Nature Reviews Urology **8**, 355 (2011); published online 14 June 2011; doi:10.1038/nrurol.2011.82

BLADDER CANCER

Predicting response to BCG

Methylation status of a select panel of genes can predict failure of bacillus Calmette–Guérin (BCG) immunotherapy, according to research carried out by Marta Sanchez-Carbayo and colleagues at the Spanish National Cancer Research Centre in Madrid. Intravesical BCG is the treatment of choice for patients with non-muscle-invasive bladder cancer who are considered to be at high risk of recurrence and progression to muscle-invasive disease. However, about a third of recipients do not respond to BCG, and the ability to identify which patients might require more-aggressive management is a major goal of bladder cancer research today.

DNA methylation is the most common epigenetic event associated with cancer development. Sanchez-Carbayo *et al*. used a novel method called methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) to identify tumor suppressor gene promoters that were differentially-methylated between BCG responders and non-responders. MS-MLPA is a semiquantitative technique that leverages PCR technology to estimate the degree of methylation of several genes in a single experiment. "Having previously optimized the MS-MLPA technique in bladder cancer cells, frozen and paraffin-embedded tumors, pairs of matching tumors and urine samples, and verified it in training and validation sets of urine specimens for bladder cancer diagnosis, we focused on addressing one of the critical clinical endpoints of bladder cancer," says Sanchez-Carbayo.

Researchers retrospectively obtained tumors from 91 patients with T1G3 bladder cancer who had undergone a 6-week course of BCG after complete transurethral resection, but not received maintenance therapy. They used MS-MLPA to analyze DNA from each tumor—checking for methylation of 25 known or suspected tumor suppressor genes—and compared the results with patient outcomes. Overall, 44% of the cohort experienced recurrence, cancer progression was reported in 19%, and 41% died over a median follow-up period of 90 months.

Methylation status of eight tumor suppressor genes was associated with response to BCG. Tumors in which *PAX6* was methylated were more likely to recur, and unmethylated *GATA5* was associated with shorter disease-specific survival. Patients whose tumors contained unmethylated *MSH6*, *RB1*, *THBS1*, *PYCARD*, *TP73*, *ESR1* or *GATA5* experienced higher rates of progression to invasive disease than their counterparts harboring methylated genes. Combining data from two, three, four, or five genes significantly improved prediction of progression. Notably, the combination of *MSH6* (commonly associated with hereditary nonpolyposis colorectal cancer) and *THBS1* (which encodes an adhesive glycoprotein that mediates cell-to-cell and cell-to-matrix interactions) could discriminate most effectively between patients likely to respond to BCG and those who might require radical intervention.

Most of the eight genes predictive of response to BCG were not previously known to be associated with bladder malignancy. As such, the mechanisms by which epigenetic regulation of their expression influences BCG response remain unknown. The authors intend to investigate further, first by validating their gene panel. Hopefully, prospective trials will follow.

Sarah Payton

Original article Agundez, M. *et al*. Evaluation of the methylation status of tumour suppressor genes for predicting bacillus Calmette-Guérin response in patients with T1G3 high-risk bladder tumours. *Eur. Urol.* doi:10.1016/j.eururo.2011.04.020