RESEARCH ARTICLE

Expression of EGFR and p53 in Head and Neck Tumors among Sudanese Patients

Mustafa Saadalnour Abusail[1], Ahmed Mohmed Ahmed Dirweesh[2], Rashid Awad Abdalla Salih[2], Ahmed Hussain Gadelkarim[2]*

Abstract

Background: The aim of this study was to assess EGFR and p53 expression in head and neck tumors among Sudanese patients using immunohistochemistry. Materials and Methods: A retrospective descriptive study was performed on 150 samples from patients diagnosed with HNCs as well as 50 from individuals with benign head and neck tumors. EGFR and p53 expression was assessed using immunohistochemistry (IHC). Results: EGFR was expressed in 126/150 (84%) of HNCS and 6/50 (12%) benign head and neck tumors where as p53 was expressed in 29/150 (19.3%) of HNCs and 2/50 (4%) of benign head and neck tumors, with significance at p values of 0.001 and 0.009 respectively. Conclusions: There is a significant association between EGFR, P53 expression and head and neck cancers among Sudanese patients, .

Keywords: Head and neck cancer - Sudanese - EGFR - p53

Asian Pac J Cancer Prev, **14 (11)**, 6415-6418

Introduction

Head and neck cancer comprises malignancies arising in the upper respiratory and digestive tracts and is a relatively frequent type of cancer (Parkin et al., 2002). Thus, the term "head and neck cancer" includes lesions at several anatomic sites, such as the lip, oral cavity, nose and paranasal sinuses, naso-pharynx, oro-pharynx, hypo-pharynx, larynx, oesophagus, salivary glands, as well the soft tissues of the neck and ear. The malignant tumors of the head and neck consist of a rather heterogeneous group of neoplasias arising in the epithelium of the upper aerodigestive tract. The most common histologic type is squamous cell carcinomas (SCC), occurring in the oral cavity, pharynx (nasopharynx, oropharynx and hypopharynx) and larynx (Lassen, 2010). Head and neck squamous cell carcinoma (HNSCC) is the seventh most commonly diagnosed cancer worldwide (Ferlay, et al., 2010) and is associated with survival rates. Its incidence varies widely among different regions. In North America and Europe, HNCs accounts for 3-4% of all cancer diagnoses. Conversely, in Southeast Asia and Africa, HNCs accounts for approximately 8-10% of all cancers (Santarelli et al., 2009). HNCs have traditionally been linked to alcohol and tobacco abuse (Goon et al., 2009). However, 15-20% of HNCs cases have no known tobacco or alcohol exposure (Gillison and Shah, 2001; Jo et al., 2009) thus, other agents, such as viruses, are being investigated. It is now evident that a significant proportion of HNSCCs are caused by HPV (Chung and Gillison, 2009).

Epidermal growth factor receptor (EGFR), it is a ubiquitously expressed transmembrane glycoprotein in the ErbB/HER family of receptor tyrosine kinase. These receptors are composed of an extracellular ligand-binding domain, a hydrophobic transmembrane segment, and an intracellular tyrosine kinase domain. Binding of natural ligands (amphiregulin and transforming growth factor alpha (TGF-α) in head and neck cancer) to EGFR results in a conformational change in EGFR. This promotes homo- or heterodimerization with other ErbB/HER family of receptors with subsequent autophosphorylation and activation of the tyrosine kinase (Ciardiello et al., 2003). This activation of EGFR leads to the initiation of intracellular signaling pathways which regulate the activation of cell proliferation, invasion, angiogenesis, and metastasis. High expression of EGFR occurs in most epithelial malignancies including head and neck squamous cell carcinoma (HNSCC) (Ciardiello et al., 2003). Over-expression or mutation of EGFR is found in 80-100% of the patients with HNCSCC, and both are associated with poor prognosis and decreased survival (Bonner et al., 2006; Hoffmann et al., 2009).

p53, it is a tumor suppressor and 'guardian of genome', plays a critical role in apoptosis, cell cycle control, DNA damage response, host resistance to carcinogens, and cellular anti-cancer mechanisms. Upon DNA damage, which usually results from chemical carcinogens, chemotherapeutic drugs, irradiation or endogenous stressors, p53-mediated pathways attempt to repair the injury through cell cycle arrest and DNA repair (Lukas et al., 2004). To eliminate threat of tumorigenesis, p53

*[1]Department of Histopathology and Cytology, Faculty of Medical laboratory Sciences, Elneelain University, Sudan, [2]Medicine, University of Hail, Kingdom of Saudi Arabia *For correspondence: Hussaingad1972@yahoo.com, he.ahmed@uoh.edu.sa*

protein triggers the intrinsic apoptosis or blocks the cell cycle permanently and induces senescence in the event that damage is too extensive (Helton and Chen, 2007). Therefore, malfunction of p53 is central to the development of most human cancers.

Genetic alteration of p53 has been shown to correlate with the development of SPM. Approximately 50% of SCCHN exhibited p53 mutation, which is similar to the frequency of p53 mutation reported for all human cancers (Brachman et al., 1992; Bénard et al., 2003). Overexpression of p53 in the first primary cancer may be a valuable marker for identifying individuals at high risk of developing SPM (Gallo and Bianchi, 1995). Moreover, p53 overexpression in tumor-distant epithelia in patients with SCCHN is associated with the risk of development of a SPM and thus could be used to identify which groups of patients are at higher risk of developing SPM (Homann et al., 2001). Therefore, the objective of this study was to assess the expression of EGFR and p53 in HNCs tissue blocks from Sudanese patients.

Materials and Methods

A total of 150 patients, 90 males and 60 females (male/female ratio, 1.5:1), aged between 12 and 85 years with mean age of 51 years, were diagnosed as having HNCs, as well as 50 samples from benign head and neck tumors were assessed for EGFR and p53 expression using immunohistochemistry. The diagnosis was based on clinical examination and histological features of the biopsy. HNCs diagnosis was verified base on Royal College of Pathologists criteria (Royal College of Pathologists, 2005). The HNCs including 144/150 (96%) squamous cell carcinomas (SCCS) and 6/150 (4%) adenocarcinoma. The sample included full coverage of patients with HNC lesions referred to our hospital within Two-year time. Ethical consent was obtained from ethical committee of the Faculty Research Board and Hospital. EGFR and P53 immunohistochemistry (IHC) was performed on formalin-fixed paraffin embedded (FFPE) tissue sections using kits from (Beijing Aide Lai Biotechnology Co., Ltd.). After antigen retrieval, sections were incubated with mouse monoclonal anti-EGFR, anti-p53 and then EnVision-System HRP anti-mouse, followed by diaminobenzidine chromogen and counterstaining with hematoxylin. During each IHC assay, proof slides were coupled with negative and positive controls provided by the manufacturer for each marker, and reactions were observed appropriately. IHC stained sections were examined under the light microscope (Olympus CHT, Optical.Co.Ltd, Japan) using 4× 10× 40× 100×, Objective and eyepieces of 10× giving a maximum magnification of 1000. EGFR staining patterns were designated as cytoplasmic or membranous where as p53 staining patterns were observed only as a nuclear staining of epithelial cells and the nuclei with clear brown color, were scored as positive. The intensity of immunohistochemical staining for both markers was scored by two investigators based on subjective evaluation of color exhibited (brown) by antigen, antibody and chromogen complex. It was scored as 0 for negative (no color), 1+ for weak (light brown color), 2+ for moderate (dark brown color), and 3+ for strong staining (very dark brown color) with 0 or 1 scores defined as negative and 2 or 3 defined as positive (Pu et al., 2009).

Table 1. Distribution of EGFR and P53, by Tumors

Markers		Tumor				Total		p value
		Malignant		Benign				
EGFR	Negative	24	(16.0%)	44	(88.0%)	68	(34.0%)	
	positive	126	(84.0%)	6	(12.0%)	132	(66.0%)	
	Total	150	(100.0%)	50	(100.0%)	200	(100.0%)	0
P53	Negative	121	(80.7%)	48	(96.0%)	169	(84.5%)	
	Positive	29	(19.3%)	2	(4.0%)	31	(15.5%)	
	Total	150	(100.0%)	50	(100.0%)	200	(100.0%)	0.009

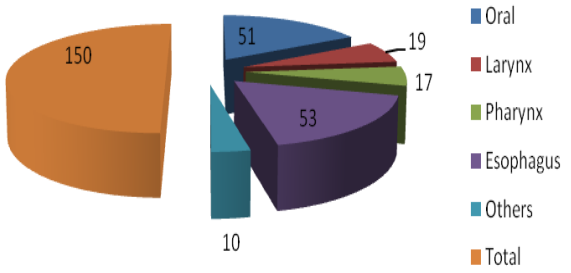

Figure 1. Description of the Study Population by Lesion Site

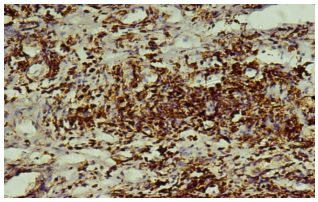

Figure 2. Pharyngeal SCC Positive EGFR Immunohistochemical Staining

Results

In the present study we investigated 150 head and neck carcinomas, obtained from different anatomical sites including oral, larynx, pharynx, esophagus and others, constituting, 51, 19, 17, 53 and 10 respectively, as shown in Figure 1.

EGFR was expressed in 126 (84.0%) of head and neck cancers (HNCs) and 6 (12.0%) of benign head and neck tumors. the expression of EGFR is significantly associated with head and neck cancers (HNCs) p value≥(0.000) as shown in Table 1, Figure 2.

p53 was expressed in 29 (19.3%) of head and neck cancers (HNCs) and 2 (4%) of benign head and neck tumors. Statistically, the expression of P53 is significantly associated with head and neck cancers (HNCS) p value≥ 0.009 as shown in Table 1.

Discussion

Worldwide there are 12,662,554 cases of all cancers diagnosed in 2008. Head and neck cancers (HNCs) comprise 8.8% of these cases. In Sudan, there were 21,860 cases of all cancers diagnosed in 2008, of which 2,942 cases were HNCs; they represent 13.5% of all cancers. The incidence rate was 13.5%, the mortality was 13% and

the males' females' ratio was 1.3:1 (Globalcan, 2008).

In regard to the association between EGFR and head and tumors, EGFR was expressed in 84% of the malignant cases in this study and 12% of the benign tumors. The expression of EGFR in head and neck cancers is statistically significant, the p value=0.022. The increased expression of EGFR in HNSCC usually linked to the gene amplification and transcriptional activation (Grandis et al., 1996; Chung et al., 2006). The loss of growth control in head and neck squamous cell carcinoma (HNSCC) is characterized by acquisition of an autocrine regulatory pathway involving the epidermal growth factor receptor (EGFR) (Grandis et al., 1997; 1998). Several studies have demonstrated that EGFR and its autocrine ligand transforming growth factor alpha (TGF-ạ) are upregulated in HNSCC (Grandis and Sok, 2004; Egloff and Grandis, 2006). Therefore, our findings support previous studies when they reported that the activation of the proto-oncogene EGFR is an early event in head and neck carcinogenesis. EGFR mRNA is highly expressed in SCCHN and contributes to the pathogenesis of this disease (Grandis et al., 1998; Soulieres et al., 2004; Lee et al., 2005). High levels of EGFR protein expression, as detected by immunohistochemistry (IHC) have been seen in up to 90% of SCCHN tumors and are associated with poor prognosis (Grandis et al., 1998; Chung et al., 2006). In a study conducted in Iraq by Sarkis et al., (2010) assessed EGFR in head and neck cancers using immunohistochemical method, they reported that EGFR was expressed in (87.5) of head and neck cancers.

EGFR overexpression and aberrant EGFR gene copy number (EGFR GCN) have been associated with poorer prognosis and disease-specific survival in SCCHN (Grandis and Tweardy, 1993; Chung et al., 2006; Temam et al., 2007). Consistent with EGFR expression as a poor prognostic factor, total EGFR and activated (phosphorylated) EGFR PY1068 were independently associated with decreased progression free survival (PFS). Although HPV infection appears to correlate with improved prognosis in SCCHN, its relationship with EGFR expression is under investigation (Fakhry et al., 2008; Rischin et al., 2009). Kumar et al. (2008) retrospectively correlated EGFR and p16 protein expression (a marker of oncogenically active HPV infection) in oropharyngeal tumors- and found that patients whose tumors expressed low EGFR and high p16 had better clinical outcomes in comparison to those whose tumors expressed high EGFR and low p16. Inactivation of pRb by HPV E7 protein results in overexpression of p16 protein, thus p16 immunostaining has served as a surrogate marker for HPV-associated SCCHN. Patients with tumors lacking both p16 expression and HPV (p16-/HPV-) had the worst disease-specific survival compared to tumors with p16+/HPV+, p16-/HPV+ or p16+/HPV- types (Smith et al., 2008). Despite the importance of HPV in the pathogenesis and prognosis of SCCHN in response to chemotherapy and radiation, the role of HPV DNA and response to EGFR inhibitors in SCCHN is unclear. a proto-oncogene tyrosine kinase receptor, is overexpressed in SCCHN, and its ligand, hepatocyte growth factor (HGF), stimulates cell proliferation, motility and invasion (Knowles et al., 2009).

In this study there was statistical significant association between P53 and head and neck cancers, the p value=0.009. Similar results were published by Gallo et al. (1995) when screened 75 cases of head and neck cancers for p53 using immunohistochemical methods, (52%) of the cases showed positive p53 immunohistochemical staining. Another study from Italy conducted by Calzolari et al. (1997) also screened p53 in 85 cases of head and cancers 35 (41.2%) of them showed positive p53 immunohistochemical staining. In India, Kumar et al. (2008) assessed p53 in 86 cases of laryngeal cancers (52%) of the cases disclosed positive p53 immunohistochemical staining. In a relation between p53 and p16 expression in head and neck tumors, some studies found positive correlation for both markers. These findings may consistent with the study by Smith et al. (2010) when they assessed 237 cases of head and neck cancers for p16 and p53, they found 13.5% of the cases were positive for the two markers. HPV infection has been demonstrated to play a role in the molecular pathways through its viral oncoproteins, E6 and E7. These proteins increase degradation of p53 and interfere with pRb function leading to upregulation of p16INK4a by loss of negative feedback control (Andl et al., 1998). P16INK4a and p53 are tumor suppressor genes and key targets in the loss of cell cycle control (Sherr and McCormick, 2002).

The study showed strong association between EGFR and p53 and head and neck cancers, and this may provides important information relating to the diagnosis, prognosis and treatment of affected patients.

References

Andl T, Kahn T, Pfuhl A, et al (1998). Etiological involvement of oncogenic human papillomavirus in tonsillar squamous cell carcinomas lacking retinoblastoma cell cycle control. *Cancer Res*, **58**, 5-13.

Bénard J, Douc-Rasy S, Ahomadegbe JC (2003). TP53 family members and human cancers. *Hum Mutat*, **21**, 182-91.

Bonner JA, Harari PM, Giralt J, et al (2006). Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *NEnglJ Med*, **354**, 567–578.

Brachman DG, Graves D, Vokes E, et al (1992). Occurrence of p53 gene deletions and human papilloma virus infection in human head and neck cancer. *Cancer Res*, **52**, 4832-3.

Calzolari A, Chiarelli I, Bianchi S, et al (1997). Immunohistochemical vs molecular biology methods. Complementary techniques for effective screening of p53 alterations in head and neck cancer. *Am J Clin Pathol*, **107**, 7-11.

Chung CH, Gillison ML (2009) Human papillomavirus in head and neck cancer: its role in pathogenesis and clinical implications. *Clin Cancer Res*, **15**, 6758-62.

Chung CH, Ely K, McGavran L (2006). Increased epidermal growth factor receptor gene copy number is associated with poor prognosis in head and neck squamous cell carcinomas. *J Clinical Oncol*, **24**, 4170-6.

Ciardiello F, Tortora G (2003). Epidermal growth factor receptor (EGFR) as a target in cancer therapy: understanding the role of receptor expression and other molecular determinants that could influence the response to anti-EGFR drugs. *Eur J Cancer*, **39**, 1348-54.

Egloff AM, Grandis J (2006). Epidermal growth factor receptor-targeted molecular therapeutics for head and neck squamous cell carcinoma. *Expert Opin Ther Targets*. **10**, 639-47.

Fakhry C, Westra WH, Li S (2008). Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J National Cancer Institute*, **100**, 261-9.

Ferlay J, Shin HR, Bray F, et al (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*, **127**, 2893-917.

Santarelli A, Lo Russo L, Bambini F, Campisi G, Lo Muzio L (2009). New perspectives in medical approach to therapy of head and neck squamous cell carcinoma. *Minerva Stomatol*, **58**, 445-52.

Gallo O, Bianchi S, Giovannucci-Uzzielli ML, et al (1995). p53 oncoprotein overexpression correlates with mutagen-induced chromosome fragility in head and neck cancer patients with multiple malignancies. *Br J Cancer*, **71**, 1008-12.

Gallo O, Bianchi S (1995). p53 expression: a potential biomarker for risk of multiple primary malignancies in the upper aerodigestive tract. *Eur J Cancer B Oral Oncol*, **31**, 53-7.

Gillison ML, Shah KV (2001). Human papillomavirus-associated head and neck squamous cell carcinoma: mounting evidence for an etiologic role for human papillomavirus in a subset of head and neck cancers. *Curr Opin Oncol*, **13,** 183-8.

Goon PK, Stanley MA, Ebmeyer J, et al (2009). HPV & head and neck cancer: a descriptive update. *Head Neck Oncol*, **1**, 36.

Grandis JR, Chakraborty A, Melhem MF(1997). Inhibition of epidermal growth factor receptor gene expression and function decreases proliferation of head and neck squamous carcinoma but not normal mucosal epithelial cells. *Oncogene*, **15**, 409-16.

Grandis JR, Chakraborty A, Zeng Q (1998). Downmodulation of TGF-alpha protein expression with antisense oligonucleotides inhibits proliferation of head and neck squamous carcinoma but not normal mucosal epithelial cells. *J Cell Biochem*, **69**, 55-62.

Grandis JR, Chakraborty A, Zeng Q (1980). Downmodulation of TGF-alpha protein expression with antisense oligonucleotides inhibits proliferation of head and neck squamous carcinoma but not normal mucosal epithelial cells. *J Cell Biochem*, **69**, 55-62.

Grandis JR, Sok JC (2004). Signaling through the epidermal growth factor receptor during the development of malignancy. *Pharmacol Ther*, **102**, 37-46.

Grandis JR, Tweardy DJ (1993). Elevated levels of transforming growth factor alpha and epidermal growth factor receptor messenger RNA are early markers of carcinogenesis in head and neck cancer. *Cancer Res*, **53**, 3579-84.

Grandis JR, Zeng Q, Tweardy DJ (1996). Retinoic acid normalizes the increased gene transcription rate of TGF-alpha and EGFR in head and neck cancer cell lines. *Nat Med*, **2**, 237-40.

Helton ES, Chen X (2007). p53 modulation of the DNA damage response. *J Cell Biochem*, **100**, 883-96.

Hoffmann TK, Schirlau K, Sonkoly E, et al (2009). A novel mechanism for anti-EGFR antibody action involves chemokine-mediated leukocyte infiltration. *Int J Cancer*, **124**, 2589-96.

Homann N, Nees M, Conradt C, et al (2001). Overexpression of p53 in tumor-distant epithelia of head and neck cancer patients is associated with an increased incidence of second primary carcinoma. *Clin Cancer Res*, **7**, 290-6.

Knowles LM, Stabile LP, Egloff AM, et al (2009). HGF and c-Met participate in paracrine tumorigenic pathways in head and neck squamous cell cancer. *Clin Cancer Res*, **15**, 3740-50.

Kumar B, Cordell KG, Lee JS (2008). EGFR, p16, HPV titer, Bcl-xL and p53, sex, and smoking as indicators of response to therapy and survival in oropharyngeal cancer. *J Clin Oncol*, **26**, 3128-37.

Lassen P (2010). The role of Human papillomavirus in head and neck cancer and the impact on radiotherapy outcome. *Radiother Oncol*, **3**, 371-80.

Lee JW, Soung YH, Kim SY (2005). Somatic mutations of EGFR gene in squamous cell carcinoma of the head and neck. *Clin Cancer Res*, **11**, 2879-82.

Lukas J, Lukas C, Bartek J (2004). Mammalian cell cycle checkpoints: signalling pathways and their organization in space and time. *DNA Repair*, **3**, 997-1007.

Parkin DM, Bray F, Ferlay J, Pisani P (2005). Global cancer statistics, 2002. *CA Cancer J Clin*, **2**, 74-108.

Pu YS, Huang CY, Kuo YZ, et al (2009). Characterization of membranous and cytoplasmic EGFR expression in human normal renal cortex and renal cell carcinoma. *J Biomed Sci*, **16**, 127-32.

Rischin D, Young R, Fisher R (2009). Prognostic significance of HPV and p16 status in patients with oropharyngeal cancer treated on a large international phase III trial. *J Clin Oncol*, **27**, 6004.

Sarkis SA, Abdullah BH, Abdul Majeed BA, Talabani NG (2010). Immunohistochemical expression of epidermal growth factor receptor (EGFR) in oral squamous cell carcinoma in relation to proliferation, apoptosis, angiogenesis and lymphangiogenesis. *Head Neck Oncol*, **25**, 13.

Sherr CJ, McCormick F (2002). The RB and p53 pathways in cancer. *Cancer Cell*, **2**, 103-12.

Smith EM, Rubenstein LM, Hoffman H, Haugen TH, Turek LP (2010). Human papillomavirus, p16 and p53 expression associated with survival of head and neck cancer. *Infect Agent Cancer*, **5**, 4.

Smith EM, Wang D, Kim Y, et al (2008). P16INK4a expression, human papillomavirus, and survival in head and neck cancer. *Oral Oncol*, **44**, 133-42.

Soulieres D, Senzer NN, Vokes EE, et al (2004). Multicenter phase II study of erlotinib, an oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with recurrent or metastatic squamous cell cancer of the head and neck. *J Clin Oncol,* **22**, 77-85.

Temam S, Kawaguchi H, El-Naggar AK, et al (2007). Epidermal growth factor receptor copy number alterations correlate with poor clinical outcome in patients with head and neck squamous cancer. *J Clin Oncol*, **25**, 2164-70.