



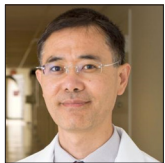
Editorial

Molecular cytopathology: The future of pathology?

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Dear Editor,

Advancements in medical technology are creating new opportunities and challenges for cytopathology. An example is the use of the high-risk human papillomavirus (HPV) test for cervical cancer screening, adding a crucial molecular test to the classic morphology-based diagnostic process.^[1] While cytopathologists have traditionally relied on visually identifying phenotypic features to diagnose tumors, including bizarre mitotic figures, enlarged hyperchromatic nuclei with irregular nuclear membranes (due to abnormal chromosomal replication), and prominent nucleoli (indicating increased protein synthesis), neoplasm is fundamentally a genetic disease. Molecular alterations in nucleic acids (DNA and RNA) and associated changes in proteins, lipids, and carbohydrates determine the tumor's phenotype and other features, such as aggressiveness and ability to metastasize.

Molecular testing is a critical tool in modern oncology, serving multiple purposes. It is used to diagnose tumors based on specific alterations, such as capicua transcriptional repressor (*CIC*) rearrangement for *CIC* rearranged sarcoma and *NUT* midline carcinoma family member 1 (*NUTM1*) fusion for *NUT* carcinoma. Furthermore, it guides treatment by identifying targeted therapy options, for example, detecting epidermal growth factor receptor (*EGFR*) mutation, anaplastic lymphoma kinase (*ALK*), receptor of oncogenesis 1 (*ROS1*), and rearranged during transfection (*RET*) fusions in lung adenocarcinoma or b-rapidly accelerated fibrosarcoma (*BRAF*) mutations in melanoma. Molecular testing can also help predict a patient's prognosis, as seen with *p53* mutations in ovarian serous carcinoma, allowing for earlier intervention in high-risk cases. Finally, highly sensitive molecular tests can monitor a patient's response to therapy, enabling timely treatment adjustments even before we can identify tumor cells under the microscope.^[2]

While molecular tests traditionally relied on extracts from surgical specimens, the field has progressed to incorporate cytopathology specimens, which are acquired through minimally invasive or non-invasive approaches. This shift enables molecular analysis of materials derived from cell blocks, smears, and centrifuged cell pellets, and the supernatants of various fluids, including urine, pleural effusion, ascitic fluid, cerebrospinal fluid, and fine-needle aspirations (FNA).^[2-9] These supernatants provide a more abundant source of cell-free nucleic acids (circulating free DNA [cfDNA] and circulating free RNA) and extracellular vesicles compared to plasma.^[2] To overcome the challenge of limited quantities, highly sensitive and multiplexed molecular assays are required for detection.^[10] These techniques encompass amplification (e.g., polymerase chain reaction [PCR]), labeling-based targeted detection (e.g., fluorescence *in situ* hybridization [FISH]), DNA/RNA sequencing (e.g., next-generation sequencing [NGS]), RNA expression analysis (e.g., microarray), and immunoassays, etc.

Compared to cytopathology, molecular approaches offer several key advantages, including the ability to analyze cell-free molecules (e.g., circulating tumor DNA) without requiring intact cells. These methods also often provide higher sensitivity and specificity. For example, molecular testing is used for FNA samples taken from indeterminate thyroid nodules classified by cytopathology. These tests analyze DNA and/or RNA, using platforms such as Afirma, ThyroSeq, and ThyGeNEXT/ThyraMIR, to better risk-stratify patients and reduce unnecessary surgery.^[7,11]

It is well-known that certain morphological features can predict molecular changes, such as the link between cervical/tonsil basaloid squamous cell carcinoma and high-risk HPV and between breast/salivary secretory carcinoma and the *ETV6-NTRK3* gene fusion.^[10,12,13] The integration of digital pathology with artificial intelligence (AI) is transforming cytopathology by enabling more effective and efficient identification and classification of carcinomas. By extracting essential information from tumor morphology and complex molecular databases, AI algorithms might predict all the pathological features based on molecular alterations. With the help of radiological studies, the diagnosis, classification, even staging of a tumor may be achieved by molecular cytopathology in the future.^[12]

One might speculate that in 50 years, carcinoma will be diagnosed with only a drop of urine and treated simply with pills. However, molecular diagnostics will not entirely replace cytopathology in the near future, though their increasing importance cannot be overstated. At present, some clinical trials already heavily rely on analyses of cfDNA from plasma specimens, a technique largely pioneered by the clinicians.^[14-16] This growing trend highlights the urgent need to integrate molecular cytopathology into our routine practice. By embracing this minimally invasive diagnostic technology of molecular cytopathology, we can position ourselves as innovators and leaders, not merely followers, in this field.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

ABBREVIATIONS

AI: Artificial intelligence
ALK: Anaplastic lymphoma kinase
BRAF: B-Rapidly accelerated fibrosarcoma
CIC: Capicua transcriptional repressor
EGFR: Epidermal growth factor receptor
FISH: Fluorescence in situ hybridization
HPV: Human papillomavirus
NGS: Next-Generation sequencing
NUTM1: NUT midline carcinoma family member 1

PCR: Polymerase Chain Reaction
RET: Rearranged during Transfection
ROS1: Receptor of Oncogenesis 1

AUTHOR CONTRIBUTIONS

SW: Conceptualizing, designing, drafting, and revising, final approval of the version to be published, ensuring that any issues related to the accuracy or completeness of any part of the work are properly investigated and resolved. The author is eligible for ICMJE authorship.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

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CONFLICTS OF INTEREST

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EDITORIAL/PEER REVIEW

To ensure the integrity and highest quality of CytoJournal publications, the review process of this manuscript was conducted under a **double-blind model** (authors are blinded for reviewers and vice versa) through an automatic online system.

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