Molecular pathology selected abstracts

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Value of identifying molecular subtypes for non-muscle-invasive bladder cancer

July 2023—Non-muscle-invasive bladder cancer accounts for the majority of bladder tumors and is typically treated with transurethral resection of the bladder tumor followed by adjuvant intravesical Bacillus Calmette-Guérin instillations. However, the long-term effectiveness of Bacillus Calmette-Guérin (BCG) is limited, and patients with recurrent or progressive disease have lower survival rates. Understanding the genetic makeup of tumors and identifying molecular subtypes associated with BCG response could provide valuable insights that aid in developing personalized treatments. The authors conducted a study in which they performed whole-transcriptome sequencing of non-muscle-invasive bladder cancers (NMIBCs) from 132 patients who had never received BCG treatment and 44 patients whose cancer recurred after BCG treatment. Based on these patients' results, the authors identified three unique molecular subtypes among the tumors—BRS1, 2, and 3. Patients with BRS3 showed increased epithelial-to-mesenchymal transition pathway activity and their tumors were enriched for mutations associated with the extracellular matrix when compared with the other two subtypes. Patients with BRS3 tumors also demonstrated a worse progression-free survival and increased activity in epithelial-mesenchymal transition (EMT) pathways. BRS1 and BRS2 tumors displayed luminal characteristics and were associated with a more favorable patient outcome after BCG treatment. The BRS3 tumor microenvironment exhibited immune-suppressive features, with higher infiltration of B cells, tumor-associated macrophages, $CD8^+$ T cells, and regulatory T cells. BRS3 tumors also showed higher intratumoral vimentin protein expression, suggesting increased immune suppression and potential treatment failure. BRS3 was associated with more aggressive biology, including enrichment of EMT, complement, IL6-JAK-STAT3, and angiogenesis pathways. Matching subtypes with clinical outcomes showed that patients with BRS3 tumors had a higher risk of recurrence after BCG therapy. BRS stratification was validated in a second cohort of 151 BCG-naïve patients who had high-risk NMIBC, and the molecular subtypes outperformed guideline-recommended risk stratification based on clinicopathological characteristics. The BRS classifier improved the clinical risk stratification of high-risk NMIBC and identified patients with BRS3 tumors who were at increased risk of progression. Post-BCG tumors that recurred were enriched for BRS3, and the gene-expression signatures of these tumors provided insight into potential therapeutic targets. Notably, BRS3 tumors displayed increased expression of immune checkpoint-related genes and *DDR2*, suggesting the potential for combination therapy with checkpoint inhibitors or anti-DDR2 treatments. The authors concluded that BRS3 tumors are associated with an aggressive phenotype and poorer survival, while BRS1 and BRS2 tumors display more favorable characteristics. Their findings highlight the importance of molecular profiling in predicting treatment response and provide potential targets for therapeutic interventions in NMIBC.

de Jong FC, Laajala TD, Hoedemaeker RF, et al. Non-muscle-invasive bladder cancer molecular subtypes predict differential response to intravesical Bacillus Calmette-Guérin. *Sci Transl Med*. 2023;15. doi:10.1126/scitranslmed.abn4118

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Use of optical genome mapping for prenatal diagnostic testing

The rate of clinically recognized pregnancy loss is approximately 15 to 25 percent, with fetal anomalies occurring in another three to five percent. Chromosomal abnormalities often cause these adverse outcomes. Therefore, guidelines recommend that all pregnant women undergo prenatal genetic screening, regardless of maternal age, underlying clinical conditions, or stage of pregnancy. In many situations, noninvasive prenatal screening is offered at 10 to 12 weeks of gestation. Noninvasive screening, which analyzes fragments of fetal DNA circulating in maternal blood, is an exquisitely sensitive method of screening for chromosomal abnormalities. However, abnormal results should be confirmed with invasive diagnostic testing, such as chorionic villus sampling or amniocentesis. Fetal tissue obtained through one of these invasive procedures is traditionally analyzed by karyotyping, FISH, or chromosomal microarray. In many clinical situations, more than one of these diagnostic tests are performed since each method may have blind spots. Karyotyping involves visually inspecting G-banded chromosomes, and its resolution is limited to 5 to 10 Mb, as these are the smallest changes that can be viewed reliably under a light microscope. FISH requires fluorescent probes that hybridize to regions of interest, so it can only detect what is covered by the probe sets. Chromosomal microarray has high resolution for submicroscopic copy number alterations, but it cannot identify balanced translocations or determine the orientation or location of copy number changes. In contrast to these conventional methods, optical genome mapping (OGM), a next-generation cytogenomic technology, can assess all types of structural variants at high resolution across the entire genome. In this method, ultralong DNA molecules are labeled with a fluorescent marker at CTTAAG sequences. These sequences occur, on average, about every 5 kb, but they vary in frequency across the genome, allowing individual ultralong DNA molecules to be identified based on fluorescent pattern. The ultralong DNA molecules are loaded into a microfluidic device in which they are linearized and flow through parallel channels to undergo imaging via a high-resolution camera. Once the pattern of fluorescent markers is analyzed across all molecules, the genome can be assembled and any abnormalities identified. The authors of this study evaluated and clinically validated the use of OGM in prenatal diagnostic testing. Using OGM, they tested 114 samples harboring 101 aberrations identified by standard-of-care cytogenetic analysis. The test had 100 percent sensitivity compared with conventional methods. The chromosomal abnormalities included 29 interstitial/terminal deletions as small as 95 kb, 28 duplications, 26 aneuploidies, six absence-of-heterozygosity regions, three triploid genomes, four isochromosomes, three marker chromosomes, one chromosome with additional material, and one translocation. Because its resolution is higher than that of conventional methods, OGM also identified 64 structural variants that potentially would be reportable. Phenotypically normal control samples were assessed to test specificity. No false-positive pathogenic structural variants were identified using OGM, demonstrating 100 percent specificity. Reproducibility at the interrun, intrarun, and interinstrument levels was also 100 percent. This study demonstrates the feasibility of using OGM for diagnostic prenatal testing, one of the most important and high-volume applications of cytogenetic analysis.

Sahajpal NS, Mondal AK, Fee T, et al. Clinical validation and diagnostic utility of optical genome mapping in prenatal diagnostic testing. *J Mol Diagn*. 2023;25(4):234–246.

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