Expression of Wnt5a in urothelial carcinoma as a potential prognostic marker

Mark Saling 1, Jordan K. Duckett 1, Scott Jenkinson 2 and Ramiro Malgor 3
1. OMSII; 2. Specialty Medicine; 3. Biomedical Sciences, HCOM
Ohio University Heritage College of Osteopathic Medicine, Athens, OH. USA

Introduction

Bladder cancer is the fifth most common cancer in the USA. An estimated 72,570 new cases were diagnosed in 2013 and 15,210 will lead to a cancer-related death. Approximately 95% of bladder cancers occur in the urothelium to cause urothelial carcinoma. Finding molecular biomarkers for urothelial carcinoma can help determine prognosis and tailor management plans.

The Wnt family of proteins has been shown to play a critical role in embryonic development, regulation of cell proliferation, motility, morphology, and cell fate. Aberrant Wnt signaling has been implicated in cancer. Wnt5a signaling, a β-catenin independent pathway has been implicated as tumor suppressor or tumor promoter for different types of cancer. Recently Wnt5a/Ror2 signaling has been described as an important pathway in epithelial-mesenchymal transition and metastatic processes. Several prognostic biomarkers have already been studied for urothelial carcinoma, including β-catenin and E-cadherin. β-catenin and E-cadherin are cell membrane proteins important for cell adhesion. β-catenin, as part of the β-catenin dependent pathway, becomes dissociated from the cell membrane and enters the nucleus to induce gene expression. Decreased expression of E-cadherin from the cell membrane has been associated with increased invasiveness.

Methods

Human urothelial carcinoma samples (n=13), were obtained during transurethral resections. The samples were immediately fixed with 10 % buffered formalin overnight. Subsequently, samples were dehydrated in sequential alcohol/xylene washes and embedded in paraffin. Tissue blocks were sectioned into 5 µm sections, analyzed using hematoxylin & eosin (H&E), and histologically graded using the WHO/ISUP consensus classification system. Specific proteins were detected as follows. Subsequent to blocking with hydrogen peroxide and 1% BSA (Sigma), human sections were incubated with antibodies against Wnt5a, E-cadherin, β-catenin, and Ror2 from Abcam, (Cambridge, England). After overnight incubation species matched secondary antibody was utilized and incubated for 1hr at room temperature. Subsequently, DAB-enhanced liquid substrate (1:10, Sigma) was added to develop the color. The slides were then counterstained with hematoxylin. In all cases, isotype-matched IgG at the same concentration was used as the negative control for the primary antibodies. Immunohistochemical staining was estimated based on intensity (intensity score) and extent (proportion score), of stained tumor cells at 400x. The Allred scoring system was used. Statistical analyses were performed with PASW Statistics 18

Results

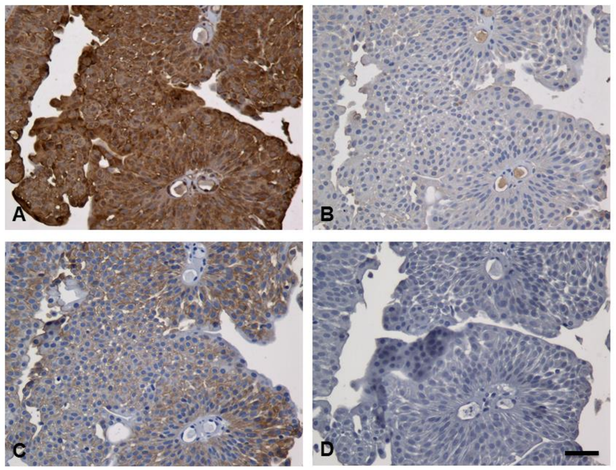

Figure 1. IHC for markers in low grade urothelial carcinoma at 200x A. Wnt5a, diffuse moderate reactivity; **B.** Ror2, diffuse weak immunoreactivity; **C.** E-cadherin, membrane strong reactivity **D.** β-catenin no reactivity. **Bar = 50 µm**

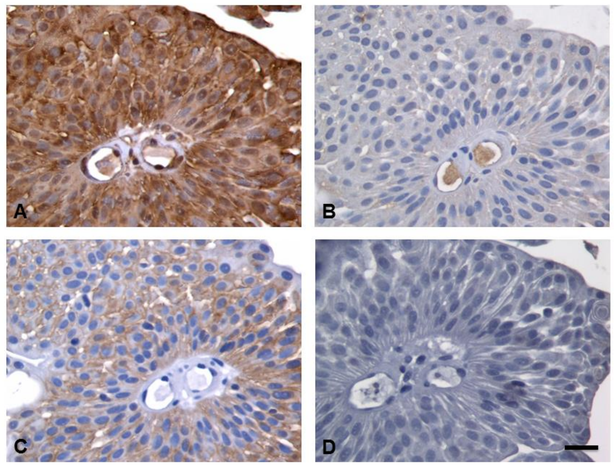

Figure 2. IHC for markers in low grade urothelial carcinoma at 400x A. Wnt5a, diffuse moderate reactivity; **B.** Ror2 diffuse weak immunoreactivity; **C.** E-cadherin, membrane strong reactivity **D.** β-catenin no reactivity. **Bar = 25 µm**

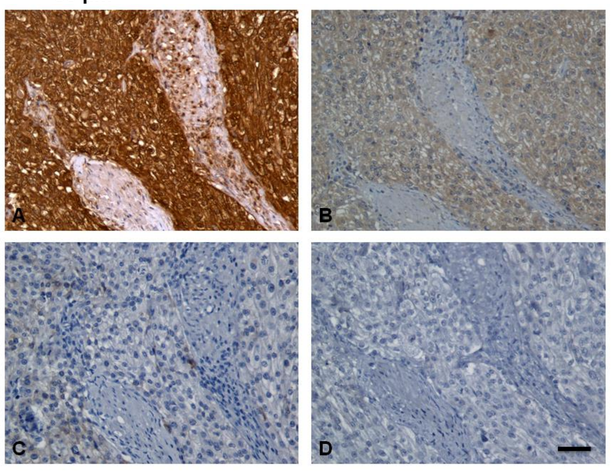

Figure 3. IHC for markers in high grade urothelial carcinoma at 200x A. Wnt5a, diffuse strong reactivity; **B.** Ror2, diffuse strong immunoreactivity; **C.** E-cadherin, weak reactivity **D.** β-catenin no reactivity. **Bar = 50 µm**

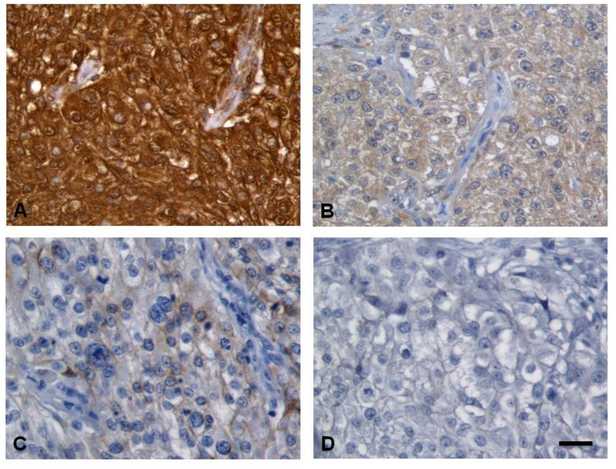

Figure 4. IHC for markers in high grade urothelial carcinoma at 400x A. Wnt5a, diffuse strong reactivity; **B.** Ror2, diffuse strong immunoreactivity; **C.** E-cadherin, weak reactivity **D.** β-catenin no reactivity. **Bar = 25 µm**

Based on the pathological reports, 6 samples were low grade and 7 high grade urothelial carcinomas. Within the 13 samples, 5 (4 high grade / 1 low grade) show muscular invasion and 3 of the invasive tumors show solid pattern, while the rest (10) show papillary aspect.

Correlations

- Correlation between tumor grade and Ror2 expression was significant at .01 level.
- Correlation between Ror2 and Wnt5a expression was significant at .01 level.
- Correlation between tumor grade and Wnt5a expression was significant at .05 level.
- Correlation between muscular invasion and tumor pattern was significant at .01 level.

Conclusion

In conclusion, our results support the previous studies that suggest Wnt5a plays a pathological role in UC. A correlation between Wnt5a /Ror2 and pathological grade suggests that Wnt5a/Ror2 signaling pathway could play a role in the aggressiveness of this cancer. The results also support their potential use as molecular biomarkers for UC. Further studies will be helpful in determining the underlying mechanism of Wnt5a/Ror2 in the pathogenesis/progression as well as their application as biomarkers for UC.

References

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Acknowledgements

Research & Scholarly Advancement Fellowship Program