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Editorial

Epigenetic biomarkers in diagnostic pathology: from analytical validity to diagnostic evidence

Vincenzo Fiorentino[1,2,*]

[1] Anatomic Pathology Unit, Department of Human Pathology in Adult and Developmental Age "Gaetano Barresi", University of Messina, 98125 Messina, Italy
[2] PhD Program in Translational Molecular Medicine and Surgery, Department of Biomedical, Dental, Morphological and Functional Imaging Sciences, University of Messina, 98125 Messina, Italy

* **Correspondence:** Email: vincenzo.fiorentino@unime.it.

Abstract: Epigenetic alterations are increasingly being evaluated as diagnostic biomarkers and risk-stratification tools in surgical pathology and cytopathology. The clinical implementation of epigenetic biomarkers requires more than the demonstration of disease-associated molecular changes. DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs may provide clinically relevant information, but their clinical value depends on reproducibility, specimen adequacy, analytical robustness, interpretability, and suitability for clinical reporting. These requirements are particularly relevant in cytopathology and molecular testing of body fluid specimens, where specimens may have low cellularity or suboptimal nucleic acid quality, and morphological interpretation may be equivocal. Urothelial carcinoma represents an informative model because urine-based methylation assays illustrate both the potential and the limitations of epigenetic translation. These assays have been explored as adjunctive tools to cytology in selected diagnostic and surveillance settings, without replacing guideline-based cystoscopic follow-up. This editorial introduces the Special Issue "Epigenetics in Diagnostic Pathology: Translational Biomarkers, Technologies, and Clinical Impact" and supports a translational approach grounded in diagnostic pathology, in which epigenetic biomarkers are evaluated according to their capacity to generate reliable diagnostic evidence in routine clinical specimens.

Keywords: epigenetics; diagnostic pathology; DNA methylation; biomarkers; liquid biopsy; molecular pathology; translational medicine

Preface

The implementation of epigenetic biomarkers in diagnostic pathology requires more than the identification of disease-associated molecular alterations. The central translational question is whether an epigenetic result can improve diagnostic interpretation, risk assessment, prognostic stratification, or patient management in a defined clinical setting.

Biomarker validation is often conducted in selected study cohorts, whereas diagnostic pathology is performed on routine clinical specimens: small biopsies, cytological preparations, cell blocks, urine samples, archival tissue, partially degraded material, specimens showing an inflammatory background, post-treatment changes, or morphologically equivocal features. A biomarker that performs well only in selected cohorts may be difficult to apply in diagnostic workflows. Conversely, a biomarker that remains informative in routine specimens may acquire clinical value when the analytical platform is appropriately matched to the diagnostic question.

Epigenetic mechanisms are relevant to diagnostic pathology because DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs provide information on gene regulation, cellular identity, differentiation state, and tumor adaptation. In cancer, epigenetic dysregulation may contribute to tumor initiation, clonal selection, immune escape, metastatic progression, and therapy resistance [1,2]. For diagnostic pathology, the practical relevance of these alterations lies in the possibility of measuring them in specimens already used in routine practice, including formalin-fixed paraffin-embedded tissue, cytological material, urine, plasma, and other body fluids [3,4].

Among epigenetic alterations, DNA methylation currently offers some of the most mature diagnostic applications. Its relative chemical stability, tumor- and lineage-associated patterns, and compatibility with limited amounts of DNA make it suitable for classification, surveillance, and risk stratification [3]. However, a methylation result is not a biological observation in isolation. It is the final output of a measurement process that includes specimen preservation, DNA extraction, bisulfite conversion or alternative methylation-sensitive approaches, amplification, sequencing or targeted detection, computational analysis, threshold definition, and clinical reporting. Each step may influence the reliability and interpretability of the final result [5,6].

At this interface, diagnostic epigenetics depends on an analytical, physicochemical, and computational measurement process: an epigenetic state must be converted into a stable, measurable, and interpretable signal. Methylation-specific PCR, digital PCR, targeted methylation panels, sequencing-based classifiers, and computational methylation profiles directly influence analytical validity, reproducibility, and clinical interpretability. For pathologists, the relevant issue is not only analytical sensitivity but the robustness of the entire process, from specimen preservation to a clinically reportable result.

The experience of central nervous system tumors illustrates the diagnostic value of this approach. DNA methylation profiling has shown that molecular classification can refine, support, or, in selected cases, revise histopathological interpretation when morphology and conventional molecular testing are insufficient [7]. This does not diminish the role of morphology. Rather, it confirms that morphology and molecular pattern recognition are most informative when interpreted in an integrated diagnostic framework.

A comparable principle is emerging in urinary tract pathology, where the diagnostic question is often clinically relevant and technically difficult. The Paris System provides a standardized framework for reporting urinary cytology, with emphasis on specimen adequacy and diagnostic categories

associated with the risk of high-grade urothelial carcinoma [8]. At the same time, urinary cytology may be limited by low cellularity, degeneration, inflammation, treatment-related atypia, or upper tract sampling difficulties. In this setting, urine-based DNA methylation assays provide a useful model for the broader evaluation of epigenetic biomarkers in pathology practice.

The clinical context remains essential. Current risk-adapted surveillance of non-muscle-invasive bladder cancer relies primarily on cystoscopy, with urinary cytology maintaining an important role, particularly in high-risk and very-high-risk disease [9]. Accordingly, methylation testing should be considered an adjunctive method rather than a substitute for cystoscopy, histology, or cytology. Its potential value lies in providing additional information that may reduce diagnostic uncertainty and support patient stratification within validated clinical pathways.

Urine-based DNA methylation assays have been evaluated for non-muscle-invasive bladder cancer surveillance and bladder cancer detection [10,11]. Upper tract high-grade urothelial carcinoma is a relevant example. In this setting, urinary cytology may be difficult, and the clinical consequences of false-negative findings in this diagnostic pathway may be significant. Comparative work on urine cytology and DNA methylation analysis in urinary samples suggested that methylation testing may add diagnostic information in patients with suspected upper tract high-grade urothelial carcinoma [12]. These data illustrate how a molecular measurement may address a defined diagnostic limitation.

The follow-up of non-muscle-invasive bladder carcinoma provides a second example, as repeated cystoscopic and cytological surveillance may be complicated by treatment-related changes, low tumor burden, or equivocal cytology. The combined use of cytology and urine-based methylation testing has been investigated in this setting, particularly in cases in which cytology alone may yield equivocal or difficult-to-classify findings [13]. The value of this approach does not depend on presenting molecular testing as superior to cytology, but on defining how the two methods may address different aspects of the same diagnostic problem. Cytology provides cellular context, including atypia, degeneration, inflammation, and tumor morphology. Methylation testing may provide a complementary molecular readout, particularly when morphological interpretation is limited by degeneration, treatment-related atypia, or suboptimal preservation, provided that sufficient analyzable material is available.

These considerations highlight a frequent point of failure in biomarker translation. It is relatively straightforward to show an association between a biomarker and a disease state. It is more difficult to define how the test should be used in the laboratory, how inadequate or borderline specimens should be managed, which threshold should trigger clinical action, and how the result should be communicated in the diagnostic report. A positive molecular result without appropriate clinical and morphological correlation may be difficult to interpret and may prompt unnecessary investigations. A negative result obtained from a specimen with insufficient cellularity or suboptimal nucleic acid quality may lead to false reassurance. An invalid result should be considered part of the assay performance profile, not only a laboratory processing issue.

For this reason, the analysis of invalid or non-informative results is central to clinical implementation. Work on methylation analysis of urinary samples in non-muscle-invasive bladder carcinoma has specifically addressed the frequency and management of invalid results [14]. Analytical validation studies of this type are essential for routine diagnostic implementation. A test with a high rate of invalid, indeterminate, or non-informative results may be difficult to implement even when its biological rationale is strong. This is especially true when adequacy criteria and clinical pathways for repeat testing are not defined.

Epigenetic biomarkers should therefore be evaluated according to the same principles expected

for any diagnostic or prognostic test. The clinical question must be precise. A biomarker intended for early detection, recurrence surveillance, tumor classification, prognostic stratification, or therapy prediction cannot be judged by the same criteria. For each assay, analytical validity, clinical validity, and clinical utility should be distinguished explicitly. A technically reproducible methylation signal does not automatically imply diagnostic usefulness, and diagnostic association does not necessarily imply clinical utility. Study design, patient selection, assay reproducibility, pre-analytical variables, thresholds, failure rates, and reporting language all matter. Guidelines for diagnostic accuracy studies and tumor marker research have emphasized transparency, validation, and clinically interpretable reporting [15,16]. Epigenetic biomarkers should meet comparable standards.

Epigenetics is not limited to DNA methylation in body fluids. Histone modifications, chromatin regulators, and non-coding RNAs may also provide diagnostic, prognostic, and therapeutic information across tumor types [17]. However, the clinical value of any molecular alteration depends not only on its detectability but also on its ability to inform a defined clinical question. A molecular alteration becomes clinically meaningful when it can be linked to a diagnostic category, a risk group, a therapeutic option, or a follow-up strategy. Without this link, even a technically robust assay may remain a research observation; with this link, an epigenetic result may contribute to diagnostic reasoning. The same principle applies to computational pathology, which may support the integration of methylation profiles, digital morphology, imaging, and clinical variables [18]. For diagnostic use, however, predictive models should provide interpretable outputs, technical reproducibility, and biological plausibility, rather than probability scores that lack sufficient diagnostic context.

Clinical implementation of epigenetic biomarkers requires predefined specimen adequacy criteria, reproducible analytical platforms, validated performance characteristics, and clinically interpretable reporting. Most importantly, the result must influence clinical management or risk assessment in a way that justifies its use.

This Special Issue is intended to address this translational pathway. Relevant contributions may include studies on DNA methylation, histone modifications, chromatin remodeling, non-coding RNAs, epigenetic therapies, liquid biopsy, cytopathology, computational pathology, and multi-omics integration. Particularly valuable will be contributions connected to diagnostic practice: biomarkers that support interpretation in morphologically equivocal cases, assays that work in limited or degraded material, methods that may refine surveillance algorithms after adequate validation, and models that provide information suitable for clinical decision-making.

Epigenetic research has expanded molecular pathology beyond sequence alterations alone. Its clinical value in pathology will depend on how clearly epigenetic measurements can be connected to diagnostic categories, risk stratification, treatment selection, and follow-up strategies. That connection requires collaboration among pathologists, molecular biologists, clinicians, bioinformaticians, and laboratory professionals.

A central task for diagnostic pathology is to define the analytical and clinical criteria by which an epigenetic measurement can be considered sufficiently robust, interpretable, and clinically relevant for inclusion in the diagnostic report.

Use of generative-AI tools declaration

The author used ChatGPT, OpenAI, solely for language editing, grammar checking, and improvement of English readability. The tool was not used to generate scientific content or formulate

conclusions. The author reviewed and approved all AI-assisted edits and takes full responsibility for the final content of the article.

Conflict of interest

Vincenzo Fiorentino is the guest editor of special issue "Epigenetics in Diagnostic Pathology: Translational Biomarkers, Technologies, and Clinical Impact" for AIMS Biophysics and was not involved in the editorial review or the decision to publish this article. The author declares no conflict of interest.

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