E-02

Low 5-methylcytosine expression as an aggressive biomarker for the urothelial carcinoma: an immunohistochemical study

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Introduction

- **Previous studies suggested that global DNA methylation is involved in breast, lung, and colon carcinogenesis. However, only a few studies showed the association between global DNA methylation and urothelial carcinoma (UC).**

Aims

- **We constructed a tissue array to elucidate the role of global DNA methylation in UC carcinogenesis.**

Martial and Methods

- **Two tissue microarrays were purchased from US Biomax, Inc. (MD, USA), including 155 tissue cores with 22 normal urothelium samples and 133 urothelium samples with UC. Global DNA methylation (5-methylcytosine; 5-MeC) was measured using the immunohistochemistry (IHC) method (H score) and image analysis (total intensity). Nonparametric analysis with Wilcoxon rank-sum test or the Kruskal–Wallis test was applied to compare the differences in 5-MeC levels and the clinical variables between the two groups.**

Table I. Clinical information of urothelial carcinoma patients and normal subjects

Variables	Normal (n = 22)	Urothelial Carcinoma (n = 133)
Age	31.64 ± 12.37	61.67 ± 12.11
Sex		
Male	11 (50.00%)	106 (79.70%)
Female	11 (50.00%)	27 (20.30%)
Tumor grade		
Low	-	76 (57.14%)
High	-	57 (42.86%)
Cancer stage		
Early*	-	101 (75.94%)
Advanced**	-	32 (24.06%)
TNM stage		
T1N0M0	-	38 (28.57%)
T2a/bN0M0	-	62 (46.62%)
T3a/bN0M0	-	33 (24.81%)

*Early stage indicates cases with cancer stages I and II.
**Advanced stage indicates cases with cancer stage III.

Table II. DNA methylation levels* for urothelial carcinoma and normal urothelium

Methods	Normal (n = 22)	Urothelial Carcinoma (n = 133)	p Value
H score	107.73 ± 64.80	74.70 ± 58.08	0.026
Total intensity	154.55 ± 92.31	104.66 ± 83.37	0.013

* DNA methylation levels were detected using two methods. H score was examined by a pathologist under a light microscope and total intensity was calculated by imaging software.

Table III. DNA methylation levels stratified by clinical variables in urothelial carcinoma cases (n=133)

Variables	H score	p Value	Total Intensity	p Value
Sex		0.99		0.90
Male	76.32 ± 61.02		106.70 ± 86.84	
Female	68.33 ± 45.09		96.67 ± 68.95	
Tumor grade		0.55		0.41
Low	75.33 ± 52.16		106.05 ± 73.41	
High	73.86 ± 65.62		102.81 ± 95.71	
Cancer stage		0.01		0.04
Early	81.14 ± 57.11		112.38 ± 82.29	
Advanced	54.38 ± 57.25		80.31 ± 83.34	
TNM stage		0.04		0.09
T1N0M0	72.11 ± 46.96		99.74 ± 66.68	
T2a/bN0M0	86.77 ± 62.67		120.32 ± 90.77	
T3a/bN0M0	55.00 ± 56.46		80.91 ± 82.10	

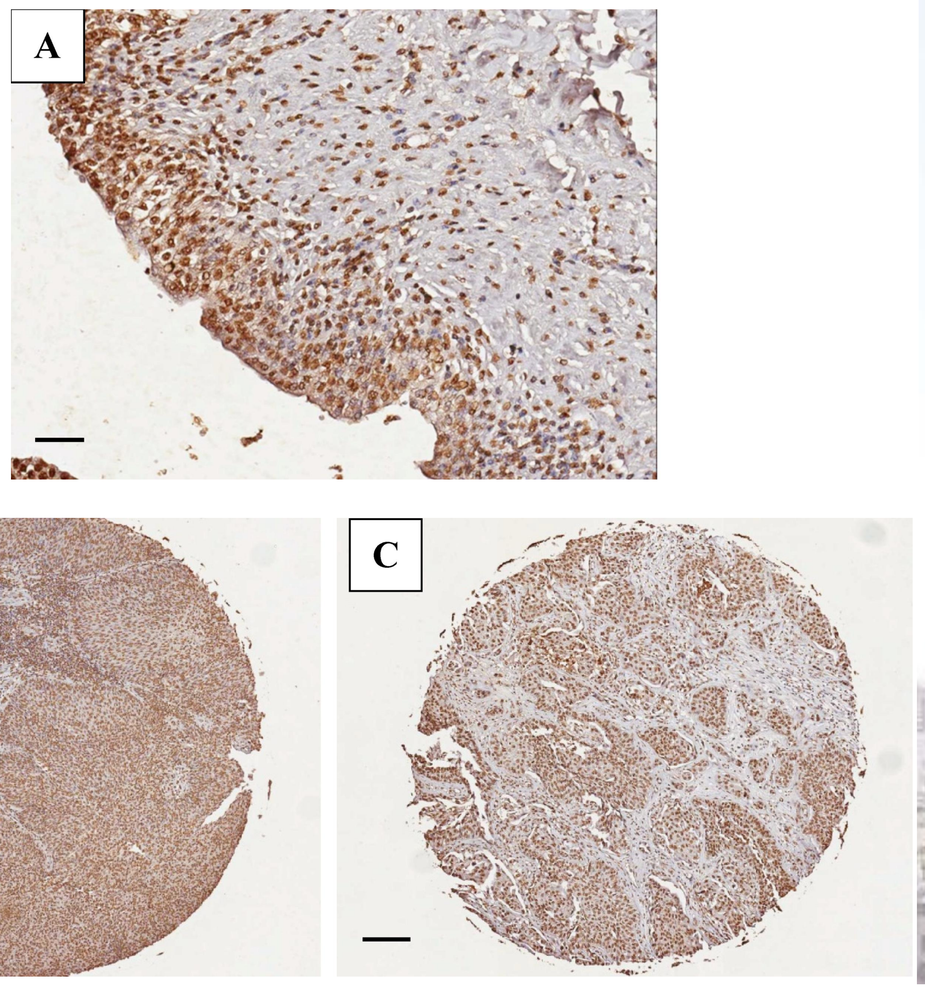

Figure 1. Anti-MC immunohistochemistry in normal urothelium and UC.
A. Normal urothelium with high expression. Bar, 100 µm.
B. Invasive UC with high expression. Bar, 400 µm.
C. Invasive UC with low expression. Bar, 400 µm.

Conclusions

- **The 5-MeC levels measured by IHC might be a good method for clinicians to evaluate global DNA methylation in UC progression.**