Molecular pathology selected abstracts

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Use of utDNA as a biomarker in muscle-invasive bladder cancer

November 2021—The standard-of-care treatment for muscle-invasive bladder cancer is neoadjuvant chemotherapy followed by radical cystectomy. Approximately 35 percent of patients who receive neoadjuvant treatment will achieve pathologic complete response (pCR). Although cystoscopy monitoring is the method of choice to evaluate tumor progression or response to therapy, this method is not always accurate for the pathological evaluation of the bladder. There is no sensitive noninvasive assay to assess minimal residual disease (MRD) after muscle-invasive bladder cancer treatment and support the use of a less-invasive bladder-sparing treatment. The authors reported on their efforts to develop a noninvasive liquid biopsy assay for urine samples employing a next-generation sequencing (NGS) method used to detect urine tumor DNA (utDNA). They conducted a cohort study to determine if utDNA collected on the day of radical cystectomy correlates with pathologic response and detects minimal residual disease in patients with localized bladder cancer. On the day of the curative radical cystectomy, paired urine and blood samples were collected from 42 patients with localized muscle-invasive cancer and 27 healthy adults. Seventy-six percent (32 of 42) of patients in the bladder cancer cohort had a confirmed diagnosis of muscle-invasive bladder cancer, 59 percent of whom had previously received neoadjuvant chemotherapy. UtDNA variant calling was performed and sequences analyzed for single nucleotide variants in the 49 driver genes most commonly mutated in bladder cancer. Only variants with duplex support, no germline match in peripheral blood, and non-silent mutations were considered for further analysis for minimal residual disease. Comparison of the strength of association between the pathologic response and residual detectable mutations in utDNA achieved a sensitivity and specificity of 81 percent. Patients with pCR had an increased number of mutations (median, two mutations), as opposed to patients with pCR (median, zero mutations). *TERT* and *TP53* were the most commonly mutated genes. Patients without pCR had a higher median utDNA level (4.3 percent) than patients who had achieved pCR (zero) and healthy adults. Variant data from healthy adults was used to determine the optimal utDNA level threshold that separates localized bladder cancer patients from healthy adults, which was determined to be 2.3 percent. The threshold classified patients with no pCR from healthy adults with a sensitivity of 81 percent and specificity of 100 percent. The threshold was used to divide MRD-positive and -negative patients. UtDNA MRD-positive patients showed worse prognosis-free survival than utDNA MRD-negative patients. Using The Cancer Genome Atlas whole genome sequencing data from 409 muscle-invasive bladder cancer tumors, tumor mutational burden was deduced by doing a linear comparison of non-silent mutations of the MRD-positive patients. The authors suggested that based on these results, targeted immune therapy could be prioritized for patients that are found to be utDNA MRD positive and have high tumor mutational burden. In summary, this study suggested that utDNA MRD detection can predict pathologic response in muscle-invasive bladder cancer. This would support more targeted clinical-management decisions and, perhaps, bladder-sparing options. UtDNA can also be used to monitor treatment and assess response. This tool may be used in the future for more personalized treatment interventions, disease monitoring, and informed decision-making. However, prospective clinical trials will be needed to validate and assess these biomarker-driven interventions.

Chauhan PS, Chen K, Babbra RK, et al. Urine tumor DNA detection of minimal residual disease in muscle-invasive bladder cancer treated with curative-intent radical cystectomy: A cohort study. *PLOS Med*. 2021;18(8):e1003732. https://doi.org/10.1371/journal.pmed.1003732

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Optical genome mapping: ability to detect constitutional chromosomal aberrations

Cytogenetic techniques, such as karyotyping, FISH, and copy number variant microarrays, are used routinely for diagnostic and prognostic purposes. However, these methods have significant limitations, such as low-quality resolution and the inability to detect structural variations (SV), mosaicisms in microarray copy numbers, and balanced chromosomal aberrations. Optical genome mapping (OGM) is a novel technique that can potentially overcome these limitations and replace the aforementioned cytogenetic assays. The technique uses a nonsequencing technology based on optical mapping that allows extremely long strands of DNA to be analyzed. The most updated version allows high-throughput whole genome imaging and its de novo assembly via a combination of microfluidics, high-resolution microscopy, and automated image analysis. The authors conducted a proof-of-principle study in which they evaluated the ability of OGM to detect simple and complex chromosomal aberrations of clinical significance. The multicenter European study involved samples from 85 people with confirmed chromosomal abnormalities, including hereditary conditions. All of the samples were assessed with the traditional cytogenetic methods of FISH, copy number variant (CNV) microarray, or karyotyping, or a combination of these techniques. Ultra-high-molecular–weight DNA was isolated from the 85 samples and processed via OGM. This was followed by linearization and imaging. Structural variants, CNVs, and other abnormalities were detected and analyzed using two distinctive pipelines—one based on coverage depth and the other based on the comparison of a de novo assembled genome map to a reference map. The authors compared the results with the conventional methods. In this study cohort, which was highly representative of a routine clinical practice in cytogenetics, the authors identified 99 chromosomal changes, including deletions, duplications, insertions, inversions, translocations, and additional complex chromosomal rearrangements, from the 85 samples. OGM detected all aberrations from the 85 samples with 100 percent concordance with cytogenetic techniques. All whole chromosome aneuploidies were detected. Unbalanced translocations were detected by the SV calling and CNV calling pipelines. The cohort contained 34 microdeletions and microduplications associated with syndromes, all of which were detected. These regions are considered to belong to the most complex sequences of the human genome. OGM resolved four cases with complex rearrangements, all of which involved people with developmental delays or intellectual disabilities, or both, and described the precise breakpoints, in contrast to karyotyping. OGM identified all significant CNVs detected previously by microarray. This study demonstrated that the OGM method can detect all classes of chromosomal aberrations and may complement or replace cytogenetic technologies. A limitation of OGM is its inability to detect the breakpoints of balanced SVs lying within large repetitive unmappable regions. However, in some cases, it detected translocations with breakpoints within pericentromeric regions that were not detected by whole genome sequencing. Another advantage of OGM is its ability to highly accurately detect balanced SVs within the entire genome. In summary, the study validated the use of OGM as a possible replacement for cytogenetics techniques in the foreseeable future. The limitations of OGM may become obsolete with future improvements to its technical and analytical aspects. The results of this study highlight the potential of OGM to serve as a cost-effective and user-friendly alternative to cytogenetic techniques for detecting chromosomal aberrations and structural variants.

Mantere T, Neveling K, Pebrel-Richard C, et al. Optical genome mapping enables constitutional chromosomal aberration detection. *Am J Hum Genet*. 2021;108(8):1409–1422.

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