7 cell layers. The basal cells are cuboidal to elongated so that the long axis of the cell is perpendicular to the basement membrane. In normal epithelium, the basal cell layer, and perhaps the one directly above it, are the only layers engaged in active cell division. For this reason, mitotic figures are the norm in the basal and parabasal layers. Mitotic figures above this level are of concern and may indicate dysplastic (pre-neoplastic) epithelial change. The epithelial cells towards the surface layer progressively flatten. The cells on the surface of the epithelium are

completely flattened, contains keratin and are therefore ideal for complete surface coverage and protection. [2]

1.2 Esophageal cancer

1.2.1 Introduction

Esophageal cancer is generally considered as one of the most aggressive carcinomas with a poor prognosis.[3] Two histologic subtypes account for more than 95% of esophageal malignancies, i.e. adenocarcinoma and SCC.[4] Annually approximately 13 200 Americans are diagnosed with esophageal cancer and 12 500 die of this malignancy.[5] Although surgical techniques and preoperative management have progressed significantly over recent years, early diagnosis and early treatment remain the pillars of long-term survival. With modern therapeutic regimes, the 5 year post-operative survival of the patients with esophageal carcinoma has improved only slightly to 20-25%.[3] In order to select an appropriate therapeutic regime and guard against both over- and under treatment, reproducible prognostic markers are needed at the time diagnosis.

Epidemiological studies identified unique features in the global distribution of esophageal SCC. The highest mortality rates are found in China accounting for 26.5% deaths in males and 19.7% in females. The incidence of SCC is significantly higher among US Black men (16.8 per 100.000) than among US White men (1.0 per 100.000). To date, high incidence areas include China (21 per 100.000), South America (13 per 100.000), Western Europe (11 per 100.000), South Africa (10 per 100.000), Japan (9 per 100.000) and the former Soviet Union (8 per 100.000).[6] However, in high-rate areas, the cancer appears almost as often in woman as in men suggesting exposure to the same causative factors.[7] The incidence and mortality from this tumor has increased rapidly over the past 25 years, [8] a trend also observed in South Africa.[9] Between 1993 and1995, 3914 new cases were

reported in males and 1943 in females in South Africa. A total of 777 deaths in females and 1.762 deaths in males were reported by the Cancer Surveillance System (CSS) in 1994. The crude incidence rate was 3.4/100 000 in females and 7.1/100 000 in males (the age standardized incidence rate for females were 4.9/100 000 and for males 11.6/100 000). [10] Incidence of esophageal cancer increases with age, with the lowest numbers before the age of 30 and the highest at the age 70.[11] One in 59 men have a life time risk of developing esophageal cancer in South Africa.[10]

Once symptoms are present (dysphagia, in most cases), esophageal SCC has usually invaded the muscularis propria or beyond and may have metastasized to regional lymph nodes or other organs.[12] At this stage irradiation, chemotherapy and surgery offer little hope of survival.[3] Patients with adenocarcinoma tend to experience heartburn leading to a consultation with their physicians, where the disease is diagnosed after the taking of an endoscopic biopsy.

Esophageal SCC is a multifactorial disease and no single etiological agent has been identified so far. Esophageal carcinoma has been reported in association with lye strictures, achalasia, Plummer-Vinson syndrome, diverticula, celiac sprue and tylosis (an autosomal dominant disorder characterized by hyperkeratosis of palms and soles). Some authors have also found an increased incidence of esophagitis, and a history of previous gastrectomy; a few cases of esophageal carcinoma have been reported in association with humoral hypercalcemia. The genetic mechanism of carcinogenesis in esophageal SCC is poorly understood. Tumor progression in SCC was found to be associated with genetic alterations, including loss of heterozygosity in chromosomes 3p, 5q, 9p, 13q, 17p, 17q and 18q and amplification of epidermal growth factor receptor (EGFR), human EGFR (HER)-2, c-myc and cyclin D1.[13] Chromosome 17q25.2-25.3 carries the autosomal dominant determinant for the esophageal disorder tylosis.[14] One study found the most frequent genetic

alteration in esophageal SCC was associated with a point mutation of the p53 gene (40-60%), which occurs at a relatively early stage of tumor development.[15] Another study found no genetic alteration but identified abnormal expression of cell to cell adhesion molecules, which are E-cadherin and β-catenin.[16] Some studies showed intercellular adherens junctions playing a pivotal role in the tumor growth, invasion, metastasis and prognosis and the suppression of cell-cell adhesiveness triggering the release of cancer cells from the primary cancer nest and confer invasive properties on a tumor.[17]

It has been found that cancers in vivo, particularly in the types where tumor cells are dissociated throughout the entire tumor mass, the cells lose their cell polarity, and infiltrate the stroma in a scattered manner. One of the most characteristic features of cultured cancer cells in vitro is loss of "contact inhibition," which reflects disordered signal transduction from the cell-cell adhesion to cell growth. Moreover, invasion and metastasis, which are the most life threatening properties of most malignant tumors, are considered to be late, but critically important, carcinogenic steps. Recently various attempts have been made to investigate the relationship between certain molecular markers and the clinical course of squamous cell carcinoma of the esophagus. The biological factors that determine differences in a patients outcome are obscure.[3, 12, 13]

In general, E-cadherin and β-catenins are strongly expressed in well differentiated cancers that maintain cell adhesion and are less invasive. Their expressions are reduced in poorly differentiated tumors that lost their intercellular adhesive properties and exhibit strong invasive behavior. Loss or reduction of the E-cadherin and β-catenin expression is known to play an important role in tumor progression and metastasis and has also been reported to be associated with a poor prognosis in many carcinomas such as stomach, colon, liver, prostate, and pancreas cancer.[18, 19]

1.2.2 General gross description

Esophageal SCC can occur in any portion of the esophagus but is most common in the middle and lower thirds in areas of normal anatomical constriction.[20] The dysplastic pre neoplastic phase, where cytologically abnormal cells are found in the epithelial lining of the esophagus, may be clinically unapparent. The carcinoma-in-situ phase, where cytologically abnormal cells are seen throughout all the layers of the epithelium, also may have minimal clinical change. Due to their low clinical levels of detection, dysplastic- and carcinoma-in-situ lesions are seldomly diagnosed microscopically. The carcinomas in their early phase are usually flat with minimal thickening of the mucosa. As the carcinoma grows it most commonly becomes exophytic forming an intraluminal mass. Esophageal obstruction is due to luminal obliteration by the tumor. Some carcinomas remain relatively flat and as they expand and outgrow their blood supply undergo areas of central necrosis.[21] Other SCCs grow circumferential and often ulcerate, with sharply demarcated margins. Polypoid forms occur and intraepithelial spread is frequent, with or without involvement of the ducts of mucosal glands. Blood vessel invasion is seen in three fourths of the advanced cases. In some cases, separate tumor nodules are seen in the wall of the esophagus or stomach (so called “intramural metastasis”).[20]

1.2.3 General microscopic description

Esophageal SCC is an epithelial malignancy with morphologic features of squamous cell differentiation without additional features suggestive of other differentiated tissues. The features of squamous differentiation, observable on routine stained sections with light microscopy, include one or more of the following: (1) Flattened polyhedral, round, or ovoid epithelial cells; (2) Intracellular keratinization; and (3) Intercellular bridges. If features of spindle cell differentiation, glandular differentiation, or basal-cell differentiation are present with the squamous features just listed, the tumors are named to reflect these distinctive features.[21] Most esophageal SCC’s are well or

moderately differentiated with typical features of SCC including: keratin pearls; intercellular bridges; single cell keratinization; and a sheet-like growth pattern.[20]

1.2.4 Early cancer and superficial carcinoma

So-called 'early cancer' of the esophagus is a clinical concept that, in terms of pathology, corresponds to invasive carcinomas strictly confined to the mucosa or submucosa, with or without lymph node spread. In the most recent edition of the guidelines for the clinical and pathologic studies on carcinoma of the esophagus,[22] the definition of early esophagus carcinoma has been changed. It is now defined as an intramucosal carcinoma without nodal or distant metastases. Superficial carcinoma is defined as carcinoma in situ, or carcinoma involving mucosa or submucosa, regardless of the presence of lymph node metastases. It is a distinctive feature of esophageal cancer that lymph node metastases is often found in superficial cancers, while it is relatively infrequent in early gastric or colon cancers.[23]

1.2.5 Variants of squamous cell carcinoma

1.2.5.1 Spindle cell carcinoma

This morphologically deceptive and unusual tumor has been referred to as spindle carcinoma, carcinosarcoma, spindle-cell SCC, or sarcomatoid carcinoma. All of these terms have distinct disadvantages. Carcinosarcoma is a distinctly different tumor with both malignant epithelial and malignant mesenchymal elements and should not be confused with spindle cell carcinoma. Spindle cell carcinoma is a polypoid, exophitic, or fungating mass that occurs in any head or neck site and surface ulceration is common. As with any ulcerated lesion, they are likely to be infected and therefore may exude pus or contain abscesses. On histology, the features of spindle cell carcinoma are: elongated epithelial cells with central, elongated nuclei. These are arranged in entangled

fascicles and intertwining bundles. The degree of hyperchromasia, enlarged nuclear to cytoplsmic ratio, mitosis and pleomorphism are strongly suggestive of a leiomyosarcoma or a fibrosarcoma. Not infrequently, foci with a storiform pattern may be seen; These may resemble a malignant fibrous histiocytoma. Vascular structures are occasionally associated with the tumor. The stroma may be heavily collagenized, presenting a hyalinized appearance or, at the other end of the spectrum, may be loose and myxoid. Multinucleated giant cells occur at times and, in the setting of hyalinized collagen, which resembles osteoid confusion with an osteosarcoma is possible.[24, 25] A poorly differentiated SCC is often associated with spindle cell areas.[20, 21]

1.2.5.2 Basaloid squamous cell carcinoma

Basaloid SCC is less common than conventional SCC of the head and neck. It is considered more aggressive than classic SCC with a tendency to involve the base of the tongue, supraglottic larynx, the pyriform fossae, esophagus, lungs, anal region, and uterine cervix. Its reputation for aggressive behavior is well earned in that, aside from widespread local invasion at the time of presentation, many patients have local metastases when the lesion becomes apparent. Men are 4 times more likely than woman to suffer this cancer, and most patients are aged 40-70 years. Almost all basaloid SCCs have areas showing features of conventional SCC. In addition, they have a follicular or lobular pattern on invasion, with peripheral, slightly elongated, palisaded cells surrounding each lobule. On serial sections, these follicles are interconnected. The lobules often contain central comedo necrosis with visible necrotic material. At other times the central material completely ‘dries out,’ giving a pseudo glandular appearance. Of interest is that a hyaline basement membrane-like material is occasionally present in the center of the islands.[26, 27]

1.2.5.3 Verrucous carcinoma

As the name suggest, verrucous carcinoma is a growth with a gross appearance of a verruca or wart. Verrucous carcinomas are often immense in size at presentation. Despite their size, however, pure forms of verrucous carcinoma rarely, if ever, metastasize. The tumors are indeed carcinomas in that they are locally destructive but are extremely well differentiated. In cytologically terms, they do not posses any of the qualities that one usually associates with malignancy. They have no increased nuclear-to cytoplasmic ratio, hyperchromatasia, increased number of atypical mitotic figures, or pleomorphism. The architecture is that of a broad, pushing, and expansile epithelial masses that extends into the stroma. Intense infiltration of mononuclear inflammatory cells at the interface of the tumor with the stroma is frequently present. These warty exophytic, extremely well differentiated tumors that are slow growing have a good prognosis.[28, 29]

1.2.5.4 Papillary squamous cell carcinoma

SCC with a papillary growth pattern occurs in the esophagus.[20] Papillary or exophitic lesions with full-thickness atypia (SCC in situ) and /or frankly invasive SCC as a component has been reported in many locations in the body. These include the skin, uterine cervix, and even the eye, as well as the larynx, oropharynx, nasal spectrum, esophagus, and nasopharynx. Papillary SCCs have been reported in both sexes and in patients aged 30-80 years. The mean age of presentation is in the sixties and women are affected less often than men. On histology, in situ or invasive papillary SCCs have similar architectures. They contain benign, fibrovascular cores with overlying squamous epithelium. The epithelial layer may be keratinizing or non-keratinizing and, with in situ lesions, full-thickness atypia is present. As usual, the atypia manifest as increased nuclear-to-cytoplasmic ratio, nuclear hyperchromatasia, angulated nuclei, various degrees of pleomorphism, increased mitotic figures (especially above the basal layers), and atypical mitoses. In addition, an unusual type

of cellular atypia, known as koilocytic atypia, is present. This particular form of cellular distortion is related to HPV infection of the epithelial cells. What is interesting about this koilocytotic atypia is that it is not expressed in all cells of papillary SCC. In fact, it is seen in fewer cells than it is in benign laryngeal papillomatosis, a human papilloma virus induced infection of the laryngeal mucosa. If the lesion is of the in situ variety, full-thickness epithelial atypia is observed without an invasive component. This is the situation as in conventional SCC in situ. The invasive variety demonstrates frank stromal invasion. This component is indistinguishable from any other form of invasive SCC.[30, 31]

1.2.5.5 Small cell carcinoma

Small cell carcinoma, neuroendocrine carcinoma and anaplastic small cell carcinoma are highly malignant esophageal tumors having morphologic features similar to those of its pulmonary counterparts. Grossly, it usually exhibits a fungating pattern of growth. Occasionally, multiple foci are found. Microspically, small cells with dark nuclei of round or oval shape and very scanty cytoplasm are seen growing in a predominantly diffuse fashion. As in small cell carcinoma of the lung, there may be rosette formation and focal mucin secretion. The prognosis is poor and most patients die within one year with generalized metastases. This tumor probably arises from the same multipotent epithelial basal cells that give rise to conventional SCC. Supporting this contention is the fact that it may be found closely intermingled with in situ or invasive SCC.[20]

1.2.5.6 Mucoepidermoid carcinoma

Although mucoepidermoid carcinoma is a neoplasm of the salivary glands, it occurs in most of the locations in the upper aerodigestive tract where SCC s and their variants occur. Mucoepidermoid carcinoma occurs over a wide range, including the pediatric population. As the name implies,

mucoepidermoid carcinoma is a malignant epithelial neoplasm with both mucus producing cells and epidermoid (i.e. squamous) cells. These cells are presents in varying ratios in different tumors. This ratio is the criterion for grading the malignancy. The higher the percentage of squamous cells, the higher the grade of the tumor.[32, 20]

1.3 Etiologic factors

1.3.1 HPV

It has been suggested that human papilloma virus (HPV) plays an etiologic role in esophageal carcinogenesis [21] as histologic changes suggestion of viral infection have been reported in up to 40% of cases.[30, 31] HPV has a predilection for squamous epithelium where they induce benign proliferative lesions such as warts on the skin, papillomas and condylomas on mucosal surfaces.[33] HPV infection, especial HPV 16, 18 and 33, has been implicated in SCC carcinogenesis because of its ability to immortalise human epithelial cells after transfection.[34, 35] The tumor virus DNA is frequently integrated into cellular genome. This intergration interrupts certain open reading frames (ORFs) of the viral genome with a loss of DNA sequence. Two ORFs, E6 and E7 however are consistently retained and expressed in tumors and tumor cell lines and are considered to be important in the development and maintenance of the malignant phenotype.[36] Viral integration can either activate or inactivate cellular genomes resulting in a failure of host-cell control of persisting viral genes.[37] Various studies aimed at establishing the importance of HPV infections in human esophageal carcinoma have been performed. Two investigations carried out in South Africa described HPV epithelial changes adjacent to carcinomas in 30% and 60% of cases respectively.[38, 39]

1.3.2 Alcohol and smoking

Alcohol and tobacco appear to be major independent risk factors, with a possible synergistic role.[40] The association between tobacco use and alcohol consumption can be rather high, especially in North America and Europe where the risk of developing SCC in drinkers is up to 25 times that of non-drinkers.[41] In some parts of the world, most notably Iran and parts of Asia, alcohol is not as strongly associated with a risk of disease development. In Japan, it has been shown that genetic factors such as polymorphism in the aldehyde dehydrogenase-2 (ALDH-2) gene, along with alcohol consumption, have been associated with an increased risk of esophageal SCC.[42] Although alcohol has not been shown to be carcinogenic, it may exert its effects by acting as a solvent for tobacco and other carcinogens or by increasing the permeability of the esophageal mucosa to these substances. The poor nutritional and dietary status of many alcoholics may also have superimposed effects.[43] In South Africa children of younger age are much prone to alcohol and drugs abuse that may later be a contributing factor in the increase in incidence of esophageal cancer. Tobacco use, either smoking (cigarette, cigars, and pipes) or chewing, independent of alcohol use, has been associated with a high risk of developing SCC. Prospective studies in the U.S and Europe have shown a two-to-six fold increase in the likelihood of developing SCC in smokers, while the chewing of tobacco, for example, in India and central Asia, present a threefold increase.[44] The smoking of opium may be an important factor in the development of SCC in other areas such as Iran.[45]

1.3.3 Dietary

A variety of dietary deficiencies have been suggested including low protein and low caloric diets or diets deficient in vitamins A, C, and E, Riboflavin, trace elements such as Zinc and molybdenum, and diets high in nitrosamines or compounds that can be converted into nitrosamines.[46] Extensive research in China and South Africa has suggested that N-nitoso compounds and their precursors are

probable etiologic factors for esophageal cancer in these high incidence areas. Several nitrosamines, including N-nitrosomethylbenzylamine (NMBA), have been isolated and identified in the diets and gastric juices collected from subjects in Linxian County in Henan province, China. In some areas of China vegetables are not picked until they are covered by molds, most notably Geotrichum- and Fusarium species, which are strongly associated with the development of SCC.[47, 48] Consumption of salt-pickled food has also been impicated in the pathogenesis of the disease.[49]

1.3.4 Non-reflux esophagitis

In high risk areas for esophageal cancer, esophagitis is common and may be seen anywhere from 60-85% of patients in screening studies. It commonly involves the middle and lower esophagus, sparing the gastroesophageal junction.[50] Appropriate controlled studies to determine whether patients without these changes would be similarly predisposed have not been carried out, so the significance of esophagitis remains uncertain.[51]

1.4 Predisposing factors

1.4.1 Celiac disease

An association between celiac disease and SCC of the esophagus has been suggested; the pathogenic mechanism may include dietary deficiency of vitamins and trace elements or increase permeability of jejunal epithelium to carinogenes.[20]

1.4.2 Previous gastric resection

Previous gastric resection may increase the risk of developing SCC in the lower esophagus. Reports mainly from Japan have demonstrated SCC arising in 3, 9 and 9, 3% of patients with gastric resection for cancer or for any reason respectively.[20]

1.5 Molecular genetics of esophageal cancer and tumor suppressor genes

As esophageal carcinogenesis is poorly understood, research is being carried out to expose the precise mechanism involved in dysplasia of esophageal precancer. It is known that tumor suppressor genes, oncogenes, and apoptotic genes are involved in the initiation and development of esophageal cancer, but to date no gene directly related to esophageal cancer has been identified.[52] Previous studies have demonstrated that p53 gene mutation occur at early stages of esophageal carcinogenesis, including mild basal cell hyperplasia and near-normal epithelia. Intra-tumor heterogeneity in the distribution of mutant p53 allele has been reported in human prostate cancer. In another study, however, p53 gene mutation was identical in all tumor areas of each case investigated. [53] The hotspots for mutation in human cancers were identified on codons 175, 245, 248, 249, 273, 282.[54] A subsequent study of esophageal SCC patient with and without family history of upper gastrointestinal tract cancer confirmed the frequent allelic loss in 14 chromosomal regions. The loss of heterogeneity (LOH) on locus 13q (D13S894) was more in common in patients with a family history of upper gastrointestinal cancer than in those without such a history. The primary finding of one study include a high level of genetic instability among the precursor lesion, four chromosomal regions (3q, 4p, 9q, and 13q) have been identified to undergo genetic change early in the neoplastic process, and a high level of intratumoral and preneoplastic genetic heterogeneity. This instability and heterogeneity exemplify the complexity of the genetic changes associated with the initiation and progression of esophageal SCC.[55]

It has been shown that both elevated and low levels of apoptosis can have a detrimental effect on the host. Recently, reports have shown that esophageal tumor cells abundantly express Fas ligand (Fasl) in vivo. Fas ligand expressed by esophageal cell lines has been shown to induce apoptosis on co-

cultured fas-sensitive lymphoid cells in vitro. Esophageal cancer cells can deplete anti-tumor lymphocytes by inducing apoptosis.[56] Telomerase activity levels have also been shown to correlate with tumor progression in several malignancies.[57]

The tumor suppressor gene Retinoblastoma (Rb), is a phospho-nuclear-protein which plays a significant role in cell cycle regulation. Hypophosphorylated Rb in the cell prevents cell progression when the cell is being assessed and upon phosphorylation the Rb protein has been found to release the E2F transcription factors that allows for the expression of important cell-cycle control genes.[58] Shi et al found that the LOH of the Rb gene correlated with the loss of pRb protein expression and associated with p53 alterations in human esophageal cancer. It was therefore suggested that associated Rb and p53 inactivation may also be the major event in the development and progression of esophageal cancer. There is also a believe that other genes in the Rb and p53 pathways contribute to the malignant transformation of the cells. In the majority of cases an alteration of p16, p15 or even both were shown to occur.[59]

1.5.1 Other genes believed to play a role in the development of esophageal cancer

1.5.1.1 Annexin 1

It has been shown by Lui et al. that the protein Annexin 1 is translocated from the plasma membrane in normal cells to the nuclear membrane in malignantt cells. It was found that Annexin 1 usually formed a consecutive typical trammel net on the plasma membrane of normal esophageal epithelia, but in esophageal cancer a great decrease was found on the cellular membrane and was highly expressed on the nuclear membrane, which was never found on normal esophageal epithelia. This data suggested that Annexin 1 translocation may be correlated with the tumorigenesis of esophageal cancer.[60]

1.5.1.2 Esophageal cancer related gene-4 (ECRG4)

Esophageal cancer related gene 4 (ECRG4) is a novel esophageal cancer related gene found to be down-regulated in esophageal SCC compared to normal esophageal tissues. It is located on chromosome 2q14.1-14.3 and contains 4 exons. ECRG4 down regulation is believed to play a role in the development of esophageal SCC. The mechanism in which it is inactivated has been demonstrated to be aberrant methylation of CpG islands in the core promoter of the ECRG4 gene.[61]

1.6 Other associations

SCC (but not adenocarcinoma) is clearly linked to low socioeconomic status.[62] Consumption of hot beverages, such as tea and fungal invasion of esophageal tissues leading to localized inflammation and irritation and have been suggested as additional promoting factors for cancers of the esophagus.[63]

1.7 Diagnosis

1.7.1 Clinical Diagnosis

The most common presenting symptoms include dysphagia and weight loss, which unfortunately are indicative of advance disease. In high-risk populations where screening programs are in place, early SCC and in situ disease are frequently asymptomatic but may sometimes present with odynophagia (possibly due to keratinization), retrosternal pain, dry throat, and back pain.[21] Paraneoplastic syndromes have been reported, with the most frequent being hypecalcaemia secondary to production of a parathyroid-like substance (PTHrP).[64, 65]

1.7.2 Clinical investigations

A barium swallow is usually the first test performed on a patient whose symptoms suggest esophageal cancer. After the patient swallowed a small amount of barium, a series of x-rays highlight bumps or raised areas on the normally smooth surface of the esophageal wall. It can also detect large, irregular areas that narrow the esophagus in patients with advanced cancer, but it cannot provide information about diseases that has spread beyond the esophagus. Endoscopy which is the diagnostic procedure that employs a thin lighted tube (endoscope) that is passed through the mouth, down the throat, and into the esophagus to examine the mucosa optically and identify abnormal areas that are subsequently biopsied. Once a diagnosis of esophageal cancer has been confirmed through biopsy, staging tests are performed to determine whether the disease has spread to tissues or organs near the original tumor or to other parts of the body. These tests may include computed tomography, endoscopic ultrasound, thoracoscopy, laparoscopy, and positron emission tomography.[66]

1.7.3 Immunohistochemical features

Esophageal SCC are invariably immunoreactive for keratins, the reaction patterns being related to the degree of differentiation of the tumor. Some poorly differentiated carcinomas stain positive for human chorionic gonadotrophin (HCG), even in the absence of morphologically demonstrable trophoblastic differentiation. Production of basement membrane components such as laminin and type IV collagen correlates with the degree of differentiation of the tumor. Epidermal growth factor receptor (EGFR) has been measured immunohistochemically and by binding assay and is found to be strongly expressed in most of the cases. Cyclin D1 is overexpressed in a high number of cases of esophageal SCC (and also of esophageal adenocarcinoma).[20] A number of test have been used to diagnose SCC of the esophagus, which include markers like keratin Cam 5.2, which strongly stain almost all SCC and most commonly more than 50% of neoplastic cells. The CK 18 component of

keratin CAM 5.2 accounts for the majority of tumor positivity, and keratin AE1/AE3, keratin 34βH11, and Involucrin also stain almost all SCC strongly in a diffuse cytoplasmic distribution. Approximately 85% to 100% of esophageal SCC reacts positively to CK19 and the staining response greatly depends on the tumor grade. Approximately 70% of well- and moderately differentiated carcinomas are reactive, most commonly in less than 50% of neoplastic cells. All poorly differentiated SCC stain with CK19, almost always in more than 50% of neoplastic cells, tiny percent of SCC are reactive with CK7 antibody, and CK5 antibody, especially the D5/16B4 clone that stains almost all SCC.[67] Although all of the markers mentioned above play an important role in the diagnosis, their expression shows no correlation with clinicopathological factors. E-cadherin and β-catenin are markers that hold great potential in explaining deteriorating prognosis during the progression of large tumors. These markers are discussed under 1.19 and 1.20.

1.8 Spread of esophageal squamous cell carcinoma

Transmural extension through the muscular layers and into peri-esophageal soft tissue is reported in about 70% of cases. The depth of invasion is also related to an increased likelihood of developing lymph node metastasis. Metastasis occurs in up to 50% of cases with submucosal involvement. In 90% of patients lymph node metastasis is common at the time of presentation and occurs early in the course of the disease, as demonstrated by the presents of celiac node metastasis in 40% of cases in which clinically no apparent evidence of metastatic disease existed. The pattern of node involvement may depend on the location of the tumor although variations occur. Tumors in the cervical region may preferentially involve the deep cervical, paraesophageal, and posterior mediastinal lymph node groups, whereas the upper and mid-thoracic tumors drain to paraesophageal, posterior mediastinal, and tracheobronchial nodes. Tumors in the lower esophagus may drain preferentially to paraesophageal, celiac, and splenic groups.[20]

1.9 Staging of esophageal cancer and prognosis

Stage 0 is the earliest stage of the disease. Cancer cells are confined to the epithelial lining of the esophagus. This corresponds with a histological diagnosis of carcinoma in situ. Stage I esophageal cancer is where cancer cells has spread slightly deeper, but still has extended to nearby tissues, lymph nodes, or other organs. In Stage IIA, the cancer has invaded the thick, muscular layer of the esophagus and may involve the connective tissue covering the outside of the esophagus. In Stage IIB, the cancer has spread to lymph nodes near the esophagus and may have invaded the deeper layers of esophageal wall. Stage III esophageal cancer has spread to tissues or lymph nodes near the esophagus or to the trachea. Stage IV has metastasized to distant organs like the liver, bones, and brain. Recurrent esophagus cancer is disease that develops in the esophagus or another part of the body after initial treatment.[68]

The overall prognosis of esophageal squamous cell carcinoma is extremely poor, the median survival after diagnosis being less than one year. The main causes of treatment failure are recurrence and metastasis of resected esophageal cancer. Factors thought to influence survival are following

1) Sex. In several series, females have fared better that males.[20]
2) Stage. In situ and intra mucosal carcinomas (Stage 0 and I) are nearly always curable, and the cure rate is significantly higher for superficial- than for deeper carcinomas.[20]
3) Lymph node metastasis. Among cases with nodal involvement, those with two or more positive nodes do worse than those with a single metastasis.[20]
4) Metastases to distant organs are also frequent, particularly to liver, lung, and adrenal glands. The tumor may also metastasize to the submucosa of the stomach, probably through the submucosal lymphatic plexus. Stage IV tumors have a grave prognosis.[20]

5) Tumor length. Data from the SEER program have indicated that tumor length is an independent predictor of mortality when controlling for depth of invasion in patients with localized disease.[20]

6) Microscopic grade (Broders). The effect of tumor grade on prognosis is controversial although some studies suggest a possible relationship to prognosis, and grade does not appear to be independently significant.[20]

7) Surgical margins. Involvement of circumferential surgical margins is associated with a high probability of local recurrence.[20]

8) DNA ploidy and proliferation indices. A correlation between aneuploidy and either survival or time to recurrence has been found in several studies (including some that performed a multivariate analysis) but not in others.[20, 21]

9) Epindermal growth factor receptor (EGFR). Over expression has been found to correlate with grade, lymph node status, and poor prognosis.[13]

10) P53. Patients whose tumors have over-expressed and/ or mutated p53 have a worse survival rate than the others.[20]

11) Patients with a marked infiltration of Langerhans cels (LC) in the tumor survived longer than those where infiltration was slight. The density of LCs therefore, serves as an indication of 'the efficiency of host defense against the carcinoma.[69]

1.10 Survival Rate

The occasional patients with early disease (Stages 0 and I) have a better chance of survival. Tumors limited to the submucosa have been found to have a 5yr survival of greater than 60% (often considerably high) which drops to 30% with the involvement of the muscularis propria and 10% with extention into the adventitial tissues. Nodal involvement has been shown to influence

prognosis. Patients without lymph node metastasis have a 5 yr survival rate of 40-49%; But with lymph node involvement this drops to anywhere from 40-22%.[20]

1.11 Treatment: surgery, chemotherapy and radiation

Esophageal cancer is a treatable disease, but it is rarely curable. Traditionally, radiation therapy has been the most common form of treatment for carcinoma of the upper two thirds of the esophagus, and surgery (in the form of esophago-gastrectomy) the usual approach for carcinoma of the lower third.[20] Primary treatment modalities include surgery alone or in combination with chemotherapy and radiation therapy. Combined modality include surgery (chemotherapy plus surgery, or chemotherapy and radiation therapy plus surgery) is under clinical evaluation. Effective palliation may be obtained in individual cases with various combinations of surgery, chemotherapy, radiation therapy, stents,[70] photodynamic therapy,[71] and endoscopic therapy with nd: YAG laser.[72] The optimal surgical procedure is controversial. One approach advocated is transhiatal esophagectomy with anastomosis of the stomach to the cervical esophagus. A second approach advocates abdominal mobilization of the stomach and transthoracic excision of the esophagus with anastomosis of the stomach to the upper thoracic esophagus or the cervical esophagus. One study concluded that transhiatal esophagectomy was associated with lower morbidity than transthoracic esophagectomy with extended en bloc lymphadenectomy; however, median overall disease-free and quality-adjusted survival did not differ significantly.[73] The removal of tumor along the lymphatic drainage by extending the node dissection may improve survival by decreasing the ratio of involved to removed nodes.[74] There is no apparently relationship between the degree of response to chemotherapy by the tumor and its degree of differentiation.[20] The overall results with any of these combination therapies remain disappointingly poor. All cases included in this study were retrieved from the files of the Dr George Mukhari hospital had no prior exposure to any form of therapy.

1.12 Gene therapeutic strategies

The management of esophageal cancer to date remains an unsolved problem. Extensive research on diagnostic markers and therapeutic analyses are carried out to generate a solution to this problem. It is believed that the cure will ultimately involve chemotherapy or radiation and a key drug that targets a specific molecule present only in the cancer cells and has a low or no toxicity effect on the normal surrounding cells.[75] Apoptosis inducing nucleotides (AINs) from CD57+HLA-DR bright-natural suppressor (57.DR-NS) cell lines were used to induce apoptosis in human esophageal cancer cells. This study revealed that AINs induce apoptosis in esophageal cancer cells through DNA strand breaks and caspase-3 activation. Research is in progress to develop an ideal anticancer agent, and several centers focus on the apoptosis generated in malignant cells. Induction of tumor cell apoptosis lack toxicity for normal cells and evade the side effects experienced in clinical trials of conventional chemotherapy.[76] The development of an antagonist to the anti-apoptotic gene, is a promising therapeutic strategy not only for esophageal cancer but various other types of cancers where this gene is highly expressed.[77] Other drugs of interest would be those targeting angiogenesis. Anti-angiogenesis drugs developed to inhibit blood and nutrient supply to the tumor cells is a potential therapeutic strategy as well. Researchers are currently working on the development of two angiogenesis molecules (angiostatin and endostatin) in combination with radiation or chemotherapy.[78, 79] A few genes with significant correlation to this disease have been found, and are currently being analyzed as potential candidates for determining prognosis and therapeutic strategies.

1.13 Histological grading of esophageal squamous cell carcinoma

Esophageal cancer is classified according to the 2002 American Joint Committee on cancer tumor-node-metastases (TNM) classification system, which takes into account the characteristics of the

primary tumor, regional nodal metastases, and distant metastatis.[68] This generic classification has long been used and serve as a guideline for treatment of carcinomas of the head and neck. Furthermore, Pathologist state their opinion on the grade of differentiation, as defined by Broders.[78] which involves the quantitative grading of cancer cells since the early 1920s. A lack of correlation between Broders' grading system and the actual prognosis of esophagus SCC has become evident. This has been explained by the phenomenon that Broders grading is based on a general microscopic opinion on the degree of differentiation of the neoplasm whereas SCC usually consists of a heterogeneous cell population with probable differences in invasiveness and metastatic behavior.[79] The classification of SCC, based on the differentiation or maturation of the tumor cell population alone, is of limited value as a basis for selection of a treatment regime as well as for prediction of the outcome of the disease.[79-81] In 1937, Jacobson et al. developed a multifactorial grading system where both tumor cell characteristics and tumor-host relations were taken into account.[79, 82] This system was successfully applied by others, but has not been widely used because it is time consuming.[83-85] Jacobson's malignancy grading system is of significant help in total assessment of SCC, taking into account the stage of invasion, vascular invasion, and the Pattern of invasion of the tissue structures of the esophagus by uncontrolled malignant cells, which are critical parameters that influence prognosis of patients. Further development of Jacobson's system have been done by Anneroth et al and Bryne et al, [79, 81] on SCC's of the oral cavity. In the original method described by Jacobson.[86] vascular invasion was added to a grading system described by Bryne at al,[81] which has been proved to have a high prognostic value. Bryne's grading consists of five morphological features: Degree of keratinization, nuclear polymorphism, number of mitoses, mode of invasion, and plasma-lymphocystic infiltration. Each of these features are scored from 1-4 according to definitions given by Anneroth et al.[79] The scores for each morphological feature were summed into a total malignancy

score. The factor with the highest prognostic value in Bryne's system is the pattern of invasion at the invasive front of the neoplasm.

1.14 Role of in situ hybridization

Hybridization histochemistry is used to locate specific messenger ribonucleic acid (mRNA) populations in tissue section. The principal tool for this technique is a labeled, recombinant deoxyribonucleic acid (DNA) or (RNA) probe with specific nucleotide sequence complementary to the mRNA of interest. This technology may be easily transferred from the research laboratory into clinical laboratories. It promises to offer an improved potential for the rapid detection of infectious disease by revealing the presence of non-mammalian DNA sequences. This greatly expands the power of cytochemistry and immunohistochemistry as a means of identifying the nature and functional status of pathologic processes as seen through the microscope. The great advantage of microscopic examination of the biopsy specimens over purely chemical analysis (polymerase chain reaction or PCR) is the ability to evaluate the tissue architecture. The localization of the probe may show an abnormal morphologic pattern that would be lost in a simple chemicals assay, and this additional advantage of in situ nucleic acid hybridization substantially adds to the capability to distinguish submicroscopic features with the aid of a light microscope. This extends our view of disease from the phenotypic- to the genotypic level.[87]

1.15 E-cadherin

1.15.1 Introduction

Cadherins are single transmembrane proteins that mediate cell to cell adhesion and include E-(epithelial), N-(neuronal), and P-(placental) cadherins [88]. E-cadherin is a calcium-dependent cell-cell adhesion transmembrane glycoprotein and maintain a normal intact epithelial cell surface. It is

anchored to the cytoskeleton via cytoplasm proteins, including alpha and beta catenin. E-cadherin is one of the most important adhesion molecules expressed in epithelial cells and is rendered as an invasion suppressor molecule.[16] When cadherin is expressed, the inactivation of other cell to cell adhesion molecules has little or no effect.[88] Binding between cadherins occurs homotypically, with identical molecules binding on adjacent cells surfaces in a 'zipper" conformation.[89] The different members of this superfamily can be organized into several sub-members according to their structural and /or functional organization. In this way, 5 different subfamilies can be considered: 1) Classical cadherins (Type I), mainly localized to adherents junctions: 2) Highly related Type II cadherins: 3) Desmosomals cadherins (desmocollins and desmogleins) that form desmosomal junctions: 4) Proto-cadherins, mainly implicated in neural development: 5) Cadherin-related proteins, like the Flamingo- and Fat-like cadherins. Adhesion junctions and desmosomes are specialized adhesive sites along the plasma membranes of the cells, which bind actin microfilaments and intermediate filaments respectively.[90-95] Although all the subclasses share similar features, such as molecular weight and calcium dependence, each subclass is distinguished by a unique tissue distribution pattern. E cadherin is found in all normal epithelia, P cadherin is expressed by trophoblast and to a minor degree in stratified squamous epithelia, and N cadherin is expressed mainly in neural tissue. Classic cadherins play an important role in tissue formation and maintenance during embryonic development, and in the induction and maintenance of normal architecture and function in adult tissues. E-cadherin localizes to zonula adherens, which are adherens junctions typically found in epithelial cells.[96, 97] This cell to cell adherent junction is a specialized region of the plasma membrane connected with cytoskeleton actin filaments, where cadherins act as Ca^{2+}-dependent adhesion molecules. The extracellular domain of E-cadherin is composed of a series of components, each comprising about 110 amino acid residues. Each of these components contains two putative Ca^{2+}-binding motifs, which are considered to play key roles in Ca^{2+}-protein and protein-protein

interactions. Cadherins mainly interact in a homophilic manner, e.g. E-cadherin binds selectively to E-cadherin, and the amino terminal 113 amino acid residues are essential for this selective adhesiveness.[96, 98]

1.15.2 Cadherin expression during development

During embryonic development, each member of the cadherin superfamily exhibits its own specific spatio-temporal expression pattern. The elegant pioneering studies of Takeichi on E- and N-cadherin expression during mouse embryogenesis established a critical role for cadherins as regulator of morphogenesis.[99] Both E-and N-cadherin are considered as prototypic cadherins in epithelial and mesenchymal tissues, respectively, and they are involved in the regulation of morphological events such as gastrulation, neurulation, cardiogenesis and somitogenesis. E-cadherin is the first adhesion molecule expressed in the mouse embryo, at the 8-cell stage, and is essential for the compaction of the morula and the subsequent organization of epithelial tissues, but it is silenced during the process of epithelial-mesenchymal-transiction (EMT) and in established mesenchymal cells.[96, 99] Loss of function studies (using functional antibodies, antisense nucleotides or transgenic knock-out mice for e-cadherin) have demonstrated that E-cadherin is crucial for early mouse development and the maintenance of epithelial morphology.[100]

1.15.3 Mechanism of E-cadherin regulation during tumor progression

Most of our current knowledge regarding the way in which cadherin expression is controlled during tumor progression is derived from the studies on E-cadherin. The involvement of E-cadherin in epithelial morphogenesis and homeostasis, and its proven anti-invasive role in carcinoma progression has stimulated interest in understanding the regulator mechanism that control E-cadherin expression under pathological circumstances. The human E-cadherin gene (CDH1) is located at the

16q22. l Locus and is comprised of 16 exons, spanning 99 kb of genomic DNA.[101] This gene (CDH1), has been found to be altered in human tumorigenesis by a different mechanism. Germline mutation in CDH1 predisposing to hereditary diffuse-type gastric cancer, whereas somatic mutations in CDH1 are demonstrated in several human carcinomas, ovarian carcinomas and signet ring cell carcinoma of the stomach. Certain tumors that display mutations in one allele of CDH1 also require deletion in the other allele, which is consistent with a two-hit mechanism for inactivation. Hypermethylation of the CDH1 promoter has been observed in some primary tumors without identified carcinomas. Transcription silencing of E-cadherin may also result from aberrant expression transcription factors that repress its promoter. The examples of such transcription receptors are, SLUD, SIP1 and E12/E47. SNAIL is located to chromosome band 20q13.1, a region frequently amplified in human cancer. In hepatocellular carcinoma (HCC), breast carcinomas, melanomas and oral SCC, an inverse correlation between SNAIL and E-cadherin expression is observed. Nevertheless, inactivation of E-cadherin does not appear to significantly increase the levels of free cytoplasmic β-catenin, probably because the excess of cytoplasmic β-catenin is rapidly removed by an intact degradation system. It has been shown that introduction of CDH1 into a cell line lacking E-cadherin and demonstrating constitutively transcription of WNT target genes, helps to sequestrate β-catenin and thus reduce the transcription of WNT target genes. However, the converse has never been proven. Loss of E-cadherin was shown not to result from constitute β-catenin/TCF transcriptional activation.[102, 103] The E-cadherin mutations reported so far occurred in 9 of the 16 exons, but the tended to accumulate in exon 6 through 10, which correspond to extracellular domain.[104] Signet ring cell carcinomas of the stomach, typical of diffuse-type cancers, were previously analysed in vivo. It was found that cellular atypia is mild in these carcinoma and their most obvious characteristic, which occurs even in the intramucosal lesion, is the complete loss of cell to cell adhesiveness resulting in destruction of the histological structure. E-cadherin genes

resulting in skipping of exon 9 were detected in the intra mucosal lesion of signet ring carcinomas and in deeply invaded areas. The incidence of mutation resulting in skipping of exon 8 or 9 in diffuse-type stomach cancers was found to be high, about 40%, but no mutation was detected in intestinal-type stomach cancer.[105]

1.15.4 Epigenetic mechanism of E-cadhein

Hypermethylation of promoter region of CpG islands has been found to be of important means in repressing tumor suppressor genes. DNA methylation is known to be involved in the development of human cancers, often characterized by a generalized hypomethylation of DNA and local hypermethylation of CpG islands in the promoters and upstream exons of many genes. The hypermethylation of CpG islands is associated with the recruitment of methyl DNA binding proteins (MBDs) and of histones deacetylase activity (HDACs), which together contribute to the compaction of the DNA in the promoter region and hence, to gene inactivation. The initial studies on CDH1 Promoter methylation not only established a relationship between E-cadherin silencing and the methylation of CpG islands in several carcinoma cell lines, but also observed that treatment with 5'-azacytidine re-activated E-cadherin expression. Furthermore, methylation of the E-cadherin was observed in primary prostate and breast tumor lesions, in contrast to normal adjacent tissues where it remained unmethylated. Since these two pioneering studies, a large amount of data regarding E-cadherin promoter methylation and tumor progression has been accumulated from a huge number of cell lines and tumors.[106] It has also been proposed that methylation of E-cadherin CpG islands can act as a second hit for transcriptional repression to silence E-cadherin in ductal breast carcinomas.[107] Despite the loss of E-cadherin observed in primary carcinomas, it has been shown that E-cadherin is frequently re-expressed at metastatic foci and even within lymph node metastases. This indicates that E-cadherin expression is dynamically regulated during tumor progression a fact that apparently

contradicts the assumed irreversibility of DNA methylation.[108] It has also been shown that phosphorylation of E-cadherin increases cadherin-β-catenin complex formation.[109] In crystal structures, this phosphorylation results in interaction with β-catenin that appear to mimic TCF binding.[110] It was also found that cadherin phosphorylation allows the cadherin to bind the monomeric, closed form of β-catenin that otherwise would be TCF selective. The observation that cadherin phosphorylation can reverse Wnt-mediated β-catenin binding selectively suggest a mechanism by which cadherin compete for the Wnt-activated form of β-catenin.[111]

1.15.5 Transcription regulation of E-cadherin silencing

Besides regulation of E-cadherin by promoter hypermethylation and/or genetic alterations, direct transcriptional control of E-cadherin has emerged in the last years as an important regulatory mechanism of E-cadherin expression. The mouse E-cadherin promoter was first isolated in 1991. Its initial characterization showed it was a TATA-less promoter containing several potential proximal regulatory elements, including a CCAAT box (-65), a GC-rich region (-30 to 58) and a palindromic elements (-70 to -90) composed of two adjacent E-boxes flanked by four inverted nucleotides called 'E-Pal'. The proximal CCAAT and GC-rich regions are required for basal E-cadherin expression and are recognized by constitutive AP2 and Sp1 transcription factors, and CAAT-binding proteins, respectively,[112-114] E-pal elements was initially described as an epithelial-specific regulator,[112] but subsequent studies showed it to be an active repressor in E-cadherin deficient cells.[113, 115] In addition, an epithelial-specific enhancer does exist in the first intron of the mouse E-cadherin gene, which is recognized by AP2 factors and other potential regulators.[113] An additional element, containing Ets-binding sites, has also been described in the mouse promoter and may contribute to the repression of E cadherin expression.[115]

A comparison of the human, mouse and dog E-cadherin promoters [116] was found to show the CAAT-box and GC-rich regions being conserved at similar locations. The proximal E-box in the mouse E-pal elements is also convened in the human and dog promoters. However, the additional E-box (E-box 3) region in E-cadherin is located at -30 in all promoters. However, the additional E-box (E-box 4) of E-cadherin downstream of the transcription initiation site in the human promoter [117] is not present in the mouse promoter. Several studies have demonstrated that E-boxes in the proximal E-cadherin promoter repress its expression. Point mutation abolishing the two E-boxes in the mouse E-pal, or E-box1 and 3 in the human promoter, produces a strong induction of E-cadherin expression. Evidence has also been presented that the E-cadherin box4 also represses E-cadherin expression,[117, 118] hence, factors able to bind to the E-boxes and repress E-cadherin expression must be present in these cells. In vivo analysis foot-printing supports this hypothesis. The E-pal or E-box 1 were specifically protected in E-cadherin deficient cells, as well as in several dedifferentiated carcinoma cell lines and fibroblasts.[113, 115] An important concept derived from such studies was that the repressors bound to proximal E-boxes were able to overcome the positive effects of constitutive factors interacting with the basal regulatory elements of the E-cadherin promoter, such as AP2 and Sp1.[115]

1.16 β-catenin

1.16.1 Introduction

Cadherin is a family of proteins including α- (102ku), β- (88ku), γ- (82ku) catenins.[119, 120]

B-catenin was first described in humans as a protein which interacts with the cytoplasmic domain of E-cadherin and with α-Catenin, anchoring the cadherin complex to the actin cytoskeleton.[121]

The primary structure of β-catenin comprises an amino-terminal domain of approximately 130 amino acids, a central region of 12 imperfect repeats of 42 amino acids known as arm repeats (since

they show homology with repeats found in the A protein of Drosophilia) and a carboxyl-terminal domain of 110 amino acids.

The amino-terminus of the β-catenin is important for regulating its stability, whereas the carboxyl terminus works as a transcriptional activating domain.[122] This cadherin-bound pool of β-catenin ultimately serves to link the cytoskeleton networks of adjacent cells, which is considered essentially for normal tissue architecture and morphogenesis.[123] Disruption of these two β-catenin pathways could be important in tumor development and progression. Previously, several reports have detected alterations of β-catenin in different neoplasm and it has been suggested that this protein plays a critical role in carcinogenesis.[124, 125]

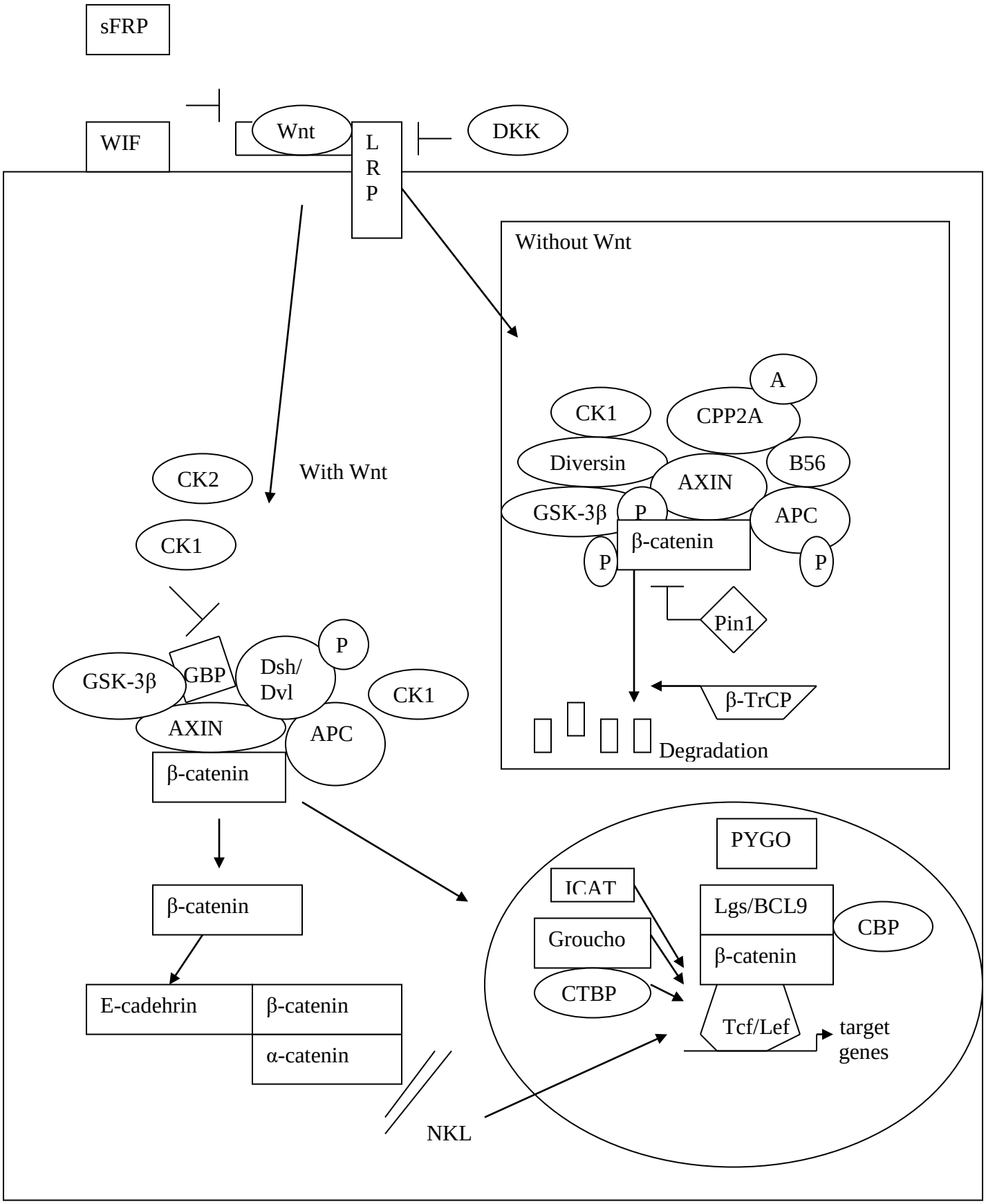
1.16.2 β-catenin regulation during tumor progression

The CTNNB1 gene encodes β-Catenins. Exon 3 of this gene is hot spot for mutations in human tumors. This exon encodes the critical Ser/Thr residues, which are sites for priming by CK1 (Ser 45) and phosphorylation by GSK_3β (Ser 33, 37 and Thr 41) and thus recognition site of β-TrCP marking β-catenin for degradation. Mutations within this exon have been found to increase the stability of the protein. Somatic mutations have been described in a wide variety of human tumors, including colorectal carcinoma, desmoids tumor, endometrial carcinoma, HCC, hepatoblastoma, intestinal gastric carcinoma, medulloblastoma, melanoma, ovarian carcinoma, pancreatic carcinoma, pilomatricoma, prostate carcinoma, SCC of the head and neck, thyroid carcinoma, and Wilms' tumor.[126] It has been shown that adenomatous polyposis coli (APC) and E-cadherin compete for the same binding region on β-catenin.[127] Is has been suggested that APC modulated the interaction between cadherins and catenin, thereby affecting the pathway through which cellular adhesion controls cell growth and differentiation. The mutant APC proteins in colon carcinomas have been

found to results in β-catenin stabilization and a significance increase of this protein within the cell may further activate β-catnin/Tcf signaling factors.[128] In the same manner, dominant activation β-catenin mutants that render the protein in sensitive to APC/GSK-3 β-mediated degradation could lead to a dysregulation of the signaling function of β-catenin and thus to carcinogenesis. When a mitotic signal is delivered by the Wnt pathway, by association of the Wg/Wnt family of secreted glycoproteins and their membrane receptors frizzled, it leads to activation of the disheveled (Dsh) protein, which is recruited to the cell membrane. The Dsh down regulates the protein complex, so that it can no longer phosphorylate β-catenin, the release of β-catenin from the phosphorylation and degradation complex promotes β-catenin stabilization and signaling. This results in an increase of free cytoplasmic β-catenin that translocates to the nucleus and directly binds the transcription factors Lef and Tcf, leading to activation of gene expression.[130, 131] B-catenin has been found to rapidly migrate into the nucleus in a temperature-dependent and WGA-sensitive manner in living cells.[132] These may suggest that β-catenin may be responsible for poor prognosis. The primary system that regulates the accumulation, intracellular localization, and functions of β-catenin involves an interplay between its interactions with the cytoplasmic tail domains of cadherins, the integral membrane proteins that mediate cell-cell adhesion, and the adenomatous polyposis coli (APC) protein, a large multifunctional protein which was firstly identified as a tumor suppressor involved in colon cancer.[133] Mutations have also been described in breast carcinoma, desmoid carcinoma, hepatoblastoma, HCCs, intestinal type of gastric carcinomas, medulloblastomas, ovarian carcinomas, pancreatic carcinomas, and thyroid carcinomas. Approximately 80% of the sporadic colorectal carcinomas contain mutations in APC. The mutation cluster region, codons 1286-1513, accounts for 10% of the coding region, but harbors 80-90% of all APC mutations. The majority of the mutations lead to a truncated protein, missing some or all of the β-catenin binding and down regulate the β-

catenin level in the cell.[134] Other findings have shown that Met activation by HGF modulates the phosphorylation of catenins, leading to changes in cellular distribution of adhesion molecules.[135]

1.16.3 The Wnt signaling pathway regulating β-catenin degradation.



The pathway with or without a Wnt signal is schematically presented on page 41.

The signaling pathway is essential in many biological processes and numerous studies of this pathway over the last years have lead to the identification of several novel components. Nevertheless, many of the mechanism involved in activation or inactivation of this particular pathway still remains to be elucidated.

In the presence of a Wnt ligand, if not inhibited by secreted antagonist, the Wnt ligand binds a Frizzled (Fz)/ low-density lipoprotein receptor related protein (LRP) complex, activating the cytoplasmic protein disheveled (Dsh in Drosophilia and Dvl in Vertebrates). Precisely how Dsh/Dvl is activated is not fully understood, but phosphorylation by casein kinase 1 (CK1) and casein kinase 2 (CK2) has been suggested to be partly responsible.[136-138] Dsh/Dvl then inhibits the activity of the multiprotein (β-catenin-Axin-adenomatous polyposis coli (APC)-glycogen synthetase kinase (GSK)-3β, which targets β-catenin by phosphorylation for degradation by the proteasome. Dsh/Dvl is suggested to bind CK1 and thereby inhibiting priming of β-catenin and indirectly preventing GSK-3β phosphorylation of β-catenin.[139] Upon wnt stimulation, Dvl has also been shown to recruit GSK-3 binding protein (GBP) to the multiprotein complex. GBP might titrate GSK-3β from the Axin and in this way inhibits phosphorylation of β-catenin. Finally, sequestration of Axin at the cell membrane by LRP has been describes [140] the overall results is accumulation of cytosolic β-catenin. Stabilized β-catenin will then translocate into the nucleus and bind to members of the T-cell factor (Tcf)/Lymphoid enhancing factor (Lef) family of DNA binding proteins leading to transcription of Wnt target genes.[133]

In the absence of a Wnt ligand, Axin recruits CK1 to the multiprotein complex causing priming of β-catenin and initiation of the β-catenin phosphorylation cascade performed by GSK-3β. Phosphorylated β-catenin is then recognized by β-transducin repeat-containing protein (β-TrCP) and degraded by the proteaosome, reducing the levels of cytosolic β-catenin. B-catenin activity is controlled by a number of binding partners that affect the stability and localization of β-catenin.

1.16.4 β-catenin and binding proteins

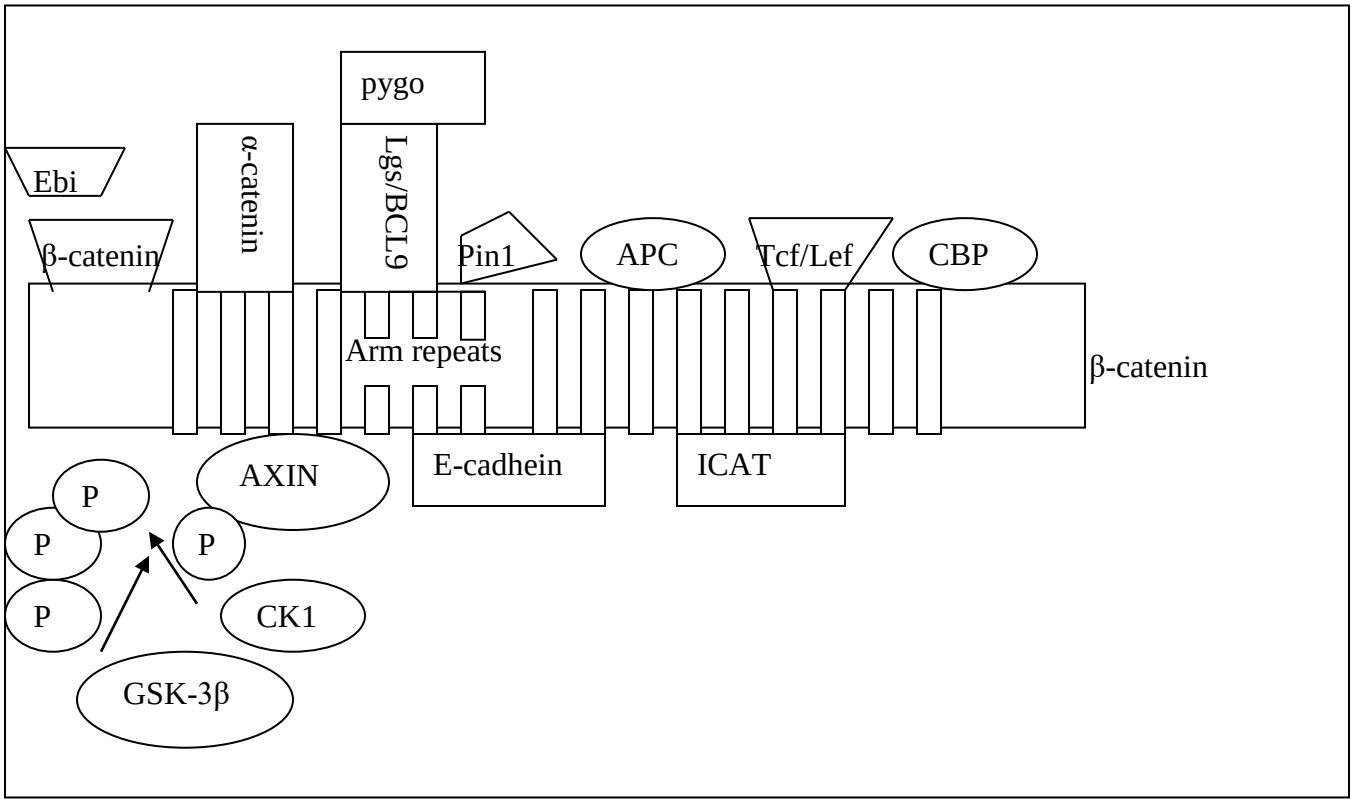


Figure 2. β–catenin and binding partners

Two ubiquitin-mediated degradation systems are involved in the catabolism of β-catenin. Both F-box proteins, β-TrCP and Ebi, recognize and bind to the same site on the N-terminal domains of β-catenin.[141] However, unlike β-TrCP, Ebi probably does not require phosphorylation of β-catenin for

recognition. Ebi works in complex with SIAH-1, a TP53 induced protein, linking activation of TP53 to the degradation of β-catenin.[142, 143] Both degradation systems require an intact APC protein.[141]

Binding of α-catenin to the N-terminal region of β-catenin [144] and E-cadherin to the Arm repeat [107] connects β-catenin to the cell. The Arm repeats domain of β-catenin mediated binding of cadherins, APC, Axin and Tcf/Lef family of transcription factors. Peptydyl-propyl cis-trans isomerase 1 (Pin 1) binds a phosphorylated Ser-Proline (Pro) motif next to the APC binding site in β-catenin and inhibits interaction between APC and β-catenin, consequently acting as a positive regulator of Wnt signaling.[145] In Xenopus, the transactivating domain of β-catenin interacts with CREB binding protein (CBP) and these synergistically stimulate transcription of Wnt target genes.[146] In mouse studies, inhibitor of β-catenin and TCF-4 (ICAT) binds the C-terminal domains of β-catenin and inhibits its interaction with TCF-4. β-catenin TCF-4 mediated transactivation of Wnt target genes is then repressed.[147]

1.16.5 Plankoglobin/γ-catenin

Plankoglobin has so far not been found mutated in human primary tumors. Only one gastric carcinoma cell line and one squamous-cell lung carcinoma cell line have been reported with mutations in this gene. Nevertheless, and in contrast to β-catenin, plankoglobin induces neoplastic transformation of rat epithelial cells in the absence of stabilizing mutations. The cellular transformation performed by plankoglobin is also distinct from β-catenin in that activation of the proto-oncogene c-MYC is required. Increased nuclear expression of plaknoglobin is seen in several human carcinomas like colorectal carcinomas, endometrial carcinomas, esophageal carcinomas and testicular germ cell tumors. The C-terminal domain that harbors the transactivating domain, is very different in plankoglobin and β-catenin and these two proteins might therefore activate different

target genes by recruiting different transcription co-factors to the plankoglobin/TCF and β-catenin/TCF complexes.[148, 149]

Interestingly, plankoglobin, also called γ-catenin, shares overall 70% amino acid identity with β-catenin and as much as 80% with the Arm repeat domain.[107] Plankoglobin binds E-cadherin, α-catenin, APC, Axin and Tcf/Lef transcription factors, and is involved in cell adhesion as well as Wnt signaling. However, differences between β-catenin and plankoglobin in these processes exist.[143]

1.16.6 α-catenin

The CTNNA1 gene encodes α-catenin, a protein involved in cell adhesion by anchoring the β-catenin-E-cadherin complex to the actin cytoskeleton. CTNNA1 has so far only been found mutated in some lung, prostate, ovarian, and colon cancer cell lines. Homozygous deletion of CTNNA1 In human lung cancer line leads to loss of cell adhesion, whereas introduction of the wild-type CTNNA1 restored normal adhesion. However, an effect of α-catenin inactivation on Wnt signaling has not been reported.[144]

1.17 Significance of the study

It was known as early as the 1940s that the mutual adhesiveness of cancer cells is significantly weaker than those of the corresponding normal cells.[150, 151] The reduced cell-cell adhesiveness allows cancer cells to disobey the social order, resulting in destruction of the histological structure, the morphological hallmark of malignant tumors.[17]

Up to date few studies in South Africa let alone Africa have been done to ascertain the behavior of the adhesion molecules γ-catenin, β-catenin, and α-catenin and as well the neuronal-cadherin,

placental-cadherin, and epithelial cadherin on neoplastic cells. However, the roles of α-catenin and γ-catenin have been shown not to play a significant role in SCC invasion and possible metastasis. Collectively the loss E-cadherin and β-catenin has been correlated with reduced survival rate and poor prognosis in patients with malignancies.[101, 149] This is one of the reasons why β-catenin and E-cadherin proteins were chosen to be investigated, because of the vast implication they have in tumorigenesis. Furthermore, the study of E-cadherin and β-catenin expression in esophageal SCC aims not only at explaining the roles of these adhesive molecules in clinicopathologic factors like histological differentiation, pattern of invasion of the vital structures of the esophageal wall, vascular invasion by malignant cells and mitotic activity of the tumor cells, but might also provide information on the expression pattern of these adhesion molecules as the cancer progresses.

Even though it has been established now that esophageal SCC has a multi-factorial etiology involving several environmental and/ or genetic factors,[152] dysregulation of the adhesion molecules generally always occurs. This study may throw more light on how the expression of these two proteins corresponds with the histological grading of the tumor.

Esophageal cancer needs a diagnostic tool for early diagnosis and also effective therapeutic strategies that ensures non-recurrence, best quality of life and an increased lifespan. The investigation of E-cadherin and β-catenin expression could reveal important information on the prognosis of SCC of the esophagus, and identify those patients at risk of invasion and metastasis.

CHAPTER 2: AIMS AND OJECTIVES

2.1 Aim

To identify the expression of the β-catenin and E-cadherin in malignant cells of a series of patients with SCC of the esophagus, and how these two markers contribute to invasiveness and poor prognosis.

2.2 Objectives

- To evaluate E-cadherin, β-catenin expression and its relation to histological grade (Broders) and at the tumor invasive front (Bryne) of esophagus
- Establish the normal expression of E-cadherin and β-catenin in ESCC and establish correlations with different stages of cancer.
- Correlation between E-cadhein and β-catenin co-expression in esophagus SCC.
- Influence of gender and age of patients as well as the location of the tumor with regard to the expression of E-cadherin and β-catenin.

CHAPTER 3: MATERIALS AND METHODS

This is a retrospective and descriptive study. The records contained in the cancer registry in the Department of Anatomical Pathology were used to retrieve cases that were diagnosed as squamous cell carcinoma (SCC) of the esophagus. The gender and age of patients as well as all available clinical data were recorded. The biopsies of the cases were reviewed by a Pathologist for the purpose of the histopathological description of Broders and Brynes grading. In order to perform the latter, only biopsies that showed the deep invasive front of the neoplasm were admitted to the study. Cases with inadequate material or data were excluded from the study. The fomalin-parafin wax tissue blocks already processed were retrieved and cut for further assessment, using immunohistochemistry and H & E stain.

3.1 Selection of subjects

This study was conducted on 44 randomly selected cases of esophageal cancer diagnosed in the Department of Anatomical Pathology at the Medunsa Campus of the University of Limpopo. The cases were diagnosed between January 1994 and December 2005 and patients were admitted to the Dr George Mukhari Hospital. The hospital serves mainly the peri-urban and rural population sample in the northern and north-western regions of South Africa and the selected patients were Black.

3.2 Control cases

Biopsies showing normal esophageal mucosa were used as controls for E-cadherin and β-catenin expression.

3.3 Histological methods

3.3.1 Hematoxylin and eosin (H&E):

The three micron tissue blocks were cut with a microtome from formalin fixed paraffin embedded tumor blocks coated with a tissue adhesive sta-on solution were placed in hotplate for 25 minutes. Thereafter, sections were deparaffinized in xylene for 5 minutes and then places in a graded series of alcohol (100%, 95%, 80%, and 70%) for rehydration and then washed in running tap water. Sections were placed in hematoxylin for 7 minutes, and rinsed in running tap water for 5 minutes, and then transferred to eosin for 5 minutes and rinsed in tap water. Section were then hydrated in a series on alcohol mentioned above, and for clearance sections were placed in two solutions of xylene for 1 minutes and then mounted.

3.3.2 Immunohistochemistry

High temperature antigen unmasking technique for immunohistochemical demonstration on paraffin sections were used. The avidin-boitin peroxidase complex method was employed as follows: The already fixed and embedded tissue samples prepared from 100 ml. neutral buffered formalin-fixed and paraffin wax were retrieved and sectioned to produce three microns sections from the paraffin wax blocks. Sections where transferred to a Surgipath Sta-On tissue section adhesive, and then incubated in 37^0C oven overnight. The tissue samples were deparaffinized in xylene three times for 5 minutes each and places in a graded series of ethanol [100%, 90%, 80%, and 70%] for 1 minutes rehydration and then washed in distilled water. To enhance antigen retrieval, sections were pretreated in a microwave oven at 850w for 12 min in 0.1mol/l citrate buffer (pH 6.1) and then cooled to room temperature. Thereafter, to block the endogenous peroxidase activity, the sections were processed using 3% H_2O_2 in methanol for 10 minutes and rinsed in phosphate buffered saline

(PBS) three times for 5 minutes. The sections were then incubated with primary antibody at room temperature (RT) for an hour with the dilution of 1: 100 that consisted of mouse monoclonal against human β-catenin (Dako Cytomation), and with the second primary antibody diluted at a ration of 1:50 which consisted of mouse monoclonal against human E-cadherin supplied by (Dako Cytomation). After primary antibody incubation the sections were rinsed in PBS for 3 minutes, and then sequentially incubated with biotinylated secondary antibody for 30 minutes at room temparature, rinsed again in PBS for 3 minutes, thereafter, section were covered with enough streptavidin-biotin-peroxidase for 30 min at room temperature, followed by rinsing with PBS for 2 minutes. The peroxidase reaction was visualized by staining with diaminobenzidine chromogen (Dako K5007) supplemented with substrate buffer for 2 minutes, and then rinsed once with PBS for 2 minutes. The sections were then counterstained with Meyer's hematoxylin for 2 minutes, then washed in running tap water for 3 minutes and run in distilled water and then mounted with Faramount aqueous media (Dako S3025).

Solutions:

0.01M Citrate buffer (pH6.0)

Add 3.84 grams of citric acid (anhydrous) to 1.8 liters of distilled water. Adjust the buffer to pH 6.0 using concentrated NaoH. Fill up to 2 liters using distilled water.

Phosphate Buffered Saline (PBS) pH 7.2

Weigh 40g of Nacl, 1g KCl, 10g of Na_2HPO_4 and 1g of KH_2PO_4 to 4 liters of distilled water. Adjust the pH to 7.2 using 0.1M HCL. Make up 5 liters with distilled water

DAB-Chromogen subtrate

2 drops of chromogen subtrate is added to 4 ml of DAB subtrate

3.3 Histological analysis

3.3.1 Hematoxilin and eosin sections

The H&E stained sections were viewed by the author and a Pathologist with a light microscope. Each case was graded according to the classic Broders Grading system [78] and devided into well differentiated (Broders grade I), moderately differentiated (Broders Grade II) and poorly differentiated (Broders Grade III). The nature of the invasive front (i.e. the deepest part of the neoplasm) was assessed according to the criteria of Bryne [80] and were designated Pattern 1 where the invasive front showed well-delineated, pushing borders. Pattern 2 show an advancing edge of the tumor that infiltrates in solid cords, bands, or strands. Pattern 3 lesions have margins that contain small groups or cords of infiltrating cells. In Pattern 4 the host tumor interface show marked cellular dissociation into small groups or even single cells. The mitotic activity on tumor cells were scored according to the original criteria decribed by Jacobsson [86] and were designated score 1 were the number of mitotic activity was between 0-1. Score 2 the number of mitotic activity ranged between 2-3 per 500 cell count, Score 3 have the mitotic activity ranging between 4-5. Score 4 the rate of mitotic activity is greater than 5 per 500 cell count. Invasion of vital structures (bloodvessels and nerves) were noted and scored as follows: Score 1 where vascular walls which shows no invasion by tumor cells. Score 2 where there are possible foci of invasion of the bloodvessels. Score 3 where the tumor cells showed invasion of a few bloodvessels. In score 4 there was numerous bloodvessel invasion by tumor cells.

3.3.2 Immunoperoxidase stains for E-Cadherin and β-Catenin

The interpretation of results was considered according to the localization of E-cadherin and β-catenin antibodies in normal esophagus. The immunohistochemical assessment of 44 biopsies of

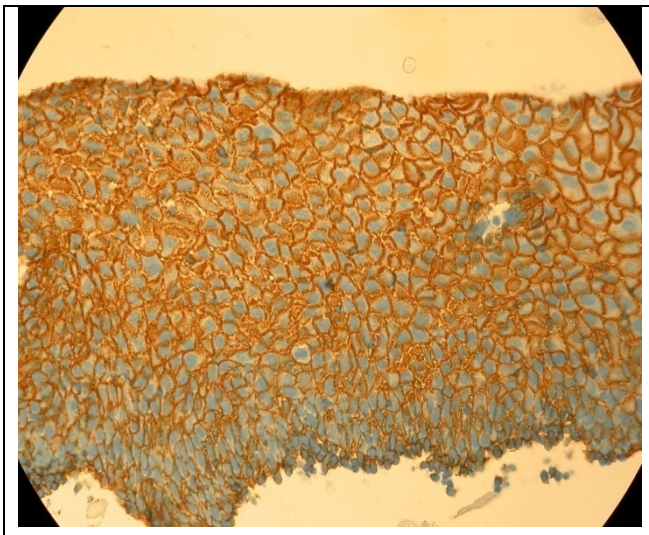
esophageal tumors taining for E-cadherin and β-catenin was compared to the staining intensity of the normal esophagus, selected at random (x400) and scored in the following categories:

- Score 0: No expression or minimal expression with less than 10% of tumor cells stained.
- Score 1: Reduced (or heterogenous) expression with less than 30% of tumor cells stained.
- Score 2: Preserved expression with more than 30% of the tumor cells stained.

3.3.2 Statistical analysis

A Statistician was consulted with the data presented in tabular format. Fisher's exact probability test analysis was used to assess the association between immunohistochemical features and clinicopathological characteristics. A P value less than 0.05 was considered significant.

CHAPTER 4: RESULTS



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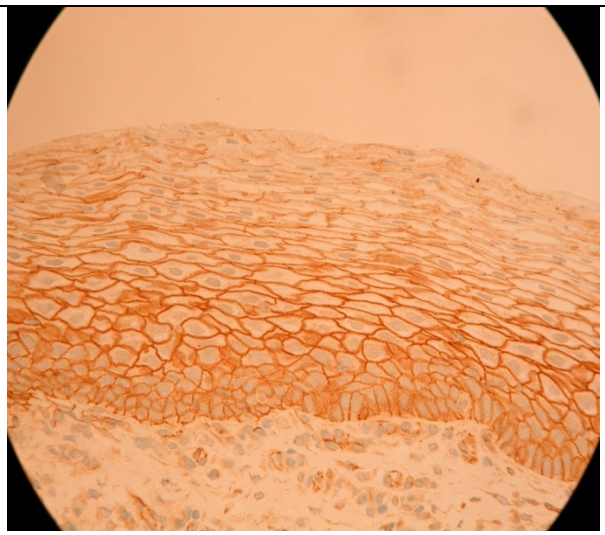


B

Figure 1 (A and B): Expression of E-cadherin and β-catenin of the normal esophagus (used as control stains). (A) The expression of E-cadherin, in normal squamous epithelium of the esophagus. (B) Normal expression of β-catenin in squamous epithelium of the esophagus

Expression of E-cadherin and β-catenin in esophageal squamous carcinoma:

Positive expression of E-cadherin and β-catenin in 44 esophageal tumors was 45.5% (20/44), and 54.5% (24/44), respectively. The reduced expression of E-cadherin and β-catenin was 47.7% (21/44) and 52.3% (23/44) respectively.

Table 1: E-cadherin and β-catenin expression in relation to Broders grading

Type	N	E-cadherin			P	β-Catenin			P
		Preserved	Reduced	Absent	r_s	Preserved	Reduced	Absent	r_s
Histological Grade									
I	1	1	0	0		1	0	0	
II	37	18	17	2		20	16	1	
III	6	1	4	1	0.20	1	5	0	0.18
β-Catenin									
Diffuse	36	19	17			20	16		
Focal	6	2	4			1	5		
Absent	2		1	1		0	0	2	
E-cadherin									
Diffuse	29	16	13			19	10		
Focal	11	4	8			1	10		
Absent	4	0	0	4		1	3		

Table II: Correlation between E-cadherin and β-catenin expression

		B-catenin		
E-cadherin	Total	Preserved	Reduced	Absent
Preserved	20	11	9	
Reduced	24	10	12	2
Total	44	21	23	

P= 0.5452

Table III: Brynes and Jacobsson grading system with the expression of E-cadherin and β-catenin

Points	N	E-cadherin		P	B-catenin		P
		Preserved	Reduced	r_s	Preserved	Reduced	r_s
Pattern of Invasion							
2	9	2	7		4	2	
3	35	10	25	1.00	17	18	0.02
Mitosis (n= 500 cell count)							
1	3	2	1		1	2	
2	4	1	3		2	2	
3	10	4	6		6	4	
4	27	14	13	1.00	11	16	1.00
Jacobson: Vascular invasion							
1	26	14 [54]	12 [46]		9 [35]	17 [65]	
2	18	7 [39]	11 [61]	0.37	12 [67]	5 [28]	0.03

Table V: E-cadherin and β-catenin expression in relation to the location of the tumor

Type	N	Brynes pattern of invasion score		E-cadherin		β-catenin	
		Patter 2	Pattern 3	Preserved	Reduced	Preserved	Reduced
Tumor Location n= 28							
Upper-1/3	2	2	0	0	2	0	2
Middle-1/3	23	4	19	12	11	11	12
Lower-1/3	3	0	3	2	1	1	2

Table VI: Expression of E-cadherin and β-catenin in relation to gender and age of patients

Type	N	E-Cadherin			β-catenin		
		Preserved	Reduced	Absent	Preserved	Reduced	Absent
Gender							
Male	27	13	12	2	13	13	1
Female	17	7	8	2	6	10	1
Age Group1							
20-35	3	2	1	0	2	1	0
36-50	11	4	7		5	6	
51-65	22	10	10	2	11	10	1
66-80	8	3	3	2	3	4	1
Total	44	19	21	4	21	21	2

Table VII: Expression of E-cadherin and β-catenin in males and females

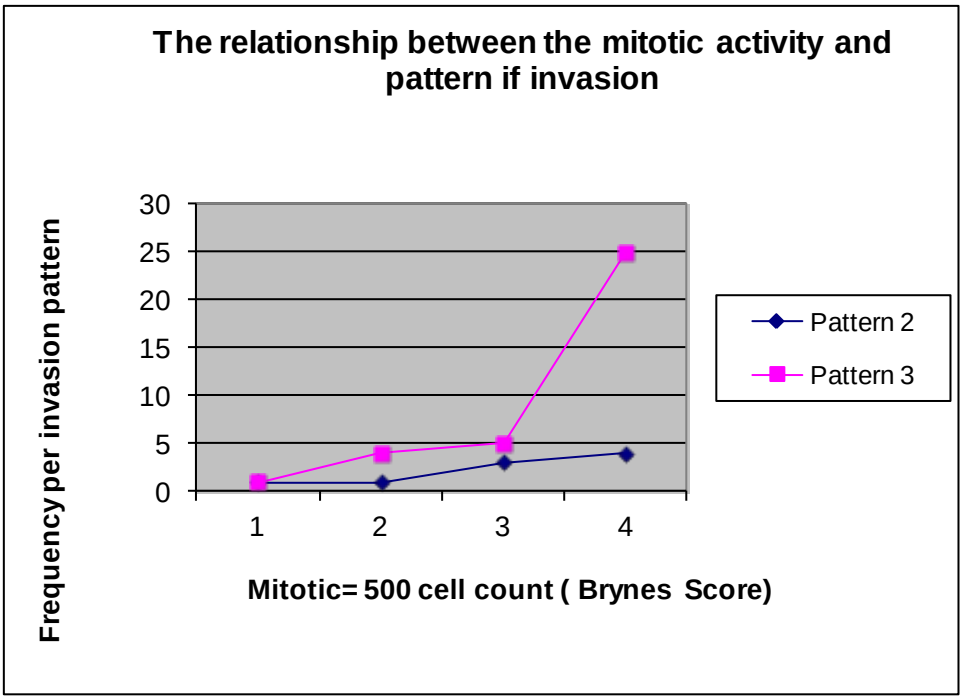
Type	N	Age Group	E-Cadherin		B –Catenin	
			Preserved	Reduced	Preserved	Reduced
Males	0	20-35	0	0	0	0
Females	3		2	1	2	1
Males	8	36-50	4	4	5	3
Females	2		0	2	0	2
Males	6	51-65	2	4	3	3
Females	8		4	4	5	3
Males	8	66-85	4	3	4	4
Females	2		1	1	0	2

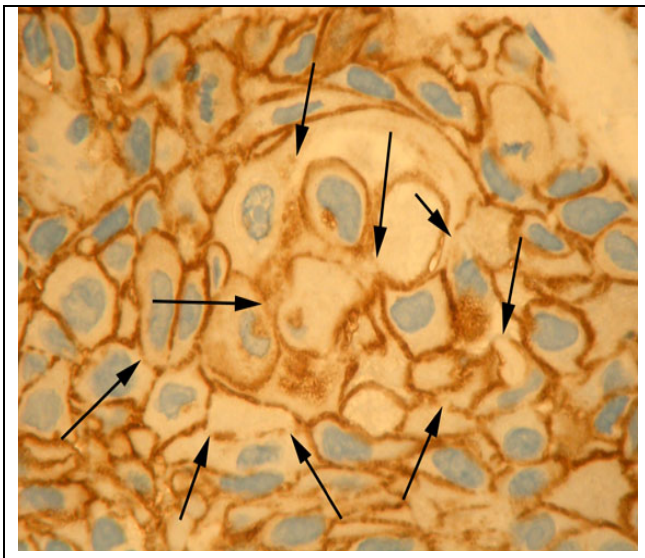
Table VIII: The relationship between mitotic activity and the pattern of invasion.

Pattern of invasion	n	Mitotic activity n= 500 cell count			
Morphological parameter		1	2	3	4
2	9	1	1	3	4
3	35	1	4	5	25

P=0.6188

Figure 2: Summary of the relationship between the mitotic activity and the pattern of invasion





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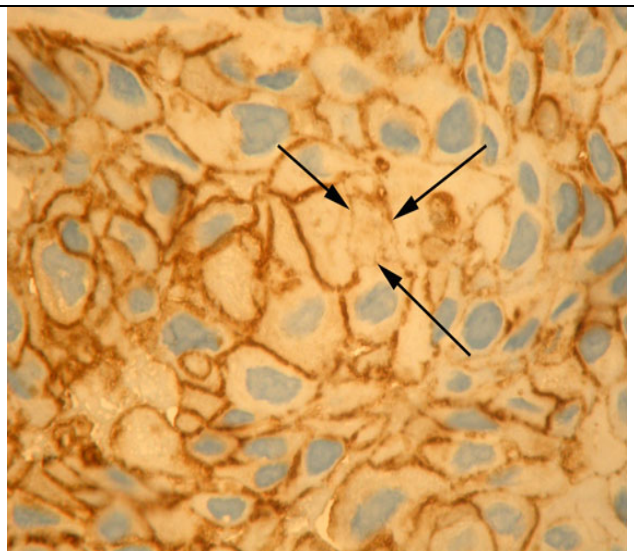

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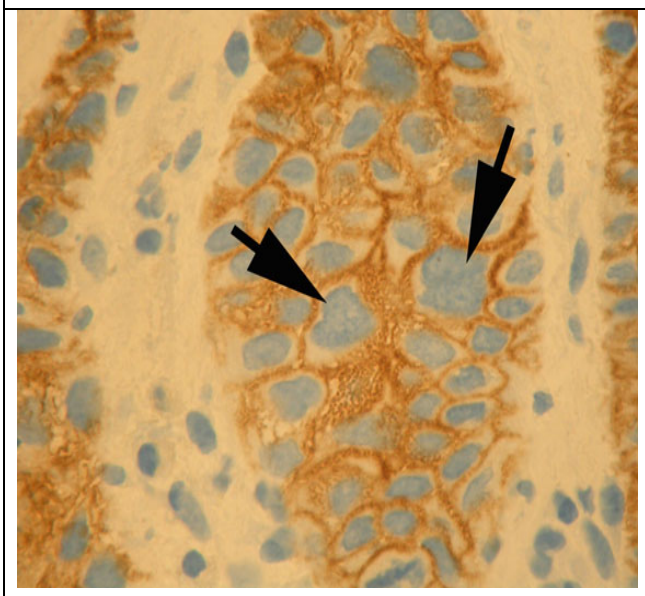

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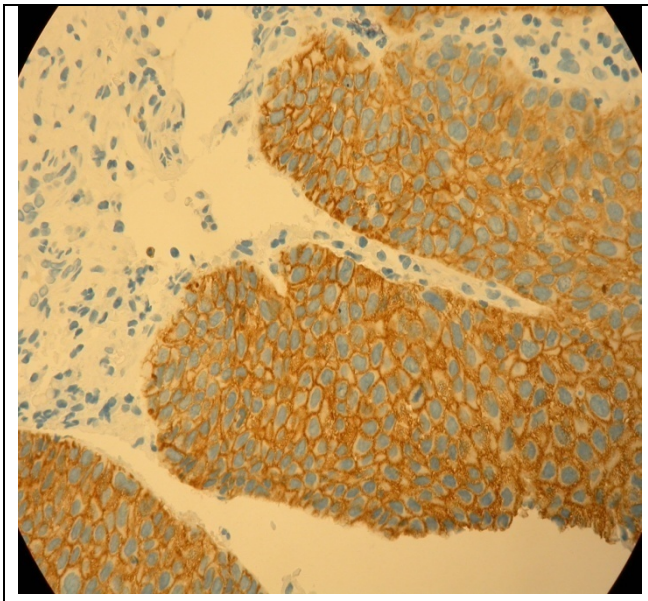
Figure 3: (A-C) squamous cell carcinoma showing intra-nuclear fusion of two cells with a loss of intra-cellular bridges (arrows).



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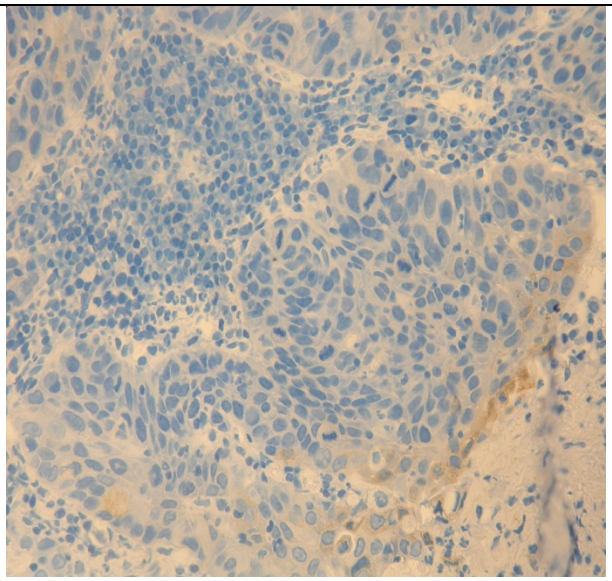

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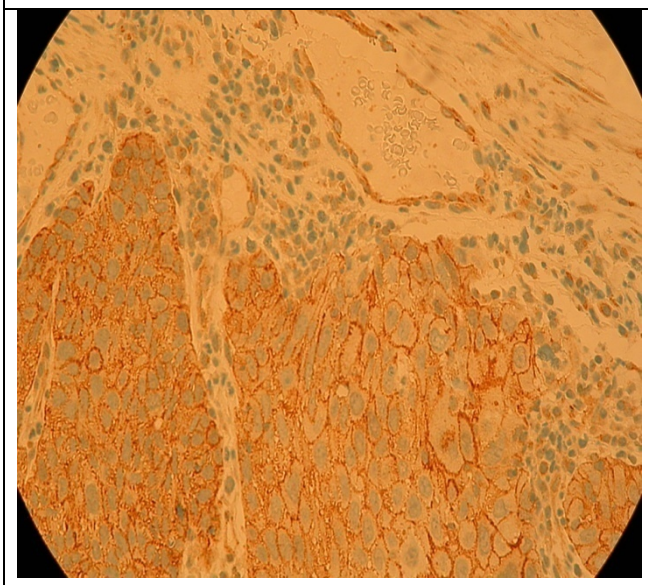

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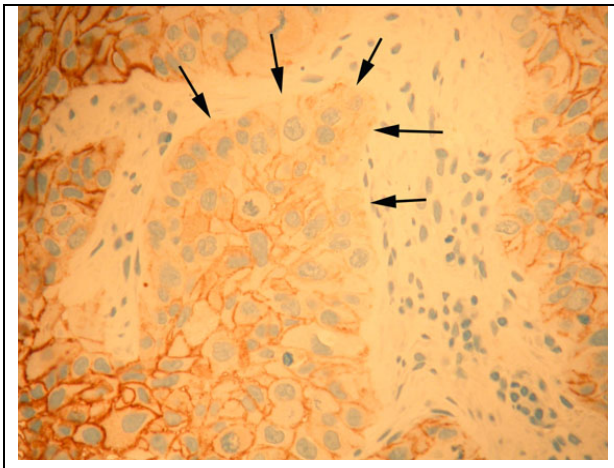
Figure 4. (A) Solid chord invasion of Pattern 2 with preserved expression for E-cadherin. (B) Solid chord invasion of Pattern 2 with reduced expression of E-cadherin with high rate of mitosis and nuclear polymorphism and the invading front. (C) Solid chord invasion Pattern 2 showing preserved expression of β-catenin at the invasive front on the neoplasm.



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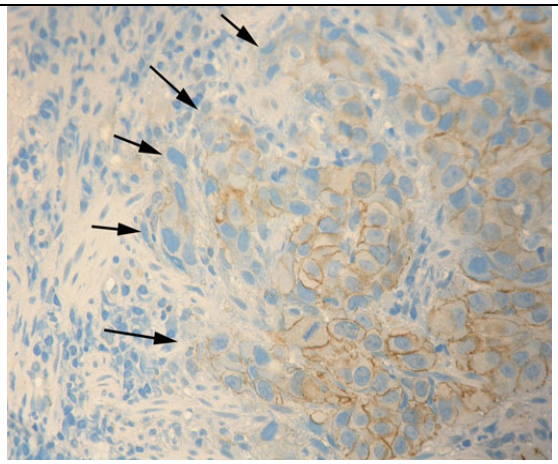

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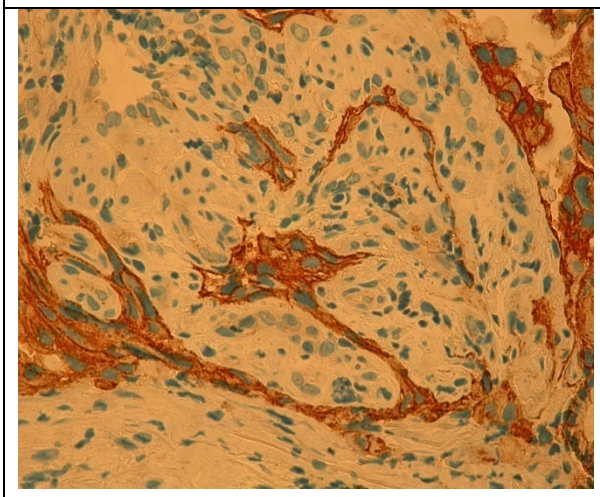

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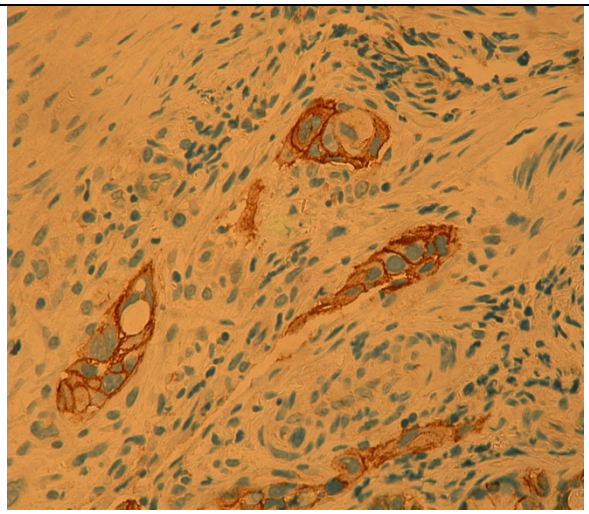

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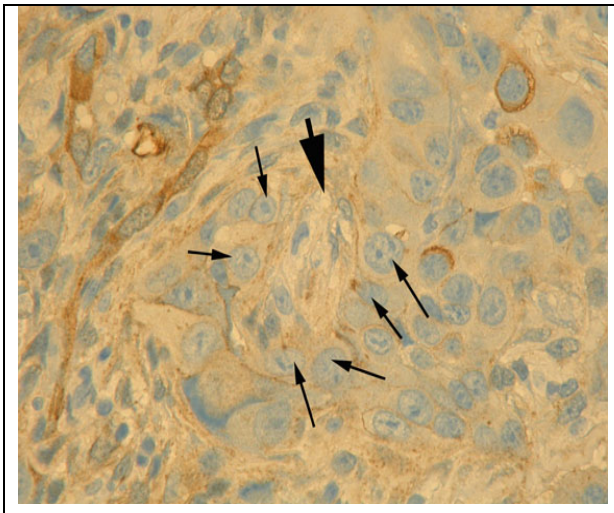

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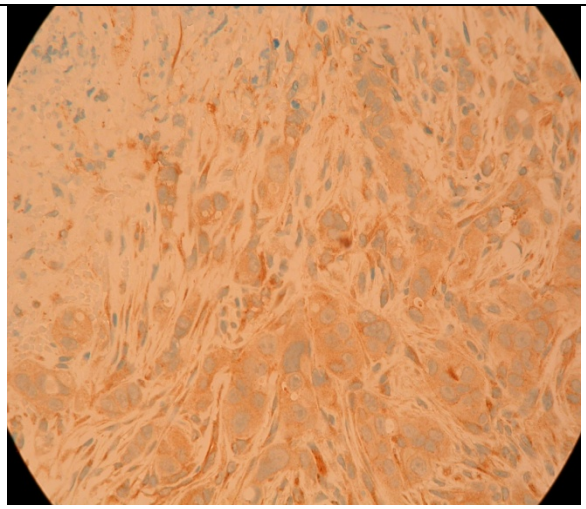

F

Figure 5. (A-to-F). Sections of squamous cell carcinoma taken from tumor cells with Pattern 3 of invading cells. (A and B). The invasive tumor front showing a reduced expression for E-cadherin. (C and D). Small group of neoplastic cell exhibiting preserved expression for E-cadherin. (E) Nerve invasion by small group of neoplastic cells of squamous cell carcinoma showing absent expression for E-cadherin. (F) infiltrating small group of neoplastic cells of squamous cell carcinoma with cytoplasmic retention of β-catenin.

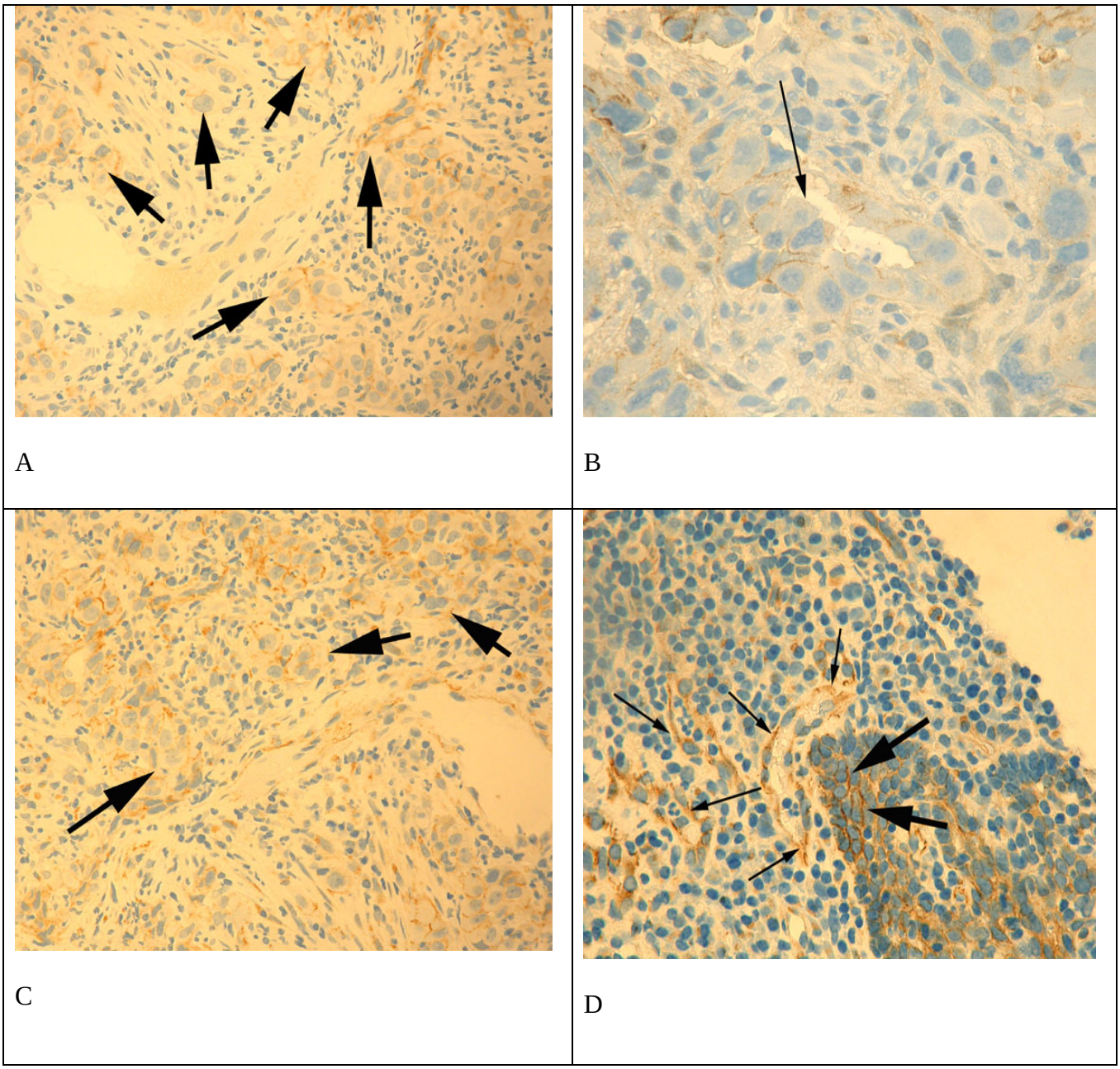

Figure 6. A) The pinching of the vascular wall by a small group of neoplastic cells with reduced expression of E-cadherin. B) Tumor cell attachment on the endothelium showing absent expression of E-cadherin. C) The pinching of the vascular wall by small group of neoplastic cells stained with β-catenin. D) The expression of β-catenin by endothelial cells and advancing tumor cells.

CHAPTER 5: DISCUSSION

All cases of SCC of the esophagus in the histopathology registry diagnosed between 1994 and-2006 of the Anatomical Pathology Laboratory serving the Dr George Mukhari Hospital were retrieved. The hospital serves the surrounding communities of Ga-Rankuwa and acts as a referral hospital for the peripheral clinics. The majority of patients are from the northern and north western regions of South Africa.

Due to budgetary limitations, 44 cases were selected randomly and subjected to the investigations. All cases were diagnosed as SCC and sufficient tissue was present in the archived wax blocks for additional sections. The biopsies were reviewed by the author and a Pathologist. There was one case of esophagectomy, and 43 were endoscopic biopsies. Application of Broders Grading system revealed one case of Broders Grade I, 37 Grade II and 6 Grade III and most tumors therefore were either moderately- or poorly differentiated. Two tumors were located in the upper third, 23 in the middle third and 3 in the lower third of the esophagus. Out of 37 Grade II SCC's, one case was classified as a papillary type SCC, a variant of SCC.

The patients included in the study consisted of 27 males and 17 females with ages ranging from 22 to 78 years. All the patients were Black and the majority came from rural villages and semi-urban areas that are generally affected by poverty and have low socio-economic status, suggesting comparable etiological determinants. At the time of presentation of the disease, the majority patients presented with severe and progressive dysphagia and weight loss. On patients whose symptoms were suggestive of esophageal cancer, a barium swallow test was performed which incorporates the ingestion of small amounts of barium followed by series of x-rays. The appearance of any bump or raised area on the normal surface of the esophageal wall indicated the presence of the disease. An endoscopic biopsy was then taken from abnormal areas for histological examination. Once a diagnosis of esophageal cancer

has been confirmed, the patients were treated according to the stage of the disease. The majority of our patients were not treated surgically because of the advanced clinical state of the disease. At this stage the majority presented with Pattern 3 infiltration as assessed with the Bryne technique. The advanced stage of most esophageal carcinomas with high Broders grading and the pattern of infiltration tend to reduce the prognosis significantly; Such patients are often treated with radiation therapy and stents. Few therefore undergo surgical resection of the tumor.

Esophageal carcinoma remains a disease of poor prognosis. Early diagnosis is compromised by the delayed onset of symptoms. By the time of surgical intervention metastases and organ infiltration have already occurred which reduces prognosis significantly and the 5-year survival rate of operative advanced esophageal SCC remains poor. Unfortunately follow up data and the subsequent accurate determination of time of survival are impossible due to the lack of contact information and residential addresses on patient files.

In 1941 Broders studied malignant neoplasm's and emphasized a correlation between histopathologic differentiation, treatment and prognosis. Tumors are assigned one of four grades according to the percentage of tumor showing incomplete differentiation [78] with poorly differentiated tumors being more aggressive, associated with metastatic potential and a poorer prognosis than their well-differentiated counterparts. The disadvantage with the Broders system is the lack of correlation with clinical outcome because most malignant human neoplasm consists of heterogeneous cell populations with probably different biological behavior patterns.[79] Poorly differentiated cells within the superficial parts of the tumor do not necessarily reflect an aggressive esophageal SCC, and grading of these parts may thus not be an indicator for the clinical behavior of a tumor.[81] Broders classification is still used in routine assessment of tumor differentiation by many pathologists today. This system of tumor grading

was used in the study and compared with the expression of E-cadherin and β-catenin during tumor progression.

In the early 1989 Bryne et al [81] assessed the most anaplastic areas in the deep invasive tumor front. Tumors are then graded based on the five morphological features: The degree of ketatinization, nuclear polymorphism, number of mitosis, plasma-lymphocytic infiltration and the pattern of invasion with the highest prognostic value at the invasive front of the neoplasm. Pattern 1 tumors have well-delineated, pushing borders. Pattren 2 tumors show an advancing edge of the tumor that infiltrates in solid cords, bands, or strands. Pattern 3 lesions have margins that contain small groups or cords of infiltrating cells. In Pattern 4 the host tumor interface show marked cellular dissociation into small groups or even single cells. His studies on SCC of the oral cavity showed a correspondence between the grading and the prognosis of a case.[80, 81] Although Brynes Grading of the invasive front of a neoplasm is more accurate in assessing the prognosis of a neoplasm, its main disadvantage lies in the requirement that the grading must be done at the deepest invasive front of the tumor mass. Superficial biopsies may therefore lead to an incorrect assessment. In this study the 44 cases selected had biopsies that met this important criterion for the application of Brynes Grading of the invasive front.

The cadherins are a family of transmembrane glycoprotein that have an important role in morphogenesis and maintenance of a different phenotyte.[101] E-cadherins mediate cell-to cell adhesion and are anchored intracellularly to the actin cytoskeleton of cell via proteins referred to as β-catenins.[127] Reduction in the expression of E-cadherin in patients with esophageal SCC was found to be strongly associated with post-operative blood borne recurrence, resulting in poorer prognosis than in those patients with normal tumor expression.. Collectively the loss of E-cadherin and β-catenin has correlated with reduced survival and poor prognosis in patients with esophageal cancer.[101, 149, 153] The

immunoperoxidase technique, which is the technique of choice to demonstrate these glycoproteins, enable microscopists to identify molecular changes associated with the deteriorating prognosis of malignant neoplasms. The present study is unique in that it attempts to compare these molecular changes with the phenotypic expression of neoplastic cell change before tumor cell dehiscence and metastasis occurs.

Esophageal SCC most commonly occurs in the middle and lower thirds of the esophagus.[21] In our study, the highest rate of ocurrence was in the middle third. No significant relationship was observed between the expression of E-cadherin and β-catenin with regard to the location of the tumor. It is our logical thoughts that the de-regulation of adhesion molecules in the esophagus particularly in the middle third of the esophagus results from the multifactorial carcinogenic factors rather than mutation of cadherin and catenin genes.

No significant differences were seen with advancing age and gender regarding the expression of E-cadherin and β-catenin. Males and females showed the same pattern of distribution for E-cadherin and β-catenin in their tumors, which was an indication that the expression of adhesion molecules during tumor progression is not gender dependent. The most affected patients were in the middle age group in both males and females which was an indication that SCC of the esophagus is a disease of the middle aged.

The normal pattern of E-cadherin and β-catenin expression has been reported in various carcinomas of epithelial tissue in origin.[101,120, 125, 148] The normal expression of E-cadherin and β-catenin in our study was mainly localized at the intra-cellular junctions (See Fig 1a and Fig 1b respectively). De-localization of E-cadherin and β-catenin was observed in the study like in others studies, where loss or

altered expression was reported.[120, 125, 148] This pattern of expression was an indication of regulatory and functional changes in the mediated cell-to-cell adhesion molecules.[95] From these patterns some of tumors cells showed a diffuse pattern, focal and other tumor cells had a disorganized pattern of expression for both E-cadherin and β-catenin.

In our investigation loss of E-cadherin was associated with invasive behavior. These findings were in accordance with other studies.[98, 120] The initiation of SCC is thought to result from a loss of intra-cellular junctions caused by a reduced expression of E-cadherin.[17, 93, 101, 124] The major function of a cell is the transcription of genes that are then later translated into proteins, which maintain normal cellular function in the body. The interruption resulting from the interference with nuclear transcriptional machinery of the cell as observed during nuclear fusion of two or more cell nucleus (as illustrated in Figure 4) hinders the production of vital proteins. The newly formed neoplastic cells assume different tumor characteristics as the tumor progress resulting in a population of different clones of cells. The alteration in the tumor cell nuclear composition results in a production and release of enzymatic proteases from the tumor cells. We now know that the proteolytic enzymes are released by tumor cells rather than stromal cells [154] which facilitates the digestion of extracellular matrix (ECM). The nuclear fusion of two or more cells was reported in previous studies as a critical event crucially involved in the initiation and progression of cancer and metastases [17, 116] and these observations were noted in superficial spreading type of SSC of the esophagus.[155] Tumor cells with nuclear fusion also showed a loss of intra-cellular junction for β-catenin resulting in its dislocation from intra-epithelial membrane to cytoplasm. These observation lead to a conclusion that β-catenin is not involved in initiating cell invasion in patients with SCC as previous reports suggested [148] but could participate in the late cascading events furthering tumor cell invasion because of its direct link between cadherin and tumor suppressor genes and oncogenes, probably exerting its influential role in these

cascading events of invasion by destabilizing the cadherin-catenin complex structure and the resulting deregulation of E-cadherin eventually contribute to a loss of its ability to act as a tumor suppressor gene.[95, 107] This observation is supported by the findings in tumors cells that showed normal expression of E-cadherin expression with no nuclear fusion of cells showed the retention of β-catenin in their intra-cellular membrane. These findings supports the clear distinction between the free cytoplasmic pool of β-catenin that is initiated through Wnt signaling pathway which leads to its ubiquitation through GSK-3β and the second accumulation of β-catenin is mediated by cadherin-binding proteins which participate in cell morphology.[143] In our result it is clear that the β-catenin accumulates as a result of E-cadherin dissociation.

Previous reports demonstrated that the reduction rate of E-cadherin expression was observed to be 66% and 88% in specimens from the patients with esophageal SCC, [120, 125] while 54.5% (24/44) of esophageal SCC examined in the present study showed reduced expression of E-cadherin. The results did not match the reported findings, because most malignant cells are polyclonal, implying the presence of heterogeneous cell population with different biologic characteristics which influence the accurate prognostication and immunoreactivity within each tumor as a marker associated with SCC differentiation.[156] The expression of E-cadherin was preserved in a tumor that was well differentiated. This observation suggested that mutation of E-cadherin gene does not occur early during SCC carcinogenesis in some tumors as it was mentioned in one study.[17] Moderately differentiated SCC showed a reduced expression of 51.4% (19/37), while Xi-Jiang Zhao et al [124] and Ying-Cheng Lin et al [119] found a reduced expression of 67% and 86% respectively. The expression of E-cadherin in poorly differentiated tumors was also observed in one case (1/6) with cytoplasmic localization of E-cadherin which indicated structural changes in cadherin-catenin complex. Several reports were available on this

phenomenon, Xi-Jiang Zhao et al [124] reported 15%, but Yi-Cheng Lin et al [119] did not observe the expression of E-cadherin.

The reduction of β-catenin expression was 69.8% in esophageal SCC in a study of Xi-Jiang Zhao et al [124] and 35.5% in Ying-Cheng Lin et al.[119, 125] In our study, 50% of β-catenin expression was considered to be reduced. No level of significance could be observed between Grade II and Grade III for the expression of both E-cadherin and β-catenin because of insignificant variables, but the observed expression for both E-cadherin and β-catenin was suggestive of a decreasing trend of expression which meant that E-cadherin and β-catenin molecules are probably responsible for tumor differentiation.

Multivariate analysis revealed E-cadherin expression was an independent factor that impacted on prognosis of the patients with esophageal SCC as determined by the Bryne grading system. These findings were consistent with the reports of Tamura et al, and recently a multicenter investigation supported this viewpoint.[125] In our investigation, we observed a lack of consistence with Broders classification with regard to tumor behavior associated with tumor differentiation with respect to E-cadherin expression. It is generally accepted that the moderately differentiated tumors are associated with a better prognosis than their poorly differentiated counterparts because of the metastatic potential of the latter. In our investigation some moderately differentiated tumors with reduced expression of E-cadherin with cytoplasmic retention of β-catenin showed an aggressive behavior with grave prognosis than some of the poorly differentiated tumors which had absent expression for both E-cadherin and β-catenin. This observation indicates a lack of consistency with the Broders grading system as a factor determining prognosis.

Previous studies did not investigate the expression of these molecules at the invasive front. In our study, the expression of E-cadherin and β-catenin at the tumor front showed the same reduction of 78% (7/9) for tumor cells with solid chord infiltration. These results suggest that the repression of E-cadherin gene occurs frequently in SSC of the esophagus. Tumor cells exhibiting small group infiltration showed a reduction of 71% (25/35) for E-cadherin and 34% (12/35) for β-catenin. Bryne Pattern 3 of invasion showed a high retention of E-cadherin expression than Pattern 2, this observation could be due as a result of multifactorial factors including: 1) tumor cell population of different clones of tumor cells: 2) mutation involved: 3) down-regulation of cadherin-catenin adhesion junctions. Interestingly β-catenin showed a low reduction at the invasive front and this retention of β-catenin clearly indicates an association between the reduced expression of E-cadherin and the retaining of β-catenin which strongly suggest that loss of E-cadherin binding may cause a redistribution of β-catenin from the cell membrane to the cytoplasm.[110]

Out of 59% of tumor cells which did not show invasion of the vascular endothelial wall, 46% (12/26) of tumor cells showed a reduced expression of E-cadherin in the our study. Xi-Jiang Zhao et al [124] reported a reduction of 61%. From 41% (18/44) of tumor cells which had invaded vascular spaces, 61% (11/18) showed a reduced expression for E-cadherin (81% was reported by Xi-Jiang Zhao et al.[124]) The expression of β-catenin in tumors with no vascular invasion showed a reduction of 65% (17/26), while 28% (5/18) was reduced in tumor cells with vascular invasion. Xi-Jiang Zhao et al [124] reported 67% for reduced expression in tumors without vascular invasion and 78% with invasion. These results obtained by Xi-Jiang Zhao et al [124] were not in accordance with our findings regarding the expression of β-catenin during vascular invasion. The results obtained strongly suggest that β-catenin participate in the late stages of metastases.

What is alarming with the esophagus SCC diagnosed in our hospital is the observation of an increase number of affected females compared to males in the period of 2004 January to 2005 December. The cause of the increased frequency of this neoplasm could be attributed to poor nutrition and infections such as HIV/AIDS. The former is related to the poor socio-economic circumstances under which most of the communities our hospital serves, lives. The prevalence of HIV/AIDS and the recent rise in carcinomas associated with the pandemic associated with co-infection by several viruses that have been proven to be oncogenic (particularly the HPV 16, 18 and 33 which have been reported to cause squamous cell carcinoma of the esophagus [39, 40]). Further investigations are needed to elucidate this observation.

This study has shown that β-catenin expression rather than E-cadherin is responsible for invasion of neoplastic cells in SCC of the esophagus. This observation was exploited further by investigating the role of E-cadherin and its invasive properties on different patterns of infiltrating cells. In our investigation, we observed two patterns of invasion of neoplastic cells in esophageal SCC. The first pattern encountered showed tumor cells infiltrating with solid chords, bands or strands (categorized as Pattern 2) and Pattern 3 was of tumors with small group or chords of infiltrating neoplastic cells.

The expression of E-cadherin and β-catenin at the invasive front has never been reported before. In our investigation, tumor cells with Pattern 1 were not seen because esophageal SCC is seldom diagnosed in the early stages of the disease. When the deep invasive grading system was applied, E-cadherin expression was reduced to 73% (32/44) on patients with esophageal SCC and the reduced protein levels were associated with the pattern of tumor cell invasion. When the expression rate of β-catenin was reduced to 43% (19/44) the protein level was associated with vascular invasion. Tumor cells with Pattern 2 showed a diffuse expression of immunostain for both the expression of E-cadherin and β-

catenin. At the invasive front this tumor cells showed a minor loss of E-cadherin from the intra-epithelial junction (Fig 4a) with uniform retention of E-cadherin on the tumor bulk maintaining cell polarity. The localization of E-cadherin at the intra-epithelial membrane shows the normal regulation of the cell-to-cell interaction, which makes the of tumor cell detachment from the primary tumor at the invasive front to be slow and this pattern correlates with general prognosis observed with tumors.[80, 81] As the tumor progress invading the ECM, there is usual repression of E-cadherin gene resulting from methylation of CpG islands in the promoter site of the gene as observed in (Fig 4b) the tumor cells at the periphery show a faint cytoplasmic expression of E-cadherin which is an indication of E-cadherin down-regulation and 78% (7/9) of the tumor cells showed this pattern. It clearly shows that tumor cells which retain E-cadherin expression at the invasive front have a better tumor behavior than their counterparts with reduced expression. When the rate of mitotic activity and nuclear polymorphism on the tumor are higher there is often reduction in the expression of E-cadherin and this are features that are indicative of biological behavior of the tumor cells during tumor progression. Of particular interest with the tumor cells which have a reduced expression of E-cadherin is the cohesion of tumor cells observed, could be resulting from a partial compensation of E-cadherin by cadherin subfamily such as cadherin-11 as it occurs during early stages of cadherin development [94, 100] and the resulting down regulation of E-cadherin mediated adhesion is responsible for a decrease in cell polarity within the tumor cells. Interestingly enough these tumor cells showed a high degree of nuclear polymorphism with high ratio of mitotic activity which is clearly an indication that the tumor cells undergo proliferation at the tumor front and we could only speculate that these tumor cells are at the verge of disintegrating and infiltrate the stroma in a scattered manner.[17]

β-catenin also plays a crucial role in the maintenance of cellular junction.[122] Out of 43% of tumor cells with reduced expression of β-catenin, 78% (7/9) also showed the same reduced expression observed with E-cadherin. Unlike E-cadherin, β-catenin showed a preserved expression in the intra-epithelial

junction in the deep margins of the invasive front (Fig 4c) with few tumor cells losing the intra-epithelial membranous expression. There is also uniform retention of expression when the tumor cells at the invasive front have low mitotic activity with moderate nuclear polymorphism. There seem to be a trend with E-cadherin and β-catenin expression with mitotic activity and nuclear polymorphism at the invasive front which is that of reduced expression.

Tumors with Pattern 3 have a general poor prognosis.[79-81] In the study, the expression of E-cadherin was reduced to 71% (25/44) which was low than tumor cells with pattern 2 invasion and this observation was attributed to by different population of cell clones at the invading front with disorganized pattern of expression resulting in different intensity of immunoreactivity with tumor cells. This phenomenon of tumor cells with this pattern of immunoreactivity was previously discussed by Bongiorno et al as a classification.[157]

Since was observed that the repression of E-cadherin occurs differently with different tumors and its retention in some of the tumors was not a surprise. These tumors are generally known to have a rate of cellular dissociation of tumor cells into focal groups of high metastatic potential.[80, 81] In deed, it was observed in the tumors that E-cadherin expression at the invasive front there is extensive down-regulation of CHD1 gene (Fig 5a) and the progressive loss of E-cadherin protein from the cell membrane leads to tumor cell detachment from the primary tumor nest (Fig 5b) and then infiltrate the stroma in a scattered manner.[17] The dissociating single tumor cells from the tumor nest are responsible for the aggressive behavior of the tumor because of their capacity to infiltrate the small capillaries and blood vessels for metastatic deposition of tumor cells to distant organs and usually such tumor cells often gain an increase in mesenchymal markers because of a high induction of EMT which frequently occurs during carcinoma invasion.[154] This observation clearly indicated the importance of E-cadherin expression at the invasive front rather than its retention by the tumor bulk. Usually the dissociating tumor nest retains expression of E-cadherin but the tumor cells detaching from this nest show a

reduced expression because the cadherin-catenin complex structure involves the interaction of more than two cells and the resulting weakening thereof leads to dislocation of the structure.[95] But few of the dissociating cells maintained the expression of E-cadherin (Fig 5c and Fig 5d) respectively. Even though it unclear what role this tumor cells posses during invasion but we speculate perhaps this tumor cells may not be participating in metastasis since the is maintenance of cell-to-cell interaction which suggest normal regulation of E-cadherin and β-catenin which translates to lesser invasive behavior of the tumor cells because of the protective role of E-cadherin as an invasive suppressor gene.[95, 107] Further investigations are needed to elucidate these observations.

Tumor cells which have completely lost the protective role of E-cadherin as illustrated (Fig 5e) are highly invasive and these cells contribute to poor prognosis because the survival rate of individual drops with invasion of underlying structures [21] as it is observed the small groups of SCC have invaded the submucosal layer pinching on the nerve.

The expression of β-catenin was reduced to 34% (12/35) and the high protein level result from the dissociation of E-cadherin from binding β-catenin which leads to its redistribution from the cell membrane to the cytoplasm (Fig 5f) which also suggest an association between abnormal expression of E-cadherin and β-catenin. The interaction of tumor cells with endothelial cells at the initial and later stages of metastatic cascade is mandatory for the passage of tumor cells into vessels at the primary site and extravasates at metastatic site.[158] During the migratory phase of tumor cells we observed a decreasing trend of E-cadherin expression, often with complete loss of expression during intravasation (Fig 6a) but there is small retention of the protein when the infiltrating cells are large than eight cells per group (Fig 6b) but the tumor cells at the invasive front pinching on vascular wall show a reduced expression of which has been the normal feature observed with E-cadherin expression at this site of the neoplasm. Interestingly enough, the expression of β-catenin during vascular invasion show

retention of the stain in the intra-epithelial membrane (Fig 6c) and these observation lead us to believe that the β-catenin might participate in the cascading events of metastasis by facilitating the binding of tumor cells to endothelial cells (Fig 6d) homotypicaly through interaction with vascular endothelial growth factor receptor (VEGF) which endothelial cell have the receptors for and the human VEGF gene promoter has been reported to contain binding sites for β-catenin/Tcf.[159, 160] These finding suggest that β-catenin after all could be responsible for poor prognosis as previously suggested [125] particularly on tumors which show high retention of the protein in the intra-cellular-junction on the invasive front.

In a previous study, the validity of mitotic count as a marker of prognosis remained controversial due to tumor heterogeneity, inter-observer disagreement, variation in the size of high power fields in different microscopes, and because cells size is not taken into consideration [81] which resulted in exclusion of mitotic count as a parameter. In the study, we observed an association between the rate of mitotic activity and the pattern of invading cells on the tumor. The proliferative activity of the tumor due to mitotic activity is a possible indicator of tumor behavior. The expression of E-cadherin and β-catenin was observed to be retained in tumor cells which had low mitotic count (Fig 4a and Fig 4c) and the reduced expression of both proteins was also observed in tumor cells with increased in mitotic activity (Fig 4c and Fig 5a). This observation lead us to speculate that the proliferative activity of the tumor might be responsible for causing repression of E-cadherin gene at the promoter site by associating with recruitment of methyl DNA binding proteins (MBDs) and histones deacetylase activity (HDACs) which together contribute to the compaction of the DNA in the promoter region and hence, to gene inactivation.[107] Tumor cells with Pattern 3 of invasion were observed to have a high degree of mitotic activity that tumor cells with Pattern 2 of invading cells (Fig 2). From this

observation we can conclude that the rate of mitotic activity on the tumor on superficial biopsies is a precise scientific indicator of tumor behavior.

CHAPTER 6: CONCLUSION

The expression of E-cadherin and β-catenin on histological grade (Broders) is associated with tumor differentiation, with preserved expression associated with well differentiated tumors and reduced expression associated with poorly differentiated tumors. There is a lack of correlation with Broders grading with clinical behavior of the tumor and with the expression of both E-cadherin and β-catenin proteins. Some moderately differentiated tumors with reduced expression of Both E-cadherin and β-catenin were more aggressive than some of the poorly differentiated tumors with absent expression both of the proteins which means the expression of these two both proteins E-cadherin and β-catenin has no prognostic significance with tumor differentiation.

The assessment of invasive tumor front revealed that the expression of E-cadherin and β-catenin by the tumor bulk is of less significance in determining tumor behavior. There is a significant trend of E-cadherin expression at the invasive tumor front being associated with tumor cell dissociation. The reduced expression of E-cadherin at the invasive tumor front is mostly associated with tumor cells with high mitotic count and high nuclear polymorphism and tumor cells with preserved expression showed a trend of low mitotic activity with nuclear polymorphism. Tumor cells with decreased expression of E-cadherin were observed showing high tumor cell dissociation contributing invasiveness associated with tumor behavior. E-cadherin is an essential epithelial marker for the assessment of tumor cell invasion and shows some minor significance in predicting tumor behavior but has less prognostic value. The preserved expression of β-catenin at the invasive tumor front lead to a conclusion that β-catenin is not involved in tumor cell invasion, but the high protein expression was linked to tumor cell attachment on endothelial thereby facilitating metastases of neoplastic cells.

CHAPTER 7: RECOMMENDATIONS

Brynes Invasive Grading system has proved to be of high prognostic value in assessing the deep margins of the tumor which gives a good indication of tumor behavior. This classification should be integrated in the assessment of patients with esophageal SCC.

The E-cadherin is an essential epithelial marker and has a significant role in the assessment of the invasive tumor front which determines tumor behavior and how tumor cell dissociates. What should be taken into account with regard to E-cadherin is its expression in the most anaplastic areas of the tumor rather than its expression in the differented part of tumor. It is essential that β-catenin as marker for squamous cell epithelial be integrated with E-cadherin as one of the panel markers in assessing SCC's because collective expression of both the β-catenin and E-cadherin indicates the regulatory abnormalities involved. It is critical to assess the nature of these abnormalities in the cadherin-catenin structure and the in situ hybridization method should be considered to elucidate the gene expression at the genomic level. This could identify the type of errors affecting the normal activity of the gene thereby enhancing therapeutic intervention.

CHAPTER 8: REFERENCES

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