Indian Journal of Genetics and Molecular Research
Volume 1 Number 2, July - December 2012

Application of Monoclonal Antibodies in Oral Cancer

Mahaboob Hasan*, MBBS, MD(Path); Lateef Ahmad Sofi, MBBS, MD(Path); Rana K. Sherwani***, MBBS, MD(Path); Nasir Salati****, BDS, MDS**

Abstract

Monoclonal anti-CD66e antibodies developed by standard methods belonging to IgG 2 subclass were applied to formalin fixed paraffin embedded tissue of oral squamous cell carcinomas, reactivity correlated with degree of differentiation , both in intensity and percentage of cells stained.

Keywords: Oral squamous cell carcinoma; Monoclonal antibodies; Immunohistochemistry; Peroxidase anti peroxidase.

Introduction

Monoclonal antibodies against CD66e have been thoroughly studied in colorectal the initial works of Gold and Freedman, its role has been studied over decades various other carcinomas, for example gastric, esophageal, medullary carcinoma of thyroid, breast carcinoma, however its role in oral carcinoma is limited.[1] Immunoperoxidase technique using peroxidase antiperoxidase immunohistochemical purpose is increasingly being used as a tool to determine whether the tumor is primary or secondary carcinoma.[2] Tsutsumi *et al* showed staining pattern varies in frozen sections and paraffin embedded tissues and antigens were more readily retrieved in frozen sections, however other studies have shown that CEA in tissue blocks remain preserved as long as ten years.[3] Allum, Stokes, Macdonald showed that monoclonal antibodies react with tonsillar mucosa, which they said was due to cross reactivity.[4]

R.B. Pai, S.B. Pai, Lalitha R.M., showed that stage 2 or more carcinomas of oral cavity stained for anti-CEA antibodies, more so in areas of necrosis.[5]

Material and Methods

11 tissue blocks of oral squamous cell carcinoma as old as 5 years were stained for CEA.

recent cases of colorectal carcinoma were used as positive controls and two sections of normal tissue were used as negative control (Table 1).

Methods

Protocol for Staining

Primary antibody used was Anti-carcinoembryonic antigen monoclonal, class IgG2 .. (BioGenex, Anti-CEA).

1. Sectioning – 3μ m thick tissue sections are taken on salinized slides.
2. Fixation – either 4-6 hours on hot plate at 50-60 ^{0}C or overnight at 37 ^{0}C.
3. Deparaffinisation – Xylene, 2 changes of 5-10 minutes each.
4. Hydration – Graded alcohol (100%,70%,50%), 5 minutes each.
5. Wash in distilled water – two washes of

Author's Affiliation: *Reader, Department of Pathology, JNMC, AMU, Aligarh, **Senior Resident, Department of Pathology, JNMC, AMU, Aligarh, ***Professor, Department of Pathology, JNMC, AMU, Aligarh, ****Lecturer, Department of Oral Pathology, ZADC, AMU, Aligarh.

Corresponding Author: Lateef Ahmad Sofi, C-7, Rizvi Apartment, Medical Road, AMU, Aligarh 202002.

E-mail: drslateef.2k7@gmail.com

Table 1

Tissue	Number of cases stained	Reactivity	Percentage reactivity
Normal tissue	2	Negative	0-5%
Inflamed tissue	4	Negative(3 out of 4)	0-5%/<15%
Colorectal carcinoma	2	Positive	90-100%
Well differentiated squamous cell carcinoma	3	Positive	75-100%
	1	positive	50-90%
Moderately differentiated squamous cell carcinoma.	3	Positive	50-90%
	1	Negative	<5%
Poorly differentiated SCC.	3	Negative	0-5%

5 minutes each.

6. Endogenous peroxide block- (H2O2 1.5 ml + methanol 50 ml) for 30 minutes, to remove background staining.
7. Two washes in distilled water for 5 minutes, each.
8. Incubation with background snipper for 15 minutes at room temperature.
9. Wash in distilled water – 2 changes of 5 minutes each
10. Antigen retrieval- wash with citrate buffer, incubate at 900C for 90 minutes.(Activates).
11. After cooling, wash with Tris buffer solution (TBS)—3 washes of 5 minutes,each.
12. Primary antibody – incubate with primary mouse monoclonal antibody against carcinoemryonic antigen at 40 ^{0}C, overnight in a humidified chamber.
13. Secondary antibody (biotinylated) - Incubate for 15 minutes at room temperature, in humidified chamber.
14. Wash with TBS (pH 7.4) – 3 washes of 5 minutes each.
15. Enzyme conjugate – incubate with streptavidin for 20 minutes at room temperature in a humidified chamber.
16. Wash with TBS (pH 7.4) – 3 washes of 5 minutes each.
17. Chromogen-– incubate with peroxidase substrate solution (Diaminobenzidine) for 1-2 mins.
18. Wash in distilled water 2 changes of 5 minutes each.
19. Counter stain with hematoxylin—30 seconds.
20. Wash in distilled water – 2 changes of 5 minutes each.
21. After drying, mount the slides with DPX.

Results

Tissue section were examined using light microscopy to determine the number of cells showing a positive reaction. Sections were classified as negative if less than ten percent, cells were positive if 10-50 percent cells were positive it was said to be 1+, 50-75% as 2+ 75-90% as over the intensity of staining was taken into consideration, sections showing 5-10% cells reactive with marked intensity were included as 1+, however less than 5% cells of any reactivity were taken as negative (Table 1, Fig 3,4).

All sections of normal tissue showed

Figure 1: Positive controlled –colorectal carcinoma

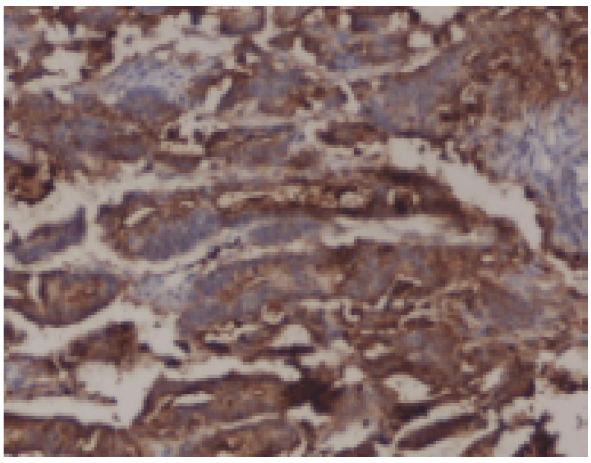
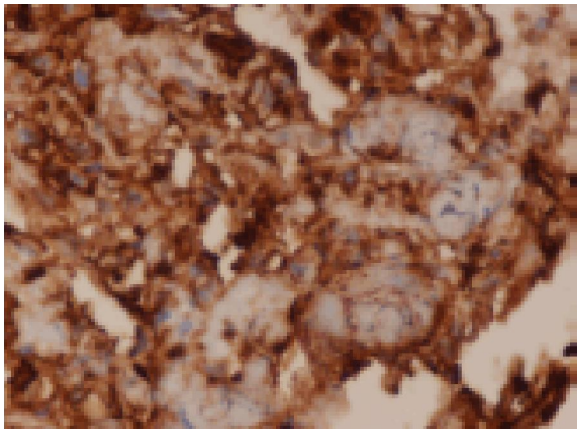


Figure 2: Well differentiated squamous cell carcinoma



negative results for monoclonal antibodies. In majority of cases inflamed tissue did not stain, however one-fourth reacted scantily. Depending upon the differentiation of carcinoma staining varied in intensity, localization and proportion of cells staining.

Well differentiated squamous cell carcinomas gave a muddy brown intracytoplasmic and membranous staining, in moderately differentiated carcinomas greater than fifty percent cells were stained, however staining was both cloudy granular and membranous, as well as golden brown intracytoplasmic (Fig 2, 3). Poorly differentiated squamous cell carcinoma did not stain, or stained with a proportion of less than 5% with membranous pattern (Fig 4). Epithelial pearls stained as eddies with paler

Figure 3: Moderately differentiated squmous cell carcinoma

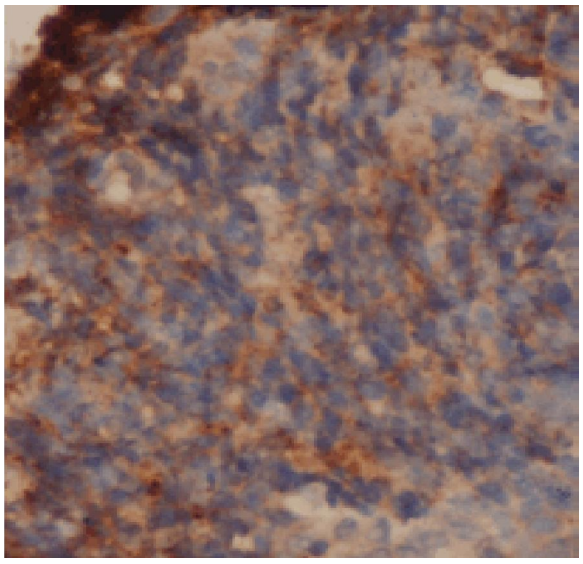
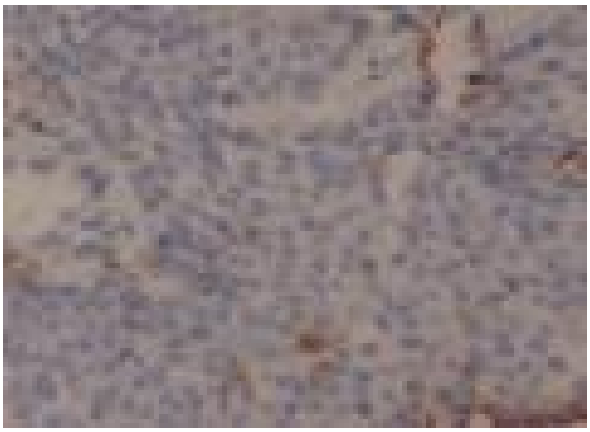


Figure 4: Poorly differentiated squamous cell carcinoma



staining as compared to the rest of malignant tissue (Fig 2). Thus comparative study of percentage cells, together with pattern indicated that carcinomas with largest number of positive cells were most differentiated.

CD66e (CEA), as a marker, has a limited role in oral cancer since usual victim of oral carcinoma is a smoker, which leads to false positive serum elevations, however tissue levels are being studied these days. Since Phil Gold and Freedman, CEA has been used as a diagnostic as well as prognostic tool in carcinomas of gastrointestinal tract; however only recently it has been used in oral squamous cell carcinoma.[6,7] Goldenberg *et al* suggested that the identification by immuno-histochemistry of tumors that express CEA could be used to determine which tumors should be monitored, however as mentioned afore that is not applicable to oral carcinoma.[8] Tsutsumi *et al* for the first time showed that staining pattern varies in frozen sections and paraffin embedded tissue, however further studies confirmed that only old specimens of paraffin embedded tissue show much variations, to obviate such confounding factor in study we used tissue sections not older than five years.[3] This study has shown the moderate to high rate of CEA expression and a direct relationship to degree of differentiation, however false positive activity in inflamed tissue may be attributed to cross reactivity with CD66a present in granulocytes, thus based on the localization of false positive can be categorized separately. Pai S.B., Pai R.B., Lalitha R.M., *et al*. showed

that oral squamous cell carcinoma express CEA with a relationship to stage of carcinoma, and staining was intense in areas of necrosis, however we did not see much variation with stage, although necrosed areas stained deeply.[5]

Conclusion

Evaluation of monoclonal antibodies using peroxidase anti peroxidase complex method employing IgG2 2 antibodies show a direct relationship with degree of histological differentiation of oral squamous cell carcinoma, albeit poorly differentiated carcinoma, which either did not react or reacted scantily.

References

1. Phil Gold, Joseph Shuster, Samuel Freedman. Carcinoembryonic antigen in clinical medicine. Historical perspectives, pitfalls and projections. *Cancer,* 1978; 42(4): 204-214.
2. Goldenberg DM, Kim EE, Delard FH, Bennet S, Primus. Radio-immunodetection of radioactive antibodies to CEA. *Cancer Res.* 1980; 40: 2984-92.
3. Tsutsumi Y, Nagura H, Watanabe K. Immunohistochemical observations of carcinoembryonic antigen (CEA) and CEA-related substances in normal and neoplastic pancreas. Pitfalls and caveats in CEA immunohistochemistry. *Am J Clin Pathol*. 1984; 82(5): 535-42.
4. WH Allum, HJ Stokes, F Macdonald, JWL Fielding. Demonstration of carcinoembryonic antigen (CEA) expression in normal, chronically inflamed, and malignant pancreatic tissue by immunohistochemistry. *J Clin Pathol.* 1986; 39: 610-614.
5. Pai SB, Pai RB, Lalitha RM, *et al*. Expression of oncofoetal marker carcinoembryonic antigen oral cancers in South India — a pilot study. *Int J Oral Maxillofac Surg.* 2006; 35: 746–9.
6. Zamchek N, Moore TL, Dhar P, Kupchik H. Immunological diagnosis and prognosis of human digestive tract cancer: carcinoembryonic antigen. *New englan journal medicine.* 1972; 286: 83-86.
7. Denk H, Tappeiner G, Echstorfer R, Holzner J H. Carcinoembryonic antigen in gastrointestinal and extra- gastrointestinal tumors and its relation to tumor cell differentiation. *Int j cancer.* 1972; 10:262-72.
8. Goldenberg DM, Sharkey RM, Primus F J. Immunocytochemical detection of CEA in conventional histological specimens. *Cancer.* 1978; 42: 1546-53.