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Original Research Article

Histochemical Demonstration of Mucins in Urinary Bladder Cancer Lesions

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ABSTRACT

Study Design and Objective: This was a retrospective descriptive study aimed to identify and classify mucins in urinary bladder cancer lesions.
Material and Methods: Fifty paraffin-embedded formalin-fixed tissue blocks of urinary bladder cancer lesions were obtained from the archives of the histopathology departments at western Riyadh hospitals. Sections of 5µ –thickness from these blocks were put on slides and stained by five different histological techniques; Alcian blue (pH0.5, pH 1.0, and pH 2.5), PAS/colloidal iron technique, and Aldehyde fuchsin/alcian blue technique.
Results: Mucin was positive in all cancer lesions; carboxylated acid mucin was positive in 35 cases (70%), neutral mucin was positive in 26 cases (52%), strongly sulphated acid mucin was positive in 8 cases (16%), and weakly sulphated acid mucin was positive in only 7 cases (14%).
Conclusion: Histochemical demonstration of mucins in bladder cancer lesions can be considered an important technique for assessment of cancer prognosis.

Key Words: mucin; bladder cancer.

INTRODUCTION

Mucins are a family of high molecular weight, heavily glycosylated proteins (glycoconjugates) produced by epithelial tissues. [1] They are characterized by their ability to form gels; therefore they are a key component in most gel-like secretions, serving functions from lubrication, cell signaling, and forming chemical barriers. [2] They often take an inhibitory role and some mucins are associated with controlling mineralization. [3] They bind to pathogens as part of the immune system. [4]

Mucins are generally put into two categories, Neutral Mucins or Acid Mucins. Increased mucin production is indicative of many cancers, including cancers of the pancreas, lung, breast, ovary, urinary bladder, colon and other tissues. [4]

Alcian Blue is a basic dye that is used in the clinical histology laboratory to demonstrate acid mucins which can be secreted by various connective and epithelial

tissue tumors. [5] The pH of the Alcian Blue staining solution is a critical factor in the staining procedure and has a direct effect on the category of mucins that can be demonstrated with the procedure. [6] The pH of Alcian Blue staining solutions should range from 0.4 to 2.5. Alcian Blue solution with a pH of 1.0 is capable of staining most sulfated acid mucins. Alcian Blue solution with a pH of 2.5 is capable of staining carboxylated mucins in connective tissues and cartilage. It is also capable of staining some weakly sulfated acid mucins like those in cervical tissue. [6]

Alcian Blue may also be used in combination with the PAS staining procedure so that both acid and neutral mucins can be demonstrated in the same tissue sample. Alcian Blue will stain acidic mucins blue and PAS will stain neutral mucins rose red. [7]

The colloidal iron staining procedure is often used in place of Alcian Blue to demonstrate acid mucins due to its greater sensitivity to acid mucins; colloidal iron stain is able to detect acid mucins in small quantities. [8]

Acid mucins contain carbohydrates with carboxylate (COO-) or -sulphonate (SO3) groups. Combination between Aldehyde fuchsin and alcian blue is used to distinguish between sulphated and carboxylated acid mucins (staining purple for sulphated mucin and blue for carboxylated mucin). [9]

The normal urinary bladder contains neither columnar nor mucus-secreting glandular epithelium. Adenocarcinoma may develop secondarily from pre-existent transitional cell carcinoma (TCC) by a metaplastic process. However, the urothelium has inherent potential to undergo several pathways of cellular differentiation. [10] Therefore, it is expected always to find mucin in metaplastic or cancerous lesions of the urinary bladder. The purpose of the current study was to demonstrate and classify mucins in formalin-fixed paraffin-embedded bladder cancer lesions of Saudi patients.

MATERIALS AND METHODS

This was a retrospective descriptive study included fifty paraffin-embedded formalin-fixed tissue blocks of urinary bladder cancer lesions obtained from the archives of the histopathology departments at western Riyadh hospitals during the period between October 2012 and March 2013.

Every tissue block was cut into six 5µ –thick sections. Each section was mounted on a new frosted-end glass slide, dried, deparaffinized, hydrated by deionized water, and stained by one of five staining methods. The first slide was stained by the H&E (Hematoxylin and Eosin) method to confirm the histopathological diagnosis that obtained from the records. The second and third slides were stained by Alcian Blue at pH 2.5 and pH 0.5 respectively to identify Weakly Sulphated Acid Mucin (blue color). The fourth slide was stained by Alcian Blue at pH 1.0 to identify Strongly Sulphated Acid Mucin (brown color). The fifth slide was stained by combined Periodic acid-Schiff's (PAS) and colloidal iron to distinguish between neutral and acidic mucins (staining magenta for neutral mucin and blue for acid mucin). The sixth slide was stained by Aldehyde fuchsin/alcian blue technique to distinguish between sulphated and carboxylated acid mucins (staining purple for sulphated mucin and blue for carboxylated mucin). The slides were then viewed and scored by using a light microscope. All quality control measures were followed carefully. Simple statistical methods were used for analysis of results and clinical data.

RESULTS

Cancer patients included in this study were 33 males (66%) and 17 females (34%), ranged in age between 13 and 80 years. Histopathological classification of cases included18 cases of Low Grade Transitional Cell Carcinoma (LGTCC), 9 cases of High Grade Transitional Cell Carcinoma (HGTCC), 19 cases of Squamous Cell Carcinoma (SCC), and 4 cases of Adenocarcinoma.

Mucin was positive in all cancer lesions, however, concentrations were variable. In cases of LGTCC (18 cases), carboxylated acid mucin was positive in 14 cases, neutral mucin was positive in 11 cases, weakly sulphated acid mucin was positive in only 2 cases, and strongly sulphated acid mucin was positive in only 2 cases.

In HGTCC lesions (9 cases), carboxylated acid mucin was positive in 4 cases, neutral mucin was positive in 5 cases, strongly sulphated acid mucin was positive in 4 cases, and weakly sulphated acid mucin was positive in only one case.

In lesions of SCC (19 cases), carboxylated acid mucin was positive in 14 cases, neutral mucin was positive in 10 cases, weakly sulphated acid mucin was positive in 2 cases, and strongly sulphated acid mucin was positive in only one case.

In Adenocarcinoma lesions (4 cases), carboxylated acid mucin was positive in 3 cases, weakly sulphated acid mucin was positive in 2 cases, strongly sulphated acid mucin was positive in only one case, and neutral mucin was negative in all cases.

In summary, carboxylated acid mucin was positive in 35/50 cases, neutral mucin was positive in 26/50 cases, strongly sulphated acid mucin was positive in 8/50 cases, and weakly sulphated acid mucin was positive in 7/50 cases (Table 1).

Table (1): Mucin Results Related to Histological Diagnosis.

Diagnosis/Type of Mucin	Weakly Sulphated Acid Mucin	Strongly Sulphated Acid Mucin	Carboxylated Acid Mucin	Neutral Mucin
LGTCC	2	2	14	11
HGTCC	1	4	4	5
SCC	2	1	14	10
Adenocacinoma	2	1	3	-
Total	7	8	35	26

DISCUSSION

The main result in this study is that carboxylated acid mucin was the most predominant type of mucin in bladder cancer lesions. It was more evident in high grade cancer. This was almost the same in the three major types of bladder carcinoma, i.e., transitional cell carcinoma, squamous cell carcinoma, and adenocarcinoma.

These results are consistent with several studies from other populations. Ebel K and Diekhans D studied nine mucus-secreting urothelial carcinomas using PAS technique. They reported that carboxylated mucin was the most prominent, followed by neutral mucin and sulfated mucin in different quantities. [11]

Barresi G and Marafioti T reported a case of intestinal metaplasia of the bladder urothelium associated with dysplastic foci and a transitional cell carcinoma with a mixture of mucins found in goblet cells. [12]

Walsh MD et al examined the presence of certain mucins in normal and malignant urothelium; they obtained sections from archival paraffin tissue blocks from 11 patients with nonmalignant urological conditions and 89 patients with transitional cell carcinomas (TCC). In normal urothelium, mucin was absent or limited, predominantly to the apical

membranes of the umbrella cell layer. Mucins were present in different quantities in all cases of TCC and significantly associated with both tumor grade and stage. [13]

The Japanese Ito H studied twenty seven bladder tumors by means of light microscopic histochemical methods for demonstration and identification of acid mucins. Alcian blue (pH 1.0), alcian blue (pH 2.5), periodic acid-Schiff (PAS) and aldehyde-fuchusin stains were performed. These stains showed that all tumor specimens, including squamous cell carcinomas, contained acid mucins. High-grade and high-stage tumors contained large amounts of sulfated mucins. [14]

Lopez-Beltran A. et al studied 25 cases of urothelial carcinoma with glandular differentiation and reported the presence of different types of mucins in all cases. [15]

CONCLUSION

From this study, it is concluded that mucins, mainly the carboxylated subtype, can be considered one of the important prognostic markers in urinary bladder cancer cases; that is because it was positive in all cases of the study and more evident with the disease progression.

REFERENCES

1. Boskey A. Biomineralization: an Overview. *Connective Tissue Research.* 2003; 44(1):5-9
2. Perez-Vilar J and Hill RL. Mucin Family of Glycoproteins. *Encyclopedia of Biological Chemistry*.2004; 2:758–764.
3. Niv Y. MUC1 and colorectal cancer: pathophysiology considerations. *World J. Gastroenterol*. 2008; 14(14):2139-41.
4. MediaLab, Inc. *Mucins Information and Courses*. http://www.medialabinc.net/mucins-keyword.aspx (accessed 28.3.2013).
5. Russell Myers. Special Stain Techniques for the Evaluation of Mucins. *microVIEWS*.2009; 18.
6. The Internet Pathology Laboratory for Medical Education. *Special Stains in Histology*. http://library.med.utah.edu/WebPath/webpath.html#MENU (accessed 29.3.2013).
7. Ekkehard Kunze, Bernd Francksen, and Harald Schulz. Expression of MUC5AC apomucin in transitional cell carcinomas of the urinary bladder and its possible role in the development of mucus-secreting adenocarcinomas. *Virchows Archiv*. 2001; 439(5):609-615.
8. Kunze E and Francksen B. Histogenesis of nonurothelial carcinomas of the urinary bladder from pre-existent transitional cell carcinomas: a histo-pathological and immunohistochemical study. *Urol Res*.2002; 30(1):66-78.
9. Newbould M and Mc William LJ. A study of vesical adenocarcinoma, intestinal metaplasia and related lesions using mucin histochemistry. *Histopathology* 1990; 17(3): 225-30.
10. Wells M and Anderson K. Mucin histochemistry of cystitis glandularis and primary adenocarcinoma of the urinary bladder. Arch Pathol Lab Med. 1985; 109(1):59-61.
11. Ebel K and Diekhans D. Mucin histochemical analysis of mucin-forming carcinomas, metaplasias of the urothelium and in persistent urachus. *Pathologe*.1996; 17(3):208-212.
12. Barresi G and Marafioti T. Mucin histochemistry and lectin binding sites in intestinal metaplasia of the

urinary bladder. *Histopathology*. 1990; 17(3):219-223.
13. Walsh MD, Hohn BG, Thong W et al. Mucin expression by transitional cell carcinomas of the bladder. *Br J Uro*.1994; 73(3):256-62.
14. Ito H. Histochemical studies of bladder tumors. *Hinyokika Kiyo*.1986; 32(3): 385-400.
15. Lopez-Beltran A, Jimenez RE, Montironi R. et al. Flat urothelial carcinoma in situ of the bladder with glandular differentiation. *Hum Pathol*. 2011; 42 (11): 1653-9.

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