RESEARCH COMMUNICATION

Diagnostic Utility of p63 (Ab-1) and (Ab-4) Tumor Markers in the Squamous Cell Carcinomas of Head and Neck

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Abstract

P63 is a gene product required in cell cycle regulation which plays vital roles in tumor differentiation. Aims of the present study were to assess the frequency, pattern, sensitivity and specificity of two p63 protein clones P63 4A4 and P63 4A4+Y4A3 in squamous cell carcinomas (SCCs). Thirty cases of head and neck region SCC diagnosed on the basis of H&E staining were examined along with 60 cases of head and neck region biopsies other than squamous cell carcinoma, negative on H&E staining, were taken as control. Immunostaining was performed on slides according to the Thermo Scientific UltraVision LP detection System. P63 4A4+Y4A3 clone is more sensitive 96.6% in comparison to 86% in P63 4A4 with having greater NPV of 98.3%. The results signify the importance of P63 4A4+Y4A3 marker over the old markers and may be used as a confirmatory marker of squamous cell carcinoma.

Keywords: Squamous cell carcinoma - immunostaining - p 63 - head and neck cancer - genotoxic stress - oligomerization

Introduction

Researchers agree on the fact that early diagnosis of the pre-cancerous lesion is important for the improvement of the survival rates (Pitiphat et al., 2002). Routine H&E staining and biopsy are the basic modalities in the diagnosis, and the gold standard for diagnosis remains the biopsy (Gath & Brakenhoff, 1999). However, H&E staining has limitation especially where morphological clues are limited or less helpful. Multiple research modalities have come forward for more accurate results, early diagnosis and determination of the stages of carcinoma. Among them one of the latest and accurate modality has been reported to be the Immunoassaying, Immunohistochemistry staining of cells or tissue sections (Coindre, 2003).

A good tumor marker must fulfill the criteria of sensitivity, specificity and clinical effectiveness (Andrea and Anette, 2001). So far no serological and biological tumor marker has yet been established for the SCC of the head and neck region (Andrea & Anette, 2001).

The p53 tumor suppressor gene is one of the most frequently mutated genes in human cancers. p53 is a sequence-specific transcription factor and plays a critical role in activating the expression of genes involved in cell cycle arrest or apoptosis under conditions of genotoxic stress. For over two decades, p53 was thought to be the only gene of its kind in the vertebrate genomes. This strong conviction, which was widely accepted in the p53 field, has now been proven to be incorrect. Two genes, referred to as p63 and p73, have been found to encode proteins that share a significant amino-acid identity in the transactivation domain (Yang et al., 1998), the DNA binding domain, and the oligomerization domain with p53 (Levrero et al., 1999).

In normal tissues, p63 was reported to be immunohistochemically detectable in basal cells of all squamous epithelia (including epidermis and hair follicles), in basal cells of urothelium, and in basal cells of prostate pithelium (Foschini et al., 2004). Squamous cell carcinomas of the skin, lung, oesophagus, and nasopharyngeal carcinoma has shown to express high levels of Np63, an important isoforms liberated by a mutated p63 gene (Chen et al., 2003). Np63 isoforms potentially suppress transactivation of p53 target genes such as p21 which induces apoptosis (Sayan et al., 2007). Furthermore, the truncated isotype p63 is detectable in most squamous cell carcinomas (including undifferentiated nasopharyngeal carcinomas) of various primary sites. The p63 over expression in these tumours is apparently due to an amplification of the p63 gene. Over-expression of p63 splice variants or isoforms is observed in many squamous carcinomas suggesting that p63 may act as an oncogene, making p63 a useful marker for the squamous carcinomas

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(Koga et al., 2003).

Two of P 63 protein clones P63 4A4 and P63 4A4+Y4A3 were used in this study in order to assess their frequency, pattern, sensitivity and specificity. The purpose of this study was to estimate the usefulness and the diagnostic yield of the p 63 markers in the squamous cell carcinomas of head and neck.

Materials and Methods

The study comprised of 90 patients, 30 cases of squamous cell carcinoma and the remaining 60 cases were control benign biopsies both from the head and neck region. It was done at Shaikh Zayed Medical Complex, department of Histopathology, on the formalin fixed, specimens of the above mentioned population. The female to male ratio was 1:1.65. The mean age of the participants was 44 ± 19.047 (SD), with age ranging from 3 years to 80 years. The SCC group was a primary sample (previously untreated), with female to male ratio of 1: 4, age averaging of 55 ± 11 (SD) and ranging from 30 -75 years. The ages in this group were further divided into 2 sub-groups, equal to or less than 65 years and greater than 65 years. Study was conducted over a period of nine months i.e., from the 1st December, 2008 to 30th August, 2009.

The total 180 slides were made i.e., 2 slides out of each specimen for the two different markers. The SCC group was receiving surgical treatment with curative intensions and were previously assessed through routine H&E staining, whereas, the case control group was from the patient undergoing routine oral surgeries. Informed consent was taken prior to all the surgeries.

Recorded data was coded and entered using SPSS statistical package version 16.0. Numerical data like the age of the patients was reported as mean and standard deviation. Range was also given along with the minimum and maximum values. However it was later on distributed in a following group: equal to or greater than 65 and below 65.

Score of each stain was determined against the different types of tissues used by applying the following criteria:

- If microscopic field has the stained cells equal to 10% or less, they were considered (–ve).
- More than 10% but less than or equal to 30% were considered single positive (+ve).
- More than 30% but less than or equal to 50% were considered double positive (++ve).
- Above 50% were considered to be triple positive (+++ve).

Nominal data like the status of case, gender, site of tissue and result of each stain was reported as frequency and percentages. Chi-square Test was used to test the association between age, gender, site of the tissue, size of the tissue and the grading of the tumor. For all analysis, p value of 0.05 or less was considered significant. Fisher exact test was applied to determine correlation between the grading of tumour and the score of the different stains. 2 x 2 tables were applied to determine the sensitivity and specificity of the three markers.

Results

30 cases were of squamous cell carcinoma and 60 controls, both from the head and neck region. The female to male ratio among SCC cases was 1: 4. The mean age among the cases was 55 ± 11 (SD), with minimum age of 30years and a maximum age of 75 years. The ages among the cases was further divided into 2 sub-groups, equal to or less than 65 years and greater than 65 years.

The scoring of each marker among both cases and control are given among the following tables. In case of both p 63 (Ab-1) and p63 (Ab-4) there is a nuclear pattern of expression, staining the basal and parabasal nucleus only. In the normal mucosal epithelium, the staining of the basal layer is stronger as compared to parabasal layer. In the suprabasal layer it is seen to be rare and very faint. Commutatively total staining is seen to be less than 10% of the total cells.

However, in SCCs different staining pattern has been observed for both the markers. It has been seen that the pattern of expression differs with the grading of the neoplasm for each marker. In case of well differentiated neoplasm the staining pattern is similar to that of the normal mucosa with positive cell comprising of 10-30% of the total cells. In moderately differentiated SCCs staining has been more intense involving the suprabasal layers and the pattern is more diffuse. In poorly differentiated SCCs, usually stained cells are greater than 50% of the total cells. P 63 (Ab-4) has shown stronger staining of the keratin pearls but is insignificant. The positive and negative scores of P 63 (Ab-4) are given in the Table1.

The positive and negative score of P63 Clone 4A4 +Y4A3 (Ab-4) is given in the Table2.

The sensitivity and specificity of each marker was determined using the above data through 2x2 tables and is given below:-

Sensitivity and specificity of p 63 4A4. (Ab-1)
Sensitivity= A/ (A+C) = 86.6%
Specificity= D/ (B+D) = 96.6%
Ve+ predictive value (PPV) = A / (A+B) = 92.8%
Ve- predictive value (NPV) = D / (C+D) = 93.5%
Sensitivity and specificity of p 63 4A4+Y4A3. (Ab-4)
Sensitivity= 96.6%
Specificity= 96.6%
PPV = 93.5%

Table 1. Positive and Negative Score of P63 Clone 4A4 (Ab-1)

Status of enrolment	P 63 (4A4)		Total
	Ve+	Ve-	
Cases	24	6	30
Control	2	58	60
Total	26	64	90

Table 2. Positive and Negative Score of P63 Clone 4A4 +Y4A3

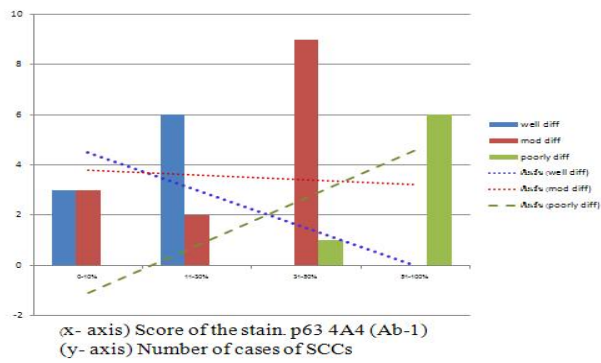| Status of enrolment	P 63 (4A4+Y4A3)		Total
	Ve+	Ve-	
Cases	29	1	30
Control	2	58	60
Total	31	59	90

Figure 1. Bar Chart with Trend Lines for the Marker p 63 4A4. (x- axis) Score of the stain. p63 4A4 (Ab-1), (y- axis) Number of cases of SCCs
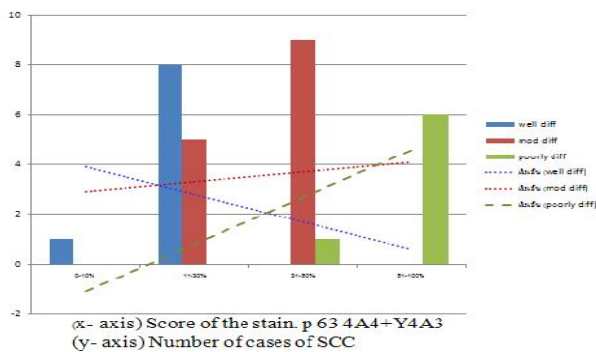

Figure 2. Bar Chart with Trend Lines for the Marker p 63 4A4+Y4A3. (x- axis) Score of the stain. p 63 4A4+Y4A3, (y- axis) Number of cases of SCC

NPV= 98.3%

Fisher exact test was applied to determine association between the score of the stain and the degree of differentiation. In both the markers the p value of less than 0.05 was calculated which is highly significant. Showing that the score of the stains are strongly associated with the degree of differentiation.

Figure 1 shows trend lines for the Marker P 63 4A4. There is a positive trend and gradient for poorly differentiated SCC to be more strongly stained as compared to well differentiated and moderately differentiated SCC which is exhibiting a negative trend.

Figure 2 shows trend lines for the Marker p 63 4A4+Y4A3. There is an increasing trend and gradient for poorly differentiated SCC to be more strongly stained as compared to well differentiated and moderately differentiated SCC. However, there is a positive trend in both moderately and poorly differentiated SCC but the slope of poorly differentiated SCC is more steep and sharp. There is positive tendency seen in P63 4A4+Y4A3 for both moderately differentiated and poorly differentiated SCC's as compared to P63 4A4 which has a positive trend for only poorly differentiated SCC. Chi square test was used to determine any association between the score of the stain, age of the patient, gender of the patient and the size of the tissue.

No association between the score of the stain, the gender of the patient and the size of the tissue was seen for both the markers. However, statically significant association has been seen between the grade 3 tumors and score (+++) of both markers with 2-sided P value to be < .0001.

Discussion

A large number of immuno-histochemical markers have been examined in the squamous cell carcinomas of head and neck but their sensitivity and specificity values have always been controversial (Rassam et al., 1995; Strong et al., 1995; Buffa et al., 2004). The present study was conducted to evaluate the sensitivity and specificity of two immuno-markers used for diagnosing Squamous cell carcinomas of the head and neck, taking H&E as a gold standard.

In healthy long, p63 stained nucleus were confined to the basal layer and were evident at the parabasal layer. Our study findings were concordant with other investigations (Jin et al., 2005; Di Como et al., 2002; Muzio et al., 2007), few studies stressed on the expression of p63 in the basal and parabasal layers and gradually decreasing the strength and with no expression in the superficial epithelial layer (Faridoni Laurens et al., 2001; Choi et al., 2002; Chen et al., 2006). However, a study was in contradiction to that of Karim Ender who reported that p63 staining was predominantly in the basal layer (Nylander et al., 2000). This difference can be due to the usage of different clone of p63 antibody in that study. In their study they used 4A4 pan-p63 MAb whereas we used 4A4 and p63 4A4+Y4A3.

In our study, we evaluated any association between the score of the stain, the gender of the patient and the size of the tissue and it was seen to be insignificant which is in consistent with the two studies conducted by L.L. Muzio et al showing no relationship of the number of positively stained cells with that of the age, sex and size of the tissue (Muzio & Santarelli 2005; Muzio et al., 2007).

Our study displayed that there is a statically significant association between the percentage of positive cells to that of the grading of neoplasms. The more the grading of the tumor, more strongly was the expression of all markers, which is cohernt with the two investigations, however these two investigations also corellated with the perineural infilteration, metastasis and survival rates (Muzio & Santarelli, 2005; Muzio et al., 2007). These factors were not seen in our study as our research was done on the incisional biopsies from the primary sites brought to the lab for routine investigation and did not include any clinical data of nodal involvement. Also the sample removed did not include the entire tissue so the perineural infilteration interpretation could not be made accurately. Furthermore, these studies were over a time span of ten years in comparison to our which was only nine months, therefore, the survival rates assessment could not be measured due to shorter time span (Muzio & Santarelli, 2005; Muzio et al., 2007).

In case of P63 4A4, sensitivity 86.6%, specificity 96.6%, PPV 92.8% and NPV of 93.5% was seen. Different studies have reported wide variation in the sensitivity of p63 4A4 for the squamous cell carcinomas ranging from 75% to 82% but it has been seen higher in our studies (Ivan et al., 2005; Muzio & Santarelli, 2005; Kargi et al., 2007). A wide range in the data of specificity is also seen for p63 4A4 ranging from 86% to 100% (Kaufmann et al., 2001; Kargi et al., 2007) but our value lies between this

range with significant value of 96.6%. These differences can be attributed to the different technical errors which each system may face during the staining procedures (Moskaluk, 2002).

In case of P63 4A4+Y4A3, sensitivity of 96.6%, specificity 96.6%, PPV 93.5% and NPV of 98.3% was seen. Interestingly, no study to our knowledge has been conducted on the Squamous cell carcinomas of head and neck, though work has been done on the prostate cancer and has proven to be as a significant marker ($p < 0.05$) (Kristiansen et al., 2008), lung cancer with sensitivity of 82% and specificity of 100% (Kargi et al., 2007) and in Basal cell carcinoma with positivity in 76% only (Bircan et al., 2006). We conclude that this clone has been studied for the first time on the head and neck SCC and has been seen to have 96.6% of both sensitivity and specificity.

Furthermore, in the present study, as in other investigations, a positive association between the p63 (4A4, 4A4+Y4A3) expression and the grade of neoplasm differentiation was noticed, supporting the use of p63 (4A4, 4A4+Y4A3) as an additional marker for diagnostic use in H&N SCC. Also if both these markers are used in combination can aid in early detection and proper diagnosis.

In conclusion:
• p63 gene plays important role in the normal cellular and carcinogenetic proliferation.
• p 63 4A4 and p63 4A4+Y4A3 markers were seen to be significant in both sensitivity and specificity and can be used for a confirmatory diagnosis of the squamous cell carcinomas.

Acknowledgements

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