REVIEW ARTICLES

Current approaches for avoiding the limitations of circulating tumor cells detection methods—implications for diagnosis and treatment of patients with solid tumors

ARTUR KOWALIK, MAGDALENA KOWALEWSKA, and STANISŁAW GÓŹDŹ

KIELCE AND WARSZAWA, POLAND

Eight million people die of cancer each year and 90% of deaths are caused by systemic disease. Circulating tumor cells (CTCs) contribute to the formation of metastases and thus are the subject of extensive research and an abiding interest to biotechnology and pharmaceutical companies. Recent technological advances have resulted in greatly improved CTC detection, enumeration, expansion, and culture methods. However, despite the fact that nearly 150 years have passed since the first detection and description of CTCs in human blood and enormous technological progress that has taken place in this field, especially within the last decade, few CTC detection methods have been approved for routine clinical use. This reflects the substantial methodological problems related to the nature of these cells, their heterogeneity, and diverse metastatic potential. Here, we provide an overview of CTC phenotypes, including the plasticity of CTCs and the relevance of inflammation and cell fusion phenomena for CTC biology. We also review the literature on CTC detection methodology–its recent improvements, clinical significance, and efforts of its clinical application in cancer patients management. At present, CTC detection remains a challenging diagnostic approach as a result of numerous current methodological limitations. This is especially problematic during the early stages of the disease due to the small numbers of CTCs released into the blood of cancer patients. Nonetheless, the rapid development of novel techniques of CTC detection and enumeration in peripheral blood is expected to expedite their implementation in the clinical setting. It is of utmost importance to understand the biology of CTCs and their distinct populations as a prerequisite for achieving this ultimate goal. (Translational Research 2017;185:58–84)

From the Department of Molecular Diagnostics, Holycross Cancer Center, Kielce, Poland; Department of Surgery and Surgical Nursing with the Scientific Research Laboratory, The Faculty of Health Sciences of the Jan Kochanowski University in Kielce, Kielce, Poland; Department of Molecular and Translational Oncology, Maria Sklodowska-Curie Institute - Oncology Center, Warszawa, Poland; Department of Immunology, Biochemistry and Nutrition, Medical University of Warsaw, Warszawa, Poland; Department of Clinical Oncology, Hollycross Cancer Center, Kielce, Poland; Department of Prevention and Cancer Epidemiology, Faculty of Health Sciences of the Jan Kochanowski University in Kielce, Kielce, Poland.

Submitted for publication November 2, 2016; revision submitted March 24, 2017; accepted for publication April 19, 2017.

Reprint requests: Artur Kowalik, Department of Molecular Diagnostics, Holycross Cancer Center, Artwinskiego 3, 25-734 Kielce, Poland; e-mail: arturko@onkol.kielce.pl.

1931-5244

http://dx.doi.org/10.1016/j.trsl.2017.04.002

Abbreviations: aCGH = array-comparative genomic hybridization; AR = androgen receptor; AR-V7 = AR splice variant 7 messenger RNA; ASGR1 = asialoglycoprotein receptor; BMDC = bone marrow–derived cells; CDH1 = Cadherin 1; CDH2 = Cadherin 2, type 1, N-cadherin (neuronal); cfDNA = cell-free DNA; COPD = chronic obstructive pulmonary disease; ctDNA = circulating tumor DNA; CTC = circulating tumor cells; DEP = dielectrophoresis; DTC = disseminated tumor cells; ECM = extracellular matrix; EMT = epithelial-mesenchymal transition; EpCAM = epithelial cell adhesion molecule; EPISPOT = EPithelial ImmunoSPOT method; FAST = fiber-optic array scanning technology; FISH = fluorescent in situ hybridization; FN1 = Fibronectin 1; HCC = hepatocellular cancer; ICAM1 = intercellular adhesion molecule; KRT = Keratin; mCRPC = metastatic castration–resistant prostate cancer; MET = mesenchymal-to-epithelial transition; M30 = neoepitope in cytokeratin 18 produced following caspase cleavage during apoptosis; NGS = next-generation sequencing; NSCLC = non-small-cell lung carcinoma; OS = overall survival; PCR = polymerase chain reaction; PD-L1 = programmed death–ligand 1; PFS = progression-free survival; PSA = prostate-specific antigen; PSMA = prostate-specific membrane antigen; qRT-PCR = real-time RT-PCR; RT-PCR = reverse transcription-PCR; SERPINE1/PAI1 = Serpin Peptidase Inhibitor, clade E (Nexin, Plasminogen Activator Inhibitor Type 1), member 1; TWIST = Twist-related protein 1, also known as TWIST1; VCAM1 = vascular cell adhesion molecule 1

INTRODUCTION

Eight million people die from cancer each year[1] and it is predicted that 13.2 million patients will do so in 2030.[2] 90% of deaths are caused by systemic disease.[1] Systemic cancer spread means the formation of metastases, that is, secondary tumors arising from cells originating from the primary tumor. Thus, metastatic cancer cells are the subject of numerous studies and are an abiding interest to pharmaceutical and biotechnology companies. These cells include circulating tumor cells (CTC) in the bloodstream and disseminated tumor cells in the bone marrow. CTC are easier to analyze on a regular basis than disseminated tumor cells which require bone marrow aspiration—an invasive and painful procedure for patients with solid tumors.[3]

The evolution of tumor cells proceeds via multiclonal expansion which causes the tumor to be composed of multiple cell subpopulations. As a result of the accumulation of up to 6 mutations,[4,5] some cells acquire the ability to metastasize. The metastasis process consists of several sequential steps: local invasion of the primary tumor cells, intravasation, extravasation, and the establishment of distant metastasis.[6] A simplified scheme of the metastatic cascade is presented in Fig 1.

During the phase of initial local invasion, substantial changes in the morphology of tumor cells occur. In the single-cell invasion pathway, epithelial cells undergo epithelial-mesenchymal transition (EMT), that is, the loss of epithelial characteristics and gain of mesenchymal characteristics.[6] EMT confers a migratory capacity and thus has been recognized as a crucial event in progression of several carcinoma types such as breast, colorectal, lung, and pancreatic cancers, as well as prostate and hepatocellular carcinomas.[7] As a result of EMT, the cube-shaped epithelial cells lose their connections with adjacent cells and are transformed into the spindle-shaped mesenchymal cells that are able to migrate and facilitate the local invasion by digesting the basement membrane and extracellular matrix.[8] Individual cells may also detach from cell clusters by ameboid migration in a collective-to-ameboid transition phenomenon or can undergo mesenchymal-ameboid transition.[7] Recent evidence suggest that in several types of sarcomas the mesenchymal-to-epithelial transition (MET) may occur.[9] Certain cancer types disseminate as single cells, while others–such as oral squamous cell carcinoma, colorectal carcinoma, melanoma, breast cancer, endometrial carcinoma, and pancreatic cancer–do so by collective cell migration.[7] Collectively migrating cells form multicellular sheets, strands, or clusters detached from primary tumors and maintain intact cell–cell junctions in deeper regions. Under certain circumstances, loss of cell junctions may be followed by single-cell detachment. Tumor cells can apparently "switch" between these various types of movement which reflects their plasticity.[6]

Accumulating data indicate the importance of the tumor microenvironment in cancer progression, which may either block or promote the development of cancer.[10] Both local (within the tumor) and systemic inflammation, which accompany cancer, may contribute to cancer progression.[11] The colonization and subsequent growth may also depend on the new site environment. Recently, increasing attention has been paid toward the role of inflammation in cancer treatment and improving the cancer patient quality of life by using anti-inflammatory agents for cancer-associated symptoms.[12] Another layer of complexity in the interplay between neoplastic cells and their microenvironment arises from inflammation being also able to stimulate and facilitate fusions of immune cells with tumor