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Review

Circulating tumour cells and circulating nucleic acids as a measure of tumour dissemination in non-metastatic colorectal cancer surgery

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Abstract

There is accumulating evidence for circulating tumour cells (CTCs) and circulating tumour nucleic acids (ctNAs) as prognostic and predictive biomarkers in colorectal cancer. Their role in the perioperative setting is evolving. These blood-borne biomarkers can potentially demonstrate tumour dissemination at time of colorectal cancer surgery and estimate the completeness of a surgical resection. CTCs and circulating ctNA levels at time of surgery, and persistent levels post-surgery, may correlate with poorer patient outcomes. These biomarkers can be utilised to refine surgical techniques to minimise tumour dissemination and determine the need for adjuvant therapy.

Keywords: Circulating tumour cells; Tumour dissemination; Colorectal cancer; Surgery

Circulating tumour cells and circulating free tumour nucleic acids

The ability to evaluate circulating tumour cells (CTCs) or circulating free tumour nucleic acids (ctNA) in blood is a highly attractive way to detect micrometastatic disease and to guide management and inform prognosis.[1] These cells are thought to be released from the primary tumour, and have the potential to form metastases. There is increasing evidence for the utility of these blood-borne biomarkers in colorectal cancer (CRC), with most studies confirming the correlation between high CTC counts or ctNA and poorer prognosis.[2]

CTC isolation techniques vary, but the best documented platforms involve immunomagnetic separation of cancer cells, which are typically nucleated, epithelial cell adhesion molecule (EpCAM) positive, cytokeratin (CK) positive and CD45 (leucocyte marker) negative.[3] CellSearch™ (Veridex LLC, NJ, USA) is the most widely used CTC enumeration platform utilising this approach, approved for clinical use by the Food and Drug Administration (FDA) in the United States. However, CTCs are thought to undergo the epithelial to mesenchymal transition (EMT), in which epithelial traits are lost in favour of mesenchymal traits. EMT is thought to be responsible for a tumour's invasive capabilities and plasticity.[4] Hence EpCAM-based antibody markers may not capture the entire spectrum of CTCs. Other methods used to isolate CTCs utilise the differential

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properties of CTCs such as their size or flow characteristics.[3]

Alternatively, detection of circulating free tumour nucleic acids has been used as a surrogate measure of the presence of tumour cells circulating in the bloodstream. This mainly involves the measurement of tumour mRNA using reverse transcription polymerase chain reactions (RT-PCR).[5] Detection of tumour-specific mutational changes, methylation patterns and ctDNA fragment sizes have also been used as surrogate measures.[6–8] It is not possible to identify the source of these ctNA with any certainty, even if they are markers of malignant potential and aggressiveness. Assays of ctNA also do not permit analysis at the single cell level. Conversely, the molecular analysis of individual CTCs is possible after isolation and holds great promise in providing a real-time profile of the evolving tumour genotype in disease that is destined to become metastatic and undergo further therapies.[9,10]

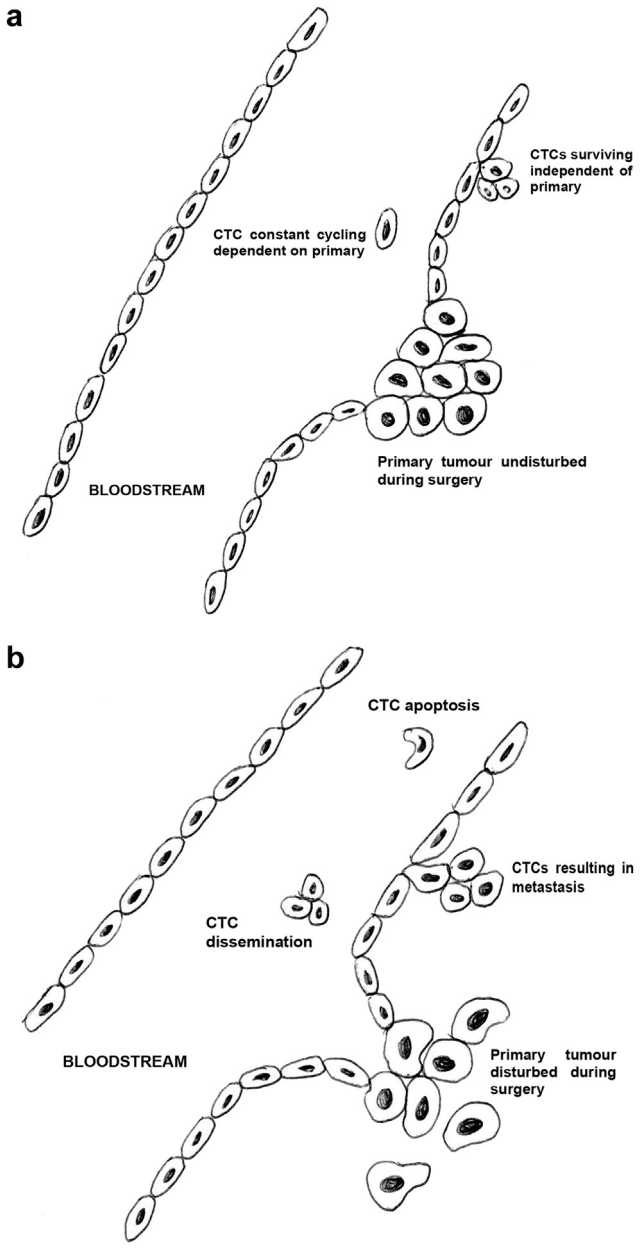

Figure 1. Schematic illustrating circulating tumour cells (CTCs) in the bloodstream. **a**. With a primary tumour that is undisturbed during surgical resection, CTCs are released and cycle through the circulation and eventually apoptose, dependent on the primary tumour to replenish CTC levels in order for equilibrium to be achieved. Some CTCs develop survival mechanisms and are viable, independent of the primary. **b**. With tumour disturbance during surgical resection, higher levels of CTCs disseminate, which eventually apoptose or develop into a viable metastasis.

Utility of CTCs and ctNAs in the perioperative setting

The principles for curative surgery include en bloc resection, no-touch technique, high ligation of vessels and systematic lymph node dissection.[11] At the time of surgery, the detection of CTCs and ctNA for CRC is thought to signify the presence of disseminated micrometastatic cells, and could therefore serve as potential biomarkers (Fig. 1). Fig. 2 illustrates the possible scenarios of CTCs at time of surgical intervention. Theoretically, CTCs must pass through the liver prior to systemic circulation; hence the presence of CTCs in tumour drainage vessels, which can be readily accessed at time of surgery, would be expected to be higher than in peripheral blood, and sampling of these vessels could potentially increase the sensitivity of CTC detection.

The ability to monitor the dynamics of these circulating biomarkers therefore, can potentially help in determining the extent of tumour cell dissemination, assess a complete microscopic 'R0' curative resection, and inform outcome and the need for adjuvant therapy. Further, these biomarkers may be utilised to compare the effectiveness of different surgical techniques such as open versus laparoscopic approaches.

CTCs and ctNA dissemination in non-metastatic surgery

Potential mRNA biomarkers such as cytokeratin 19 and 20 (CK19, CK20), carcinoembryonic antigen (CEA), human telomerase reverse transcriptase (hTERT) and guanylyl cyclase (GCC) have been extensively investigated in the perioperative setting in CRC patients (Table 1). Cancer-specific mutant ctDNA, such as *KRAS* mutations, and p16 promoter methylation have also been investigated in this setting.[12–14] In general, the data are conflicting, with some studies demonstrating tumour cell dissemination and some demonstrating differences in these circulating biomarkers with different surgical techniques. A prospective study of 42 patients undergoing curative surgery for CRC with either open or laparoscopic surgery showed increased CEA mRNA, as measured by quantitative RT-PCR in the inferior mesenteric vein blood measured immediately after tumour mobilisation.[15] Peripheral blood measured on skin closure showed decreased detection of both CK20 and