International Journal of Research in Medical Sciences
Sundaram S et al. Int J Res Med Sci. 2016 Sep;4(9):4172-4175
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20162955

Research Article

Analysis of MUC4 expression in the prostatic adenocarcinoma and its pathological implications

Sandhya Sundaram[1]*, Simon Durai Raj C.[1], Krishnakumar R.[1], Santhosh D.[1], Sangeetha Narashiman[2], Prathiba D.[1]

[1]Department of Pathology, Sri Ramachandra Medical College & Research Institute, Porur, Chennai, India
[2]Department of Oral Pathology, Sri Ramachandra Medical College & Research Institute, Porur, Chennai, India

Received: 24 February 2016
Accepted: 22 March 2016

***Correspondence:**
Dr. Sandhya Sundaram,
E-mail: sandsrid@gmail.com

ABSTRACT

Background: Expression of various types of mucins has been documented in several malignancies and seems to play a sentinel role in some epithelial malignancies. MUC1 and MUC4 are two transmembrane mucins that are involved in cell signalling events that drive the proliferation of cells. Therefore, mucins may be important biomarkers for early diagnosis and targeted therapy due to their frequently altered expression pattern in carcinomas. The objective of the study was to determine the expression pattern of MUC-4 in prostate cancer and to correlate the expression with clinicopathological parameters.
Methods: Thirty cases of prostate carcinomas of different grades were selected from the case files of our institute for the study. Immunohistochemistry was done on all cases using monoclonal antibodies against MUC- 4 by the avidin biotin technique. The expression and pattern of staining were analyzed .Staining pattern in the adjacent benign areas were also noted.
Results: Our study showed that expression of MUC4 was considerably down regulated in prostate cancer tissues when compared to normal or benign prostatic tissue. Few cases of prostate cancer showed a moderate staining while majority cases of adjacent normal areas were moderate to strongly positivity.
Conclusions: The results of our study implies that MUC4 is down regulated in prostate cancer and this may be of significance for diagnostic application and tumor progression in prostate cancer. Since MUC4 is known to modulate HER2/ErbB2 signalling it may prospectively prove to be useful in prostate cancer therapy.

Keywords: Muc-4 expression, Prostate cancer, Immunohistochemistry

INTRODUCTION

Worldwide, prostate cancer is the second most common cancer and fifth leading cause of death from cancer among men. In 2012, 1.1 million men were diagnosed with prostate cancer, which accounts for 15% of the cancers among men worldwide and in the United States, it accounts for 29% of cancer diagnosed.[1,2] Remarkable differences were observed in the incidence of prostate cancer globally. The rates are highest in Australia, New Zealand, Northern America and in Western and Northern Europe. In Asians prostate cancer was uncommon, but due to changes in dietary factors or other lifestyle, the incidence of prostate cancer seems to be increasing.[1,2] In India, prostate cancer is the second leading site of cancer among men in cities like Delhi, Kolkata, Pune and Trivandrum, third in Bangalore and Mumbai. In Chennai, prostate cancer is the fourth most common cancer among men.[3]

Metastatic prostate cancers are lethal and resistant to current conventional therapies. The mechanism involved in the progression of prostate cancer is unclear. Localized prostate cancers are treated by radical prostatectomy or

radiation. Advanced cancer either recurrent or metastatic, initially respond to androgen ablation therapy, but eventually the cells are no longer responsive to such therapy, resulting in a lethal cancer termed androgen-independent prostate cancer.[4,5]

Mucins are a group of glycoproteins, involved in the protection and lubrication of epithelial surfaces and also engage in signal transduction pathways that regulate morphogenesis.[6,7] A total of 21 human mucin genes (MUC1-MUC4) have been identified and subclassified into three groups: transmembrane, gel forming or secreted and soluble mucins.[4,8-10] Transmembrane mucins contribute to epithelial cell-cell interactions and secreted mucins form a physical barrier to provide protection for epithelial cells.[4,8] In addition, mucin control many cellular processes, including growth, differentiation, transformation, adhesion, invasion, and immune surveillance.[8,11] However, in cancer cells and during progression, the mucin molecule becomes altered.[4]

Previous studies have shown that MUC4, a transmembrane mucin, is expressed by epithelial cells in a variety of tissues.[10-12] MUC4 is normally expressed by the luminal epithelial cells of the stomach, colon, lung, trachea, cervix, and prostate.[10,12-14] An over expression of MUC4 is observed in pancreatic, lung, breast, colon, and ovarian malignancies, suggesting its pathologic significance and the association of MUC4 with the poor prognosis of the pancreas, lung, and bile duct cancer patients has also been reported.[9,11,15-19] In the present study, we have determined the expression of MUC 4 in prostatic adenocarcinoma and its correlation with clinico-pathological data.

METHODS

Sample collection

We randomly selected 30 patients who had undergone trans rectal ultra sound guided biopsy or transurethral resection of the prostate at Sri Ramachandra Medical College and Research Institute, Chennai and with a proven diagnosis of prostate adenocarcinoma. Haematoxylin and Eosin stained slides for each cases were reviewed and Gleason's score were assigned. During review, multiple blocks were identified based on the presence of adequate tumor and the representative nature of the overall grade. Tumors were classified as high grade when the combined Gleason's score was 7 or high and as low grade when the combined score was less than 7. Sections from colon carcinoma were used as control. All the specimens were fixed in formalin and embedded in paraffin blocks.

Immunohistochemistry

Immunohistochemistry was done in 3-4 µm thickened sections using Mouse anti-human MUC4 (Biogenex Laboratories, USA) by the avidin biotin technique. After deparaffinization, the antigenic determinant sites were retrieved in citrate buffer with steam for 10 minutes. Endogenous peroxide was blocked with 3% hydrogen peroxide. The sections were probed with primary antibody MUC4 for 1 hour at room temperature and incubated with secondary antibody. The binding of antibody was detected by 3, 3'- diaminobenzidine after the incubation with HRP conjugate which forms a brown colour. Finally the slides were counter stained with Mayer's hematoxylin. Similarly process was done with colon carcinoma as positive control for MUC4 antibody immunostaining. Stained sections were evaluated under the light microscope and intensity of the staining was classified semi-quantitatively.

RESULTS

Among the 30 cases studied, the ages were ranged between 60 to 90 years with the mean age of 75 years. Of the 30 prostatic adenocarcinoma cases 19 (63.4%) were high grade (Gleason's score ≥7) and 11 (36.6%) were low grade (Gleason's score <7). Most common presentation was urinary retention. MUC4 immunostaining showed cytoplasmic and membrane positively in normal prostate glands (Figure 1). Among 30 cases 26 (87%) cases showed negative staining for MUC4, 3 (10%) cases of low grade carcinoma showed moderate to intense positivity while 1 (3%) case showed moderate positive staining (Figure 2). However, statistical analysis of MUC 4 expression between high and low grade tumors did not show any significance (p=0.087). Interestingly, the micro vessels within the tumor cell showed positivity for MUC4. Areas of prostate intraepithelial neoplasia (PIN) within the tumor also showed positive expression for MUC 4.

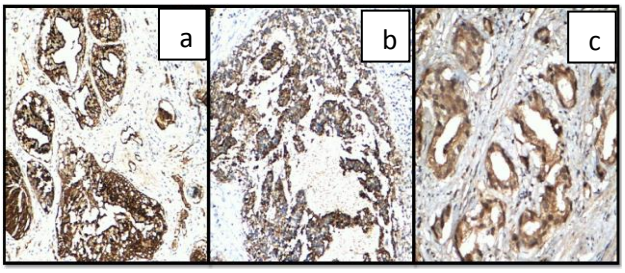

Figure 1: (a) Normal prostate glands showing cytoplasmic and membrane positivity for MUC4 immunostaining x100; (b) Colonic carcinoma (control) showing positive staining for MUC4 x100; (c) Low grade prostate carcinoma positive for MUC4 x200.

DISCUSSION

Multiple malignancies like pancreatic, ovarian, gastric, lung and breast shown abnormal expression, localization and glycosylation of mucins as their characteristic events. Among the various mucin family members, MUC1 and MUC4 are highly involved in tumour growth, tumour-

stromal interactions, intracellular and extracellular signalling, metastasis and resistance to immunity and chemotherapeutic agents. In fact, the down-regulation of MUC4 may reverse the chemo resistance in pancreatic cancer. In spite of several studies on the oncogenic potential of MUC4 in various cancers, not much detail was available in prostate adenocarcinoma, particularly in the Indian population.

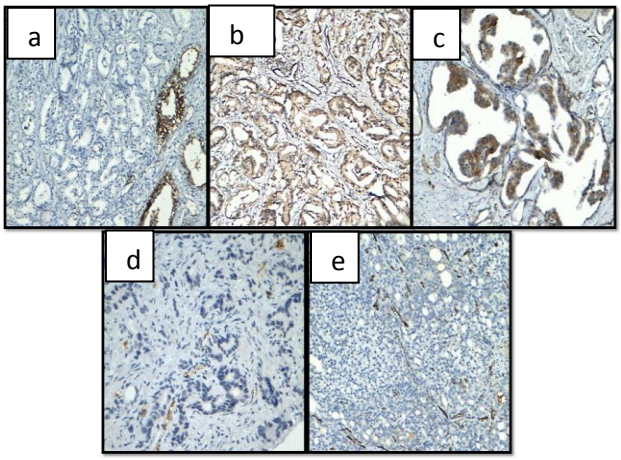

Figure 2: (a) Negative staining for MUC4 in prostate cancer areas. Adjacent normal prostate glands showing strong positivity x100; (b) Moderate positive staining for MUC4 in prostate carcinoma, Gleasons score 7 x100; (c) Areas of prostatic intraepithelial showing focal positivity for MUC4 x200; (d) High grade prostate carcinoma showing negative staining for MUC 4 antigen x100; (e) High grade prostate carcinoma showing negativity for MUC4. Micro vessels within the tumor show positivity (internal control) x100.

In the present study we examined the expression of transmembrane protein MUC4 in low and high grade prostate carcinoma tissue and also determined its expression in adjacent normal prostate glands. A significant down regulation of MUC4 was observed in prostatic carcinoma tissues compared to normal prostate tissues. Strong staining pattern was observed in all the non-neoplastic prostate cancer cases. Interestingly, intense expression of MUC4 was observed in micro vessels within the tumour. Only three cases of low grade prostate carcinoma with Gleason's score of less than six showed moderate to intense MUC4 cytoplasmic staining while one case of high grade prostate cancer showed moderate to weak staining for MUC4 antibody. The remaining eighteen cases showed loss of MUC4 expression.

Singh et al also observed the lower MUC4 expression in prostatic adenocarcinoma tissue and higher in adjacent normal/benign prostate tissue. When Singh et al treated the prostate cancer cell lines with histone deacetylases and DNA methyl transferases inhibitors; he observed the increased expression of MUC4. Singh et al suggested the epigenetic mechanism might be regulating the MUC4 expression during pathogenesis of prostate cancer. Further, positivity was seen in areas of PIN indicating that loss of MUC 4 occurs as lesions progresses.[14]

Contrary to our study, Senapati S et al observed MUC4 over expression in gastric cancer tissues than adjacent normal tissues. He also found that MUC4 over expression was associated with an aggressive phenotype of gastric cancer cells and also increases the activation of ErbB2 oncoprotein.[20] A similar over expression has also been documented in pancreatic cancers where MUC4 may have a potential use as a diagnostic molecular marker.[11]

However our findings in a limited number of cases suggest that, contrary to other known tumors, MUC4 is down regulated in most prostatic carcinomas. Further studies need to be carried out in larger samples for elucidating its specific role and to interpret the mechanism of MUC4 down regulation in prostate cancer. MUC4 immunohistochemistry analysis may be a useful in studying the mucin expression pattern in prostate cancer and might be a promising tool in designing therapy related to regulation of MUC4.

CONCLUSION

In conclusion, our study suggests that MUC4 down regulation may be of significance in defining the mechanistic basis of prostate cancer and may be related to tumor progression. Further studies are necessary to interpret the molecular mechanism and effect of MUC4 loss on development of prostate carcinoma.

ACKNOWLEDGEMENTS

Authors would like to thank Mr. Ravishankar for statistical analysis of the study and Akila Selvi Rajaretnam for helping in preparing the manuscript.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11. Lyon, France: International Agency for Research on Cancer. 2013.
2. Kumar V, Abbas AK, Aster JC. Robbins and Cotran Pathologic Basis of Disease Ninth ed: Elsevier. 2015.
3. Jain S, Saxena S, Kumar A. Epidemiology of prostate cancer in India. Meta Gene. 2014;2:596-605.
4. Cozzi PJ, Wang J, Delprado W, Perkins AC, Allen BJ, Russell PJ. MUC1, MUC2, MUC4, MUC5AC

and MUC6 expression in the progression of prostate cancer. Clinical & experimental metastasis. 2005;22(7):565-73.
5. Saraon P, Jarvi K, Diamandis EP. Molecular alterations during progression of prostate cancer to androgen independence. Clinical chemistry. 2011;57(10):1366-75.
6. Hollingsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. Nature reviews Cancer. 2004;4(1):45-60.
7. Hudson MJ, Stamp GW, Chaudhary KS, Hewitt R, Stubbs AP, Abel PD. Human MUC1 mucin: a potent glandular morphogen. The Journal of pathology. 2001;194(3):373-83.
8. Kufe DW. Mucins in cancer: function, prognosis and therapy. Nature reviews Cancer. 2009;9(12):874-85.
9. Saitou M, Goto M, Horinouchi M, Tamada S, Nagata K, Hamada T. MUC4 expression is a novel prognostic factor in patients with invasive ductal carcinoma of the pancreas. Journal of clinical pathology. 2005;58(8):845-52.
10. Chaturvedi P, Singh AP, Batra SK. Structure, evolution, and biology of the MUC4 mucin. FASEB J. 2008;22(4):966-81.
11. Andrianifahanana M, Moniaux N, Schmied BM, Ringel J, Friess H, Hollingsworth MA. Mucin (MUC) gene expression in human pancreatic adenocarcinoma and chronic pancreatitis: a potential role of MUC4 as a tumor marker of diagnostic significance. Clinical cancer research: an official journal of the American Association for Cancer Research. 2001;7(12):4033-40.
12. Buisine MP, Devisme L, Copin MC, Durand-Reville M, Gosselin B, Aubert JP. Developmental mucin gene expression in the human respiratory tract. Am J Respir Cell Mol Biol. 1999;20(2):209-18.
13. Lopez-Ferrer A, Curull V, Barranco C, Garrido M, Lloreta J, Real FX. Mucins as differentiation markers in bronchial epithelium. Squamous cell carcinoma and adenocarcinoma display similar expression patterns. Am J Respir Cell Mol Biol. 2001;24(1):22-9.
14. Singh AP, Chauhan SC, Bafna S, Johansson SL, Smith LM, Moniaux N. Aberrant expression of transmembrane mucins, MUC1 and MUC4, in human prostate carcinomas. The Prostate. 2006;66(4):421-9.
15. Davidson B, Baekelandt M, Shih Ie M. MUC4 is upregulated in ovarian carcinoma effusions and differentiates carcinoma cells from mesothelial cells. Diagn Cytopathol. 2007;35(12):756-60.
16. Hanaoka J, Kontani K, Sawai S, Ichinose M, Tezuka N, Inoue S. Analysis of MUC4 mucin expression in lung carcinoma cells and its immunogenicity. Cancer. 2001;92(8):2148-57.
17. Singh AP, Chaturvedi P, Batra SK. Emerging roles of MUC4 in cancer: a novel target for diagnosis and therapy. Cancer research. 2007;67(2):433-6.
18. Tamada S, Shibahara H, Higashi M, Goto M, Batra SK, Imai K. MUC4 is a novel prognostic factor of extrahepatic bile duct carcinoma. Clinical cancer research: an official journal of the American Association for Cancer Research. 2006;12 (14 Pt 1):4257-64.
19. Tsutsumida H, Goto M, Kitajima S, Kubota I, Hirotsu Y, Wakimoto J. MUC4 expression correlates with poor prognosis in small-sized lung adenocarcinoma. Lung Cancer. 2007;55(2):195-203.
20. Senapati S, Chaturvedi P, Sharma P, Venkatraman G, Meza JL, El-Rifai W. Deregulation of MUC4 in gastric adenocarcinoma: potential pathobiological implication in poorly differentiated non-signet ring cell type gastric cancer. British journal of cancer. 2008;99(6):949-56.

Cite this article as: Sundaram S, Simon DRC, Krishnakumar R, Santhosh D, Narashiman S, Prathiba D. Analysis of MUC4 expression in the prostatic adenocarcinoma and its pathological implications. Int J Res Med Sci 2016;4:4172-5.