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Molecular Profiling of Isolated Tumour Cells in Colorectal Cancer: Prognostic and Predictive Value

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Abstract

Colorectal cancer remains a leading cause of cancer-related morbidity and mortality worldwide, and accurate staging is critical for guiding treatment decisions. Lymph node involvement serves as one of the most decisive prognostic indicators, distinguishing stage II from stage III disease and influencing the use of adjuvant chemotherapy. Within this framework, the classification of metastatic deposits in lymph nodes has been refined into macrometastases, micrometastases, and isolated tumour cells (ITCs). While macrometastases and micrometastases have well-established clinical implications, the biological and prognostic relevance of ITCs remains an area of uncertainty. Conventional histopathological examination and immunohistochemistry have been unable to consistently demonstrate that ITCs influence survival or recurrence in colorectal cancer. However, molecular profiling technologies, including genomic sequencing, transcriptomic analysis, epigenetic studies, and single-cell approaches, offer a new opportunity to evaluate whether ITCs are biologically inert or represent seeds of metastatic potential. This paper provides a comprehensive literature-based review of ITCs in colorectal cancer, with particular emphasis on the application of molecular profiling techniques to clarify their prognostic and predictive significance. The discussion extends to emerging technologies such as artificial intelligence, digital pathology, and liquid biopsy, which may integrate with ITC profiling in the future. Finally, challenges, limitations, and future research directions are outlined. Molecularly characterizing ITCs may transform their current ambiguous clinical status into a defined biomarker guiding staging, treatment, and surveillance in colorectal cancer.

Keywords: *Colorectal cancer, isolated tumour cells, micrometastases, lymph node staging, molecular profiling, genomic alterations, transcriptomics, epigenetics, single-cell sequencing, immunohistochemistry, prognostic biomarkers*

Introduction

Colorectal cancer (CRC) is the third most common malignancy worldwide and remains a major cause of cancer-related deaths despite advances in screening, surgical management, and systemic therapy (Yang et al., 2023). Prognosis and therapeutic planning in CRC depend heavily on tumour staging, particularly nodal status. The presence or absence of lymph node metastasis divides stage II from stage III disease in the American Joint Committee on Cancer (AJCC) staging system, with direct implications for the recommendation of adjuvant chemotherapy. Patients with stage III disease derive a well-established survival benefit from systemic therapy, whereas the value of chemotherapy in stage II disease is less clear and typically restricted to patients with high-risk features (Mukherji et al., 2022). Consequently, accurate detection of lymph node metastases is essential for both staging and patient management.

Histopathological assessment of resected lymph nodes is the current gold standard for detecting metastases. Traditionally, metastases are classified by size, with macrometastases defined as deposits larger than 2.0 mm, micrometastases as deposits between 0.2 and 2.0 mm, and isolated tumour cells as single cells or clusters smaller than 0.2 mm (Zhao et al., 2024). While macrometastases are easily

identified on routine haematoxylin and eosin (H&E) sections, micrometastases and ITCs may require immunohistochemistry (IHC) or deeper sectioning for detection. Importantly, micrometastases have been associated with increased risk of recurrence and adverse outcomes in multiple studies (Yang et al., 2023), whereas ITCs have not consistently demonstrated prognostic significance. As a result, major guidelines generally classify patients with ITCs as node-negative, recording them as pN0(i+) (Mukherji et al., 2022).

Despite this conservative classification, ITCs remain a subject of debate. Some studies suggest that ITCs may contribute to systemic recurrence (Kong et al., 2017), while others argue they are clinically irrelevant artefacts of dissemination with limited metastatic potential. This controversy raises the question of whether morphology alone is sufficient to characterize ITCs or whether advanced molecular techniques are required to uncover their true biological properties. With the advent of next-generation sequencing, transcriptomics, epigenetic profiling, and single-cell methodologies, it is now possible to analyze rare cell populations such as ITCs at unprecedented resolution (Zhao et al., 2024). Such approaches may reveal whether ITCs are biologically inert remnants of tumour shedding or whether they represent viable precursors to metastasis.

This paper explores the emerging field of molecular profiling of ITCs in colorectal cancer. It reviews existing evidence on the genomic, transcriptomic, and epigenetic features of ITCs, discusses their potential role in prognostication and therapy prediction, and considers how novel technologies may enhance their detection and interpretation. By critically examining the literature, this paper aims to clarify the potential of ITCs as clinically meaningful biomarkers and to chart a course for future research in this evolving field (Teng et al., 2024).

Isolated Tumour Cells in Colorectal Cancer: Clinical and Biological Context

The classification of ITCs as separate from micrometastases emerged from the need to distinguish truly minimal tumour cell deposits from clusters with more established metastatic behavior. ITCs are typically detected only by immunohistochemistry, often using pan-cytokeratin antibodies, and are not usually visible on routine H&E examination (Mukherji et al., 2022). In colorectal cancer, reported detection rates of ITCs vary widely, largely depending on the number of sections examined and the use of IHC.

The clinical significance of ITCs remains controversial. Early studies suggested that their presence did not correlate with recurrence or survival, leading to the decision to classify patients with ITCs as node-negative in both North American and European guidelines (Yang et al., 2023). However, subsequent studies have reported conflicting findings, with some suggesting an association between ITCs and adverse outcomes (Kong et al., 2017). This inconsistency may reflect underlying biological heterogeneity within ITCs themselves. Some ITCs may represent biologically quiescent cells incapable of establishing metastases, while others may possess genetic and molecular features conferring metastatic potential (Zhao et al., 2024). Morphology alone cannot resolve this heterogeneity, and molecular profiling has emerged as a critical tool to address this uncertainty.

Molecular Profiling of Isolated Tumour Cells

Molecular profiling refers to the use of genomic, transcriptomic, epigenetic, and proteomic analyses to characterize tumour cells at a deeper biological level than morphology or immunohistochemistry can provide. In the context of ITCs, molecular profiling aims to answer two key questions: first, whether ITCs are clonally related to the primary tumour and capable of metastatic spread; and second, whether ITCs

carry specific molecular alterations that predict recurrence or treatment response (Yang et al., 2023; Kong et al., 2017).

Genomic studies have demonstrated that ITCs often share key driver mutations with the primary tumour, including alterations in APC, KRAS, TP53, and BRAF. The presence of such mutations indicates that ITCs are not benign artefacts but true tumour cells disseminated to the lymph nodes (Zhao et al., 2024). However, the detection of shared mutations does not necessarily establish metastatic potential, as many tumour cells may disseminate but fail to survive or proliferate.

Transcriptomic profiling offers additional insights. Expression studies suggest that ITCs may exhibit gene expression patterns associated with stemness, epithelial-to-mesenchymal transition (EMT), and immune evasion. Markers such as CD44 and ALDH1 have been detected in ITCs, implicating a stem-like phenotype with potential for metastatic colonization (Mukherji et al., 2022). EMT-related genes, which facilitate invasion and migration, have also been observed. Importantly, transcriptomic heterogeneity among ITCs suggests that only a subset may be biologically aggressive, potentially explaining the inconsistent prognostic impact of ITCs across studies (Yang et al., 2023).

Epigenetic profiling, including DNA methylation and microRNA expression, has provided further evidence of biological activity. Aberrant methylation of tumour suppressor genes and deregulated expression of microRNAs such as miR-21 and miR-200 have been associated with ITCs, supporting their role in tumour progression (Kong et al., 2017). MicroRNAs are particularly relevant as they can modulate EMT, apoptosis resistance, and immune interactions.

At the protein level, ITCs have been shown to express molecules that enable immune evasion, such as PD-L1. This finding aligns with the hypothesis that ITCs persist in lymph nodes by escaping immune clearance, potentially serving as dormant reservoirs capable of later reactivation (Teng et al., 2024).

Prognostic and Predictive Value of ITCs

The prognostic value of ITCs in colorectal cancer remains unresolved. Some studies have shown that ITCs correlate with increased risk of recurrence, particularly in stage II patients who otherwise appear to have favourable prognoses (Yang et al., 2023). However, other studies have failed to replicate these findings, leading to the prevailing classification of ITCs as clinically insignificant (Mukherji et al., 2022).

Molecular profiling has the potential to clarify this ambiguity. By identifying high-risk molecular subtypes of ITCs, researchers may be able to distinguish aggressive deposits from biologically inert ones (Zhao et al., 2024). For instance, ITCs harbouring mutations in KRAS or BRAF, or exhibiting stemness and EMT gene expression, may have higher metastatic potential. Similarly, ITCs within microsatellite instability-high (MSI-H) tumours may display distinct immune interactions, raising questions about their responsiveness to immunotherapy (Kong et al., 2017).

From a predictive standpoint, ITCs may carry actionable mutations relevant to therapy selection. The detection of KRAS or NRAS mutations in ITCs could indicate resistance to anti-EGFR therapy, while MSI-H status may suggest benefit from immune checkpoint inhibitors (Yang et al., 2023). Incorporating ITC molecular profiles into clinical decision-making could therefore enhance personalized therapy strategies.

Emerging Technologies

Recent technological advances are reshaping how ITCs can be studied and integrated into clinical practice. Single-cell sequencing enables the analysis

of ITCs at unprecedented resolution, capturing heterogeneity that bulk sequencing cannot reveal (Zhao et al., 2024). This technology allows for the identification of rare subclones with metastatic or drug-resistant phenotypes.

Digital pathology and artificial intelligence offer another avenue for ITC detection. AI algorithms trained on large datasets can identify occult tumour cells on digital slides more consistently than human pathologists. Coupling AI detection with molecular profiling could standardize ITC identification and stratification across institutions (Mukherji et al., 2022).

Liquid biopsy represents a complementary approach, as circulating tumour DNA and circulating tumour cells may reflect the biology of ITCs. Correlating ITCs with liquid biopsy findings could enable non-invasive monitoring of minimal residual disease and recurrence risk (Kong et al., 2017).

Challenges and Limitations

Despite the promise of molecular profiling, significant challenges remain. Isolating ITCs for molecular analysis is technically demanding due to their rarity. Methods such as laser capture microdissection or flow cytometry require specialized expertise and equipment. Furthermore, the cost of single-cell sequencing and advanced profiling may limit feasibility in routine practice (Teng et al., 2024).

Interpretive challenges also exist. Not all molecular alterations are clinically actionable, and distinguishing prognostically relevant ITCs from biologically inert ones requires large-scale validation. Ethical considerations must also be addressed, as patients may be exposed to overtreatment if molecularly aggressive ITCs are identified but never manifest clinically significant disease (Yang et al., 2023).

Future Directions

Future research must focus on large, multicenter studies integrating molecular profiling of ITCs with long-term clinical outcomes. Such studies will clarify whether ITCs should remain classified as node-negative or whether a subset warrants reclassification as node-positive based on molecular risk factors. Development of cost-effective, standardized workflows for ITC isolation and analysis will also be essential for clinical translation. Integration of molecular profiling with AI-based pathology and liquid biopsy could provide a comprehensive framework for risk stratification and surveillance (Zhao et al., 2024; Mukherji et al., 2022).

Conclusion

Isolated tumour cells represent one of the last unresolved questions in colorectal cancer staging. While histopathology has consistently failed to demonstrate their prognostic significance, molecular profiling offers the possibility of uncovering their true biological potential. Genomic, transcriptomic, epigenetic, and proteomic studies suggest that ITCs may harbour aggressive features, including driver mutations, stemness, EMT-related expression, and immune evasion markers. However, not all ITCs are biologically equal, and their clinical relevance likely depends on molecular context. By distinguishing inert from aggressive ITCs, molecular profiling could refine staging systems, inform adjuvant therapy decisions, and personalize surveillance strategies. The integration of ITC profiling with emerging technologies such as single-cell sequencing, artificial intelligence, and liquid biopsy heralds a new era in the understanding of

minimal residual disease in colorectal cancer. Ultimately, molecular profiling may transform ITCs from a pathologic curiosity into a clinically meaningful biomarker guiding precision oncology.

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