Bladder cancer: Validating what we've got

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Cite as: *Can Urol Assoc J* 2013;7:33-4. http://dx.doi.org/10.5489/cuaj.236

Urinary biomarkers are urgently needed to improve the care and reduce the cost of managing bladder cancer, the most expensive solid cancer to treat on a per patient basis. One of the main problems with most current bladder cancer markers is the struggle to identify both high- and low-grade cancers, most likely due to differing molecular pathways. Indeed, given the very divergent biology between chromosomal stable p53 wild type low-grade disease and the genetically unstable high-grade bladder cancer, it looks somehow unlikely that a "one size fits all" urinary marker will emerge. Numerous urine-based markers have been investigated; some have better sensitivity than cytology, but frequently with lower specificity. None of these markers so far are indisputable parts of daily practice.[1] Also, since ideal biomarkers for bladder cancer should be urine-based (non-invasive), stable, sensitive, specific and cost-effective, the current available markers are all lacking in one or more of these requirements.

MiRNAs are short non-coding RNA molecules that post-transcriptionally modulate protein expression. They are extremely stable and this is very important when considering urinary markers. Aberrant miRNAs expression, either up- or down-regulation, has been linked to cancer by acting as oncogenes or tumour suppressor genes. Microarray platforms have been developed for analysis of miRNA expression. Different pathological bladder cancer subtypes (low grade vs. high grade) show distinct miRNA gene expression signatures as demonstrated by the landmark studies of Catto and colleagues.[2] Specific miRNAs have been shown to act either as tumour suppressors (miR-17-5p, miR-126, miR-221, miR-99a/100) or as oncogenes (miR-21, miR-26a, miR-29c, miR-30c, miR-30e-5p). High-grade bladder cancer is characterized by miRNA up-regulation, including miR-21 that suppresses p53 function. In contrast, in low-grade disease, there is down-regulation of many miRNA (i.e., loss of miR-99a/100 leading to up-regulation of FGFR3 before its mutation). The miR-200 family (miR-200a, -200b, -200c, -141 and -429) and miR-205 are frequently silenced in high-grade bladder cancer and have been implicated in epithelial to mesenchymal transition and tumour invasion.[2]

In the present study by Snowdon and colleagues,[3] based on 8 patients and 5 healthy controls, miRNA-125b showed an average 10.42-fold decrease in the cancer samples compared to the control samples ($p < 0.01$) and miR-126 showed an average 2.70-fold increase in the cancer samples compared to the controls ($p = 0.30$).

It is not completely clear how miRNA-200 performed here in the limited number of samples analyzed. Based on previous publications and given its functions, one would have expected this marker to perform reasonably well in high-grade bladder cancer.[4] For instance, Wang and colleagues recently quantified the urine sediment and supernatant levels of microRNA (miRNA) targets related to epithelial-mesenchymal transition in 51 patients with bladder cancer and in 24 controls.[5] They found that patients with bladder cancer had depressed levels of the miR-200 family, miR-192, and miR-155 in the urinary sediment.

The sensitivity and specificity of the model in Snowdon and colleagues' study were 80% and 100%, respectively.[3] The sensitivity of cytology on the same bladder cancer cases was 20% only. This sensitivity seems extremely low especially for high-grade cases. With 1 case of carcinoma in situ (CIS), 3 cases of low-grade transitional cell carcinoma (TCC) (1973 WHO grade 2) and 4 cases of high-grade TCC (1973 WHO grade 3), one would have expected the CIS to be positive and probably 3 out of 4 high-grade cancers also, leading to an expected overall sensitivity of around 50%.

The usefulness of a large spectrum of miRNAs has been recently investigated in 68 patients with bladder cancer and

53 age-matched controls, quantifying a total of 15 miRNAs by real-time polymerase chain reaction.[6] Interestingly enough, miRNAs were found to be very stable within urinary cells despite adverse handling. Individually, miR-1224-3p had the best individual performance with specificity, positive and negative predictive values and concordance of 83%, 83%, 75% and 77%, respectively. The combination of miRs-135b/15b/1224-3p detected bladder cancer with a high sensitivity (94.1%), a specificity of 51% and was correct in 86% of patients (concordance). However, two invasive cancers (3%) would have been missed.

Urine epigenomics as labelled by Sánchez-Carbayo is clearly a hot topic in bladder cancer, but obviously requires additional studies and especially validation.[7]

Clearly Snowdon and colleagues outlined it; although their results are promising, these preliminary findings require more investigation. The keyword as always will be cross-validation on different cohorts, using different techniques and finding the same miRNAs as the urinary biomarkers of interest. The big challenge is to reconcile different results and different miRNAs when analyzing different patient cohorts.

Too often different groups end up finding different miRNAs as their most promising biomarkers leaving more questions open than bringing definitive answers.[8] This remains a challenge and an important hurdle before these markers can be brought into clinical practice.

Validation, validation, validation...

Competing interests: None declared.

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