**5-Hydroxy-Methyl-Cytosine Content is Strongly Associated with Degree of Histological Differentiation in Penile Squamous Cell Carcinomas**

Michael C. Haffner, MD; George J. Netto, MD; William G. Nelson MD, PhD; Srinivasan Yegnasubramanian MD, PhD; Kristen L. Lecksell, BS; Antonio L. Cubilla, MD; Alcides Chaux, MD

**ABSTRACT**

**Background:** Cytosine methylation represents an important epigenetic modification that plays a crucial role in normal differentiation as well as carcinogenesis. Recent evidence suggests that methylated cytosine (5mC) can become oxidized to 5-hydroxymethylcytosine (5hmC) in mammalian genome. We have recently shown that global 5hmC levels are greatly reduced in invasive adenocarcinoma. Here we evaluate the status of 5hmC in squamous cell carcinoma of the penis and explore the association of 5hmC with the degree of tumor differentiation.

**Design:** Thirty-eight formalin-fixed paraffin-embedded archival cases of penile squamous cell carcinoma were selected to build a tissue microarray (TMA). Each tumor was sampled 39 times. TMA spots were scanned using the APERIO system and uploaded to the TMAJ platform (http://tmaj.pathology.jhmi.edu). In total, 147 individual TMA spots were evaluated. Histological grade was assigned in each spot. Global 5hmC levels were assessed using a 5hmC specific antibody and standard immunohistochemical techniques. Extent of 5hmC expression was estimated in each spot as a percentage of positive cells. Association between 5hmC extent and grade was evaluated by the Kruskal-Wallis test and trends were confirmed using Cuzick's test.

**Results:** Normal squamous epithelium showed strong staining for 5hmC. In invasive tumors, extent of 5hmC expression showed a strong association with tumor differentiation. 5hmC extent decreased with increasing histologic grade (Figure 1). Differences were significant (P = .0001), as well as trend across ordered categories (P < .0001).

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**Conclusion:** In penile squamous cell carcinomas, global 5hmC levels significantly decrease with increased histologic grade. Our finding suggests that alterations in 5hmC expression are associated with carcinogenesis and are inversely correlated with tumor differentiation in penile squamous cell carcinoma.