Analysis of tumor DNA in urine as a highly sensitive liquid biopsy for patients with non-muscle invasive bladder cancer (NMIBC).

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Noninvasive detection of urine tumor DNA (utDNA) has the potential to augment the diagnosis and management of bladder cancer. We sought to identify whether patient specific molecular events identified initially in the primary tumor could be detected in their utDNA. We interrogated both genetic and epigenetic events by a number of different technologies. Single nucleotide variants (SNVs) were analyzed by Next Generation Sequencing (NGS) on both the Ion Torrent platform (using amplicon enrichment) and the Illumina platform (via hybridization capture). Thirteen differentially methylated regions (DMRs) in the genome were analyzed by methylation-specific quantitative polymerase chain reaction (MS-qPCR). The cohort included patients with newly diagnosed NMIBC and those undergoing surveillance cystoscopy following a prior diagnosis of NMIBC. DNA was isolated from fixed paraffin embedded (FPE) primary tumors, matched blood buffy coat (BC), urine sediment (US) and, in some cases, clarified urine (CU-a.k.a. urine supernatant). Tumor specific molecular events were identified by comparing primary tumor tissue to BC (for SNVs with NGS) or to pooled data from urine DNA from healthy controls (for DMRs with MS-qPCR). Initially, 8 patients were evaluated with all three technologies. A larger cohort of 66 patients undergoing surveillance for NMIBC was then analyzed using MS-qPCR and a subset was also analyzed using NGS on the Ion Torrent platform. Tumor specific DMRs and/or SNVs were detected in tumor tissue from 73 of 74 patients. In the 8 patients assessed using both NGS platforms, SNVs were found in the primary tumor for 8/8 (Ion Torrent) and 6/8 (Illumina) patients, and both NGS platforms identified utDNA in 4 of 5 biopsy confirmed NMIBC patients. In patients with sufficient DNA to be assessed with all three technologies, in both US and CU, there was 100% agreement for all positive/negative calls for utDNA. Targeted genomic regions methylated in NMIBC tissue were seen to be methylated in the corresponding utDNA at the time of diagnosis or recurrence. In the cohort of 66 patients undergoing surveillance for NMIBC using MS-qPCR, a negative predictive value for high risk recurrences of 95% (with a prevalence of 10%) was achievable. These results provide evidence that both genetic and epigenetic events are evident in utDNA and reflect the presence and genetics of the NMIBC. This proof of concept study demonstrates the potential for a personalized non-invasive means to detect and monitor bladder cancer, thus making a valuable contribution to the field of precision medicine.