Molecular Pathology Selected Abstracts, 7/14

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Is there a role for molecular diagnostics in bladder cancer?

Bladder cancer is the fourth most common cancer in men in the United States and is associated with high rates of cancer morbidity and mortality worldwide. Multiple subtypes of bladder cancer have been identified, the most common of which is urothelial cancer. Once diagnosed, treatment depends on the pathologic grade and stage of the bladder tumor. For superficial tumors, therapy includes transurethral resection and instillation of chemotherapeutic or proinflammatory agents directly into the bladder. However, for tumors that deeply invade the wall of the bladder, patients undergo radical cystectomy and receive chemotherapy if metastases are present, although outcomes for this population remain relatively poor. In attempts to improve therapy, several recent efforts have been undertaken to better characterize molecular alterations associated with bladder cancer and to use this information to develop targeted molecular therapies. Recently, the Cancer Genome Atlas project examined 131 cases of muscle-invasive urothelial carcinoma and analyzed data on whole genome and exome DNA sequencing, DNA copy number, complete mRNA and microRNA expression, DNA methylation, and protein expression and phosphorylation. Mutations were consistently found in 32 genes, including p53 (mutated in 49 percent of bladder tumors), PIK3CA (20 percent), RB1 (13 percent), FGFR3 (12 percent), and TSC1 (eight percent). Integrating these data, the investigators identified major pathways that are often dysregulated in muscle-invasive bladder cancer. Alterations in the p53/Rb pathway, including mutations in CDKN1A and CDKN2A, occurred in 93 percent of tumors. Additional altered pathways that affected cell proliferation and survival included the PI3K/Akt/mTOR and RTK/RAS signaling cascades in the majority of tumors. Several proteins associated with these pathways, including FGFR3, ERBB2 (HER2), EGFR, and ERBB3, are particularly amenable to targeted molecular therapies. Finally, the study identified epigenetic changes in bladder cancer. Chromatin remodeling and histone modification was affected in 89 percent of bladder tumors, more than in any other cancer studied, and the SWI/SNF complex that regulates gene expression was affected in 64 percent of tumors. Overall, this study found that 69 percent of the bladder tumors examined had potential molecular therapeutic targets. It is likely that the findings of this study will help to elucidate the molecular pathogenesis of bladder cancer and will be especially useful as clinical trials are developed to examine molecular therapies in urothelial carcinoma.

The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature.* 2014; 507:315–322.

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Potential improvements to noninvasive prenatal testing

Noninvasive prenatal testing (NIPT) is becoming a widely adopted method to screen for fetal chromosomal aneuploidies in at-risk pregnancies and is gaining momentum for all pregnancy risk categories. NIPT assays are based on cell-free (cf) fetal DNA present in maternal blood plasma. The methods used by commercially available tests involve quantitating plasma DNA sequences from specific genomic regions to determine if an aneuploidy (that is, trisomy 21, 13, 18, and sex chromosome aneuploidies) is present in the fetus by using massively parallel DNA sequencing technologies and sequence-based tag counting algorithms. A hallmark for circulating cfDNA is that the molecules are short fragments of less than 200 bp. The authors conducted a study in which they showed that it

is possible to estimate fetal fraction of cfDNA in maternal plasma by measuring the overall size distribution of maternal plasma DNA. The authors also showed that fetal chromosome dosage can be estimated by measuring the increased or decreased proportion of short fragments from the aneuploid chromosome in maternal plasma. In the study, cfDNA size was measured by both paired-end massively parallel sequencing reads and microchip-based capillary electrophoresis as determined by a bioanalyzer. After testing various cutoffs, the authors determined that a cutoff of 150 bp gave the best performance for fetal aneuploidy detection. One benefit of using size-based measurement of maternal plasma DNA is in assessing fetal cfDNA fraction, which is critical for NIPT accuracy. Current methods use Y-chromosome markers, polymorphic markers, and DNA methylation markers to assess fetal cfDNA fraction. An advantage of using size-based measurements is both the relevance to all pregnancies and the availability of maternal plasma DNA size distribution that is inherent in next-generation sequencing read data. A second area where size-based measurements may improve current NIPT assays is in detecting sex chromosome aneuploidies. In 18 test cases (10 monosomy X, eight euploid), size profiling accurately called all samples (100 percent sensitivity and 100 percent specificity), whereas the tag counting method had 90 percent sensitivity and 100 percent specificity. Although the sample size used in this study was relatively small, it lays the foundation for ways to improve sensitivity and specificity in current NIPT. One can envision incorporating both current tag counting methods with fragment size profiling to more accurately detect fetal chromosomal aneuploidies from cfDNA present in maternal plasma.

Yu SC, Chan KC, Zheng YW, et al. Size-based molecular diagnostics using plasma DNA for noninvasive prenatal testing. *Proc Natl Acad Sci U S A.* 2014;111:8583–8588.

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