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ARTICLE

p53 and Cyclin A Protein Expression in Squamous Carcinoma of the Oesophagus

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The aim of this study was to explore the relationship between p53 and cyclin A immunostaining in squamous carcinomas of the oesophagus. It has been shown that both these proteins are over-expressed in poorly differentiated endometrial carcinomas. Fifty oesophagectomy specimens were analysed for p53 and cyclin A immunoexpression. This was correlated with patient age and gender and tumor stage and grade. Forty-two percent of cases were p53 positive, while 94% of the squamous cancers expressed cyclin A protein. Neither protein showed any statistically significant correlation with clinicopathological parameters. This study has demonstrated that only 42% of oesophageal squamous carcinomas from South Africa express p53 protein, while the vast majority (94%) express cyclin A protein. Neither of these proteins showed any relationship to each other or any clinical feature or the tumor grade or stage. (Pathology Oncology Research Vol 5, No 3, 193–196, 1999)

Keywords: oesophagus, squamous carcinoma, cell cycle, p53, cyclin A

Introduction

Squamous cell carcinoma (SCC) of the oesophagus remains a major cause of morbidity and mortality in several geographical locations. Southern Africa is considered to be a "hot-spot" for this particular malignancy. Indeed, SCC of the oesophagus is the commonest cancer encountered in Black males in this region. Dietary factors such as vitamin deficiencies, soil poor in trace elements, like molybdenum and selenium, and ingestion of carcinogens harboured in poorly preserved foods, are all thought to be of pre-eminent importance in the pathogenesis of oesophageal SCC in Southern Africa. It is said that differences in causal agents (alcohol and tobacco in North America and Europe, dietary factors in Asia, the Far East and Southern Africa) and the diverse genetic profile, are responsible for regional variations seen in SCC of the oesophagus.[1]

Abnormalities of the cell cycle are important in the process of carcinogenesis and several key proteins are involved in maintaining the integrity of the normal cell cycle. Once these proteins are aberrantly expressed, abnormal cell cycling and hence, the malignant state, ensues. Two such proteins are p53 and cyclin A. *p53* is the prototype tumor suppressor gene and its protein is linked to cell regulation. Abnormalities in the *p53* gene and protein have been associated with several malignancies. Cyclin A protein is expressed just before the beginning of DNA synthesis, gradually increases until prophase and decreases by metaphase.[2] It is an important check mechanism in the G1-S transition of the cell cycle. Recently, it has been shown that both p53 and cyclin A immunoexpression is associated with high grade endometrial carcinomas.[3]

The purpose of this study is, therefore, to analyse p53 and cyclin A immunoexpression in SCC of the oesophagus and see if a similar relationship between these two proteins and tumor grade and stage exists in oesophageal SCC.

Materials and Methods

Fifty oesophagectomy specimens were accessed from the files of the Department of Pathology, University of Natal School of Medicine. Histological grading was done on all the tumors, which were conventionally placed into

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one of the following, three categories: well, moderately or poorly differentiated. The tumors were staged according to the UICC/TNM system.

Immunohistochemistry was performed on the formalin fixed, paraffin embedded tissue. Three μm sections were picked up onto poly-L-lysine coated slides and incubated for 10 minutes at 60°C. Sections were cleared through xylene and rehydrated with descending grades of alcohol. Microwave antigen retrieval was accomplished by placing slides in 0.01 buffered sodium citrate at pH 6.0 at 85° for 10 minutes in a H2500 microwave processor (Energy Beam Sciences, Inc., Massachusetts). After microwaving, sections were cooled for 10 minutes. After transfer through two changes of PBS at pH 7.4, the slides were incubated in 3% H_2O_2 at room temperature for 5 minutes. The sections were incubated with cyclin A (6E6, Novocastra Laboratories, Newcastle-upon-Tyne, UK, dilution 1 in 60) and p53 (DO7, Dakopatts, UK, dilution 1 in 25) for 4 minutes in a domestic microwave (Sharp Carousel, R7280, 650W). The sections were then incubated with biotinylated link antibody in the microwave at 10% power output for 3 minutes and 30 seconds. After washing in PBS, sections were incubated with peroxidase labelled streptavidin in the microwave for 3 minutes and 30 seconds. Sections were then incubated with substrate-chromogen (DAB), and after washing with distilled water, counterstained with Mayer's haematoxylin for 3 minutes. Finally, sections were rinsed in ammoniated water, rinsed in running tap water, dehydrated in alcohol and xylene, and coverslipped with DPX mountant.

All negative cases were repeated and appropriate positive controls (a case of oesophageal SCC known to be cyclin A positive and a colorectal cancer known to be p53 positive) were run in parallel. Omission of the primary antibody was used for negative controls. Nuclear immunolabelling was regarded as a positive reaction.

Scoring of immunoreactivity – The number of positive nuclei in a high power field were divided by the total number of nuclei present in that field, and this was expressed as a percentage. All the cells in the biopsies were counted, while 25 to 50 high power fields were assessed in the resection specimens, depending on the amount of tumor represented on a particular slide. A mean of the number of fields counted was then taken. An arbitrary score was then assigned as follows: < 5% positivity: negative: 6–25% positivity: +1; 26–50%: +2; > 50%: +3.

Statistical analysis was performed using the Chi squared and Fisher's exact tests. p values <0.05 were deemed significant.

Table 1. **Clinicopathologic features and immunohistochemical profile**

Age	*Sex*	*Stage*	*Grade*	*p53*	*Cyclin A*	*Age*	*Sex*	*Stage*	*Grade*	*p53*	*Cyclin A*
42	M	IIA	WD	-ve	+3	44	F	IIB	WD	-ve	+3
54	M	IIB	WD	+4	+1	41	M	IIA	WD	-ve	+3
44	F	IIA	WD	-ve	+1	54	F	III	MD	-ve	+3
49	M	IIA	WD	+4	+1	61	M	IIA	WD	+4	+1
28	F	IIA	PD	-ve	+2	68	M	IIB	MD	+1	-ve
62	M	IIB	MD	-ve	+3	38	M	IIA	WD	+4	+1
59	M	III	PD	-ve	+3	63	F	IIB	MD	+2	+3
71	M	IIA	WD	+4	+3	61	M	IIA	WD	+4	+1
43	M	III	WD	-ve	+1	33	M	IIA	WD	-ve	+3
55	M	IIA	WD	-ve	+3	54	M	IIA	MD	-ve	-ve
68	M	IIA	WD	-ve	+2	50	F	IIA	WD	-ve	+2
68	F	III	WD	+4	+2	53	M	IIA	WD	-ve	+2
58	M	IIA	PD	+4	+1	50	M	III	WD	-ve	+3
77	M	IIA	WD	-ve	+4	49	M	IIA	WD	-ve	+1
48	F	III	PD	-ve	+3	45	F	IIA	WD	-ve	+1
58	M	IIA	WD	-ve	+2	29	M	IIA	WD	-ve	+2
28	M	IIA	WD	+4	+2	54	F	IIA	MD	+4	+3
54	M	IIA	WD	+4	+1	58	F	IIA	PD	-ve	-ve
54	M	IIA	PD	-ve	+1	49	M	IIA	WD	+4	+3
63	M	IIB	WD	+4	+2	70	M	IIA	WD	+3	+1
65	M	IIA	WD	-ve	+3	54	M	IIA	WD	+3	+4
48	M	IIA	WD	-ve	+3	62	M	III	WD	+3	+1
52	M	IIB	WD	+4	+2	43	M	IIA	MD	-ve	+2
53	F	IIB	PD	-ve	+2	71	F	IIA	WD	+4	+2
54	M	IIA	WD	+4	+2	52	M	III	PD	-ve	+1

WD = well differentiated, MD = moderately differentiated, PD = poorly differentiated

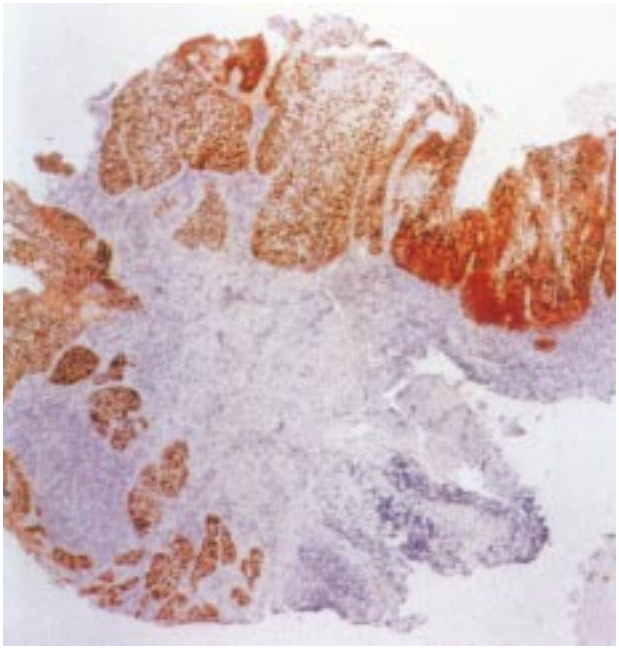

Figure 1. *p53 immunohistochemical staining of the surface dysplastic squamous epithelium as well as the invasive component. The vast majority of tumor cells are positive. (x100).*

Results

Table 1 summarizes the results. Of the 50 cases 37 were in males and 13 in females, ranging in age from 28 to 77 years (average 51 years). Thirty four cases were placed into stage IIA, 8 in stage IIB and a further 8 in stage III. As regards tumor differentiation, 35 cases were well, 7 moderately and 8 poorly differentiated.

p53 immunohistochemistry – Overall 21 of the 50 cases showed immuno-positivity. Analysing this further, 20 of the positive cases contained more than 50% of the tumor displaying nuclear p53 staining *(Figure 1)*. The dysplastic epithelium overlying the tumor showed Similar staining to the invasive component. Thus, the staining obtained with p53 was clear cut, with cases either being strongly positive or totally negative.

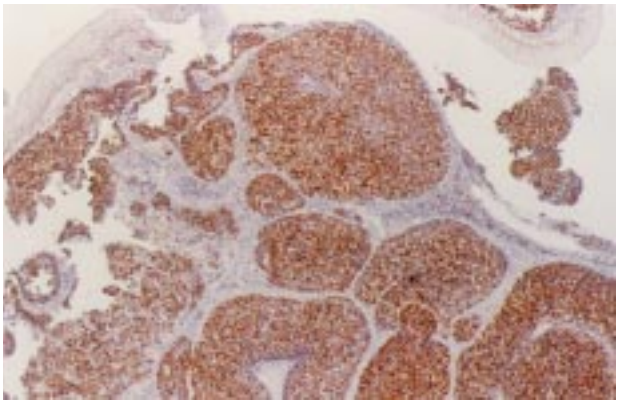

Figure 2. *Invasive squamous carcinoma showing intense immunopositivity for cyclin A. (x100).*

Cyclin A immunohistochemistry – Forty seven of the SCCs demonstrated some cyclin A positivity *(Figure 2)*. Eighteen of these 47 cases contained more than 50% positive tumor cells.

p53/cyclin A staining versus clinicopathological parameters – When p53 and cyclin A immunoreactivity was analysed against patient age and gender, tumor differentiation and stage, no statistically significant p values were obtained. Furthermore, when p53 was evaluated against cyclin A, a statistically significant relationship was not observed.

Discussion

p53 abnormalities, at both a protein and gene level, have been observed in SCC of the oesophagus from different geographical areas. The *p53* gene has been found to display mutations in 33% to 50% of oesophageal cancers.[4-6] Immunohistochemical demonstration of p53 protein varies from 87.2% to 67.2% to 53%.[7-9] In the current study, only 42% of cases showed p53 immunopositivity. This difference in staining percentages may occur for two reasons. Firstly, different p53 monoclonal antibodies were used in the different studies. For instance, Wang et al and Shimaya et al used the monoclonal antibody, PAb 1801,[7,9] whilst in the study under discussion, DO7 was used. Allied to this could be several technical considerations such to differences in antigen retrieval and fixation protocols. These latter factors would be expected to have less of an impact as standardization of protocols and techniques are becoming more commonplace. Secondly, there may be true geographic variation in the frequency of p53 abnormalities at both the protein and gene level. This may be directly or indirectly related to causal factors, that is, tobacco and/or alcohol as opposed to dietary carcinogens. The study by Hollstein et al examined oesophageal SCCs with a strong association with cigarette smoking and alcohol.[4] The *p53* mutations that were found were distributed over the mid region of the gene. Furthermore, they found differences in the nature of the base substitutions in SCC of the oesophagus and other cancers of the gastrointestinal tract. Thus, the differences in p53 expression seen in the various published series may be the result of exposure to different genotoxic environmental factors. Like the study of Sarbia et al, the current study did not find any correlation between p53 immunostaining and age, gender, tumor stage or histological grade.

We have demonstrated that the majority of SCC of the oesophagus express cyclin A. However, unlike other mem-

bers of the cyclin family of proteins, such as cyclin D1, cyclin A expression did not correlate with any clinicopathological parameter. It has been shown previously that cyclin D1 is expressed in the minority of SCC of the oesophagus from South Africa.[10] However, cases with lymph node spread tended to be positive for cyclin D1 suggesting that these tumors may have a greater propensity for spread.[10] With regard to cyclin A, a similar finding was not apparent and a relationship with p53 protein was also not observed.

Thus, this study demonstrates that in oesophageal SCC from South Africa, 42% of cases express p53 protein and 94% of cases are positive for cyclin A. Neither of these proteins showed any relationship with each other, patient age and gender, nor tumor grade and stage.

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