



# Radiation Biology and Circulating Tumor Cells

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The metastatic process is multifactorial, and the biological mechanisms and microenvironmental factors that drive tumor cells to leave the primary site and colonize distant secondary sites are areas of active research. An excellent 2017 review, “Emerging biological principles of metastasis,” describes the known biological programs that promote the metastatic dissemination of cancer cells (1). Metastatic cells acquire genetic and epigenetic alterations and phenotypic changes that promote invasion into circulatory systems, a process described as epithelial-mesenchymal transition (2). Single tumor cells or multicellular aggregates derived from the primary site enter the circulation and are capable of maneuvering through capillaries to seed metastatic tumor colonies (3). The question of whether the mobilization of circulating tumor cells (CTCs) during, and as a result of, cancer therapy can be a cause of metastasis was recently discussed by Martin et al (4) from the Peter MacCallum Cancer Centre.

A number of isolation and identification methods have been developed for CTCs; however, further standardization is needed (5). Currently, only the CellSearch system (6) has been approved by the Food and Drug Administration for clinical use. Although CTCs are rare, enumerating CTCs to assess the metastatic potential or tumor aggressiveness is clinically appealing because it is less invasive than conventional tissue biopsy (7, 8), and its feasibility allows for the monitoring of treatment response using longitudinal assessments. Despite technical concerns, the study of CTCs is providing new insights into cancer progression. In this Oncology Scan, we consider 4 studies addressing the mechanisms of cancer metastasis that focus on CTCs and also an alternate approach using circulating tumor–released DNA.

**Giannou et al. *NRAS* destines tumor cells to the lungs. *EMBO Mol Med* 2017. (9)**

**Summary:** The study by Giannou et al (9) considered the mechanisms of cancer cell mobilization from the primary

tumor to the target organs and the development of metastases. They investigated the hypothesis that distinct classes of genes drive subpopulations of cells with distinct organ-specific tropisms (9). The investigators studied the role of *NRAS* mutations in the development of lung metastasis from subcutaneous tumors grown in mice. Using sophisticated molecular biology methods and *Cxcr1*- and *Cxcr2*-deficient transgenic mice, the investigators reported that mutant or overexpressed *NRAS* is required for tumor cells of various tissues of origin to metastasize to the lungs (9). They demonstrated that this is not due to the growth potential derived from *NRAS* but by tumor-secreted interleukin-8–related chemokines that signal to cognate CXCR1 receptors on pulmonary endothelium and CXCR2-expressing myeloid cells to facilitate pulmonary homing of CTCs.

**Comment:** Although their study was preclinical and used mouse models of spontaneous lung metastasis to investigate the pulmonary homing of CTCs, the data suggest that a clinical strategy using targeted chemotherapeutics to inhibit chemokine receptor signaling might prevent lung metastasis in various tumor types that express mutant or overexpressed *NRAS*. This statement is supported in their report by proof-of-principle experiments that showed the CXCR1/2 antagonist navarixin prevented the initiation of lung tumor colonies by circulating *NRAS* mutant tumor cells, providing further evidence in support of therapeutically targeting CXCR1/2 (10). These data support the hypothesis that a single oncogene can cause lung metastasis, because *NRAS* mutations/gain are common in a range of cancers that commonly home to the lungs.

**Adams et al. Sequential tracking of PD-L1 expression and RAD50 induction in circulating tumor and stromal cells of lung cancer patients undergoing radiotherapy. *Clin Cancer Res* 2017. (11)**

**Summary:** The tracking of CTCs to provide prognostic assessments of disease is becoming established. Adams

et al (11) have extended this concept by examining the role of cancer-associated circulating stromal cells, most notably cancer-associated macrophage-like cells, because these cells are associated with tumor cell invasion. In their study, the investigators used the DNA double-strand break repair protein Rad50 as a marker of radiation damage to identify circulating cells originating from the irradiated tumor. They combined this with an assessment of programmed cell death ligand 1 expression to investigate the role of immunomodulation in metastasis in CTCs, CTC subtypes undergoing epithelial-mesenchymal transition, and tumor-associated macrophages. Circulating cells were assayed before and after therapy, and compared with the findings from primary tumor biopsy specimens from 41 lung cancer patients undergoing radiation therapy. The data indicated that the circulating cancer-associated macrophage-like cells did originate from the primary lung tumors.

**Comment:** The study by Adams et al (11) has demonstrated the potential of dynamically tracking biomarkers of treatment response for both tumor and stromal cells using blood-based assays. The novelty of their study was the use of RAD50 foci formation to quantifiably track clear radiobiological changes in lung tumors, and the possibility of tracking the radiation response of tumors during therapy to complement other biological assays to monitor the treatment response (12). Although their study needs further validation, it has demonstrated that the use of repeated liquid biopsies compared with repeated tumor biopsies offers the potential for a more practical and less invasive technique to clinically investigate the mechanisms of cell tumor metastasis and assess the clinical benefit of programmed cell death ligand 1/programmed cell death 1 therapies.

**Kalinich et al. An RNA-based signature enables high specificity detection of circulating tumor cells in hepatocellular carcinoma. *Proc Natl Acad Sci U S A* 2017. (13)**

**Summary:** In an elegant series of experiments, Kalinich et al (13) reported that RNA quantitation can provide a sensitive and specific CTC assessment for the early detection of hepatocellular carcinoma (HCC), although large-scale validation is still required. The innovation in their study was the use of a high-throughput microfluidic CTC-iChip to deplete hematopoietic cells from the blood, thereby enriching for CTCs and maintaining the integrity of CTC RNA (13). This allowed for a high-throughput, tissue lineage-specific digital polymerase chain reaction (PCR) assay. They described the identification and validation of 10 liver-specific transcripts that were used to identify HCC-derived CTCs in more than one-half of untreated patients with HCC but in only 3% of patients with nonmalignant liver disease at risk of developing HCC. Positive CTC scores declined in the treated HCC patients. This PCR-

based approach overcomes many of the specific limitations of CTC quantification that use antibodies and microscopic validation, which can be extremely time consuming and variable. Importantly, the PCR approach proved significantly more sensitive than the serum alpha-fetoprotein measurements frequently used to monitor responses. The RNA lineage-based analysis approach also overcomes the limitation of circulating tumor-derived DNA (ctDNA)-based genotyping, which often lacks information on its tissue of origin. The investigators concluded that this approach has considerable promise for the early detection of different cancer types, providing tissue-specific transcripts can be identified.

**Comment:** A number of methods have been developed to assess and characterize CTCs in different cancer types to define the metastatic potential (7, 14-16). Kalinich et al (13) have described for the first time a viable and high-throughput screening method for early detection and monitoring of treatment response for HCC using CTCs. Moreover, this concept overcomes the limitations of time-consuming antibody staining and microscopic scoring to discount contaminating white blood cells, such as those required for other CTC assessment techniques.

**Abbosh et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. *Nature* 2017. (17)**

**Summary:** Abbosh et al (17) demonstrated the use of ctDNA rather than CTCs as a predictor of recurrence. Similar to CTCs, ctDNA has proved useful for detecting and monitoring cancer progression from plasma samples (18). The aim of their large multicenter collaborative study was to use a tumor-specific phylogenetic approach to profile plasma-borne ctDNA from early-stage non-small cell lung cancer (NSCLC) patients recruited to the TRACERx clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01888601) identifier NCT01888601) with the aim of identifying NSCLC relapse (17). The experimental approach was to track clonal and subclonal single-nucleotide variants, in pre- and postoperative samples, to investigate the clonal/subclonal fidelity of ctDNA. The study identified independent predictors of ctDNA release and demonstrated a correlation between the computed tomography-defined tumor volume and the mean clonal plasma variant allele frequency of single-nucleotide variants in ctDNA-positive NSCLC cases, as defined using a patient-specific multiplex-PCR next-generation sequencing approach. Longitudinal profiling demonstrated that ctDNA was also associated with tumor relapsed and even implicated a driving mutation in relapse disease in 1 case. The multiplex-PCR next-generation sequencing platform demonstrated high sensitivity. However, the investigators indicated the current technology limits detection to tumors sized  $\geq 10$  cm<sup>3</sup>, although they predicted that the resolution will improve as the technology evolves.

**Comment:** The study by Abbosh et al (17) has advanced our understanding of how tumors respond to therapy and how longitudinal studies of liquid biopsies can be used to monitor treatment response. Their study also identified independent predictors of ctDNA release. It has further established the feasibility reported by others (12) of using ctDNA as a surrogate for tumor recurrence and provided a platform for identifying “druggable” biological features of recurrent disease (17).

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