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CIRCULATING TUMOR CELLS: WHERE WE LEFT OFF?

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Cancer metastasis and recurrence are the leading causes of cancer-related death. Tumor cells which leave the primary or secondary tumors and shed into the bloodstream are called circulating tumor cells (CTC). These cells are the key drivers of cancer dissemination to surrounding tissues and to distant organs. The use of CTC in clinical practice necessitates the deep insight into their biology, as well as into their role in cancer evasion of immune surveillance, tumor resistance to chemo- radio- and immunotherapies and metastatic dormancy.

Aim. The purpose of the work was to review the current knowledge on the CTC biology, as well as the prospects for their use for the diagnosis and targeted treatment of metastatic disease.

Methods. The work proposed the integrative literature review using MEDLINE, Biological Abstracts and EMBASE databases.

Results. This review summarizes and discusses historical milestones and current data concerning CTC biology, the main stages of their life cycle, their role in metastatic cascade, clinical prospects for their use as markers for the diagnosis and prognostication of the disease course, as well as targets for cancer treatment.

Conclusions. Significant progress in the area of CTC biology and their use in cancer theranostics convincingly proved the attractiveness of these cells as targets for cancer prognosis and therapy. The effective use of liquid biopsy with quantitative and phenotypic characteristics of CTCs is impeded by the imperfection of the methodology for taking biological material and by the lack of reliable markers for assessing the metastatic potential of CTCs of various origins. The variety of mechanisms of tumor cells migration and invasion requires the development of complex therapeutic approaches for anti-metastatic therapy targeting CTCs. Efforts to address these key issues could help developing new and effective cancer treatment strategies.

Key words: circulating tumor cells; circulating tumor microembols; metastasis; epithelial-mesenchymal transition; minimal residual disease.

Cancer is the second leading cause of death in the world after cardiovascular diseases. Experts estimate that cancer rates have risen to 2.7 million cases (all types of cancer except non-melanoma skin cancer) and led to 1.3 million deaths in 2020 in the European Union [1]. The Cancer Statistics 2020, published in the peer-reviewed journal of the American Cancer Society CA: A Cancer Journal for Clinicians, reported 180,690 cancer cases and 6,065,20 deaths, which is about 4,950 cases and more than 1,600 deaths daily [2]. In Ukraine, incidence of cancer per 100 000 population is ~384.7 cases according to the data from Bulletin of National Cancer Registry of Ukraine (No 21).

The main cause of death in ~80% of cancer cases is the development of relapses and metastases. This actualizes the search for reliable markers for the assessment and prediction of the course of oncological pathology, as well as for the development of methods and means of prevention and treatment of cancer recurrence and metastatic

disease. Attractive candidates for the role of such markers are tumor cells that separate from the primary node and circulate in the blood and lymphatic vessels — the circulating tumor cells (CTC). That is why CTC are called “pioneers” of tumor origin, which are responsible for the metastatic spread of cancer.

The study of the CTC biology is important both in terms of improving the knowledge about the biology of cancer, and applying the acquired knowledge in clinical practice for the development of targeted treatments for cancer. The problem of studying the CTC dates back to antiquity, i.e., since the time of Hippocrates, when such pathology as cancer was first established, the search for effective treatments of which is still relevant today. The importance and prospects of this scientific area is evidenced by the long period of its study with the growing number of publications in the scientific literature and the involvement of specialists in various fields of biology and medicine: from cytologists, geneticists and biochemists to biophysicists, molecular biologists and immunologists.

This review summarizes and discusses historical milestones and current data concerning CTC biology, the main stages of their life cycle, the significant role of CTC in metastasis, the methods of their study and clinical prospects for their use as markers for the diagnosis and prognostication of the disease course, as well as targets for cancer treatment.

Historical milestones in the study of circulating tumor cells

The ability of a malignant neoplasm to spread systematically was first mentioned in ‘Humoral Theory’ of Hippocrates — the “father of medicine”, who described not only the term “cancer” but also singled out its ability to grow, be surrounded by blood vessels, and its immune infiltration (Fig. 1) [3].

The long period from the death of Hippocrates to the Middle Ages was famous for a number of works devoted to the study of tumors and the problem of cancer treatment, which are still questionable in their usefulness. It was not until the middle of the 18th century that Henri Le Dran (1685–1770), a leading French physician, realized that cancer was not only a systemic disease, as Hippocrates believed, but was local with gradual progression. In 1757, Le Dran suggested that

Fig. 1. **Chronological periodization on the study of circulating tumor cells from ancient times to the present**

From left to right: Hippocrates (460–370 BC), Henri Le Dran (1685–1770), Joseph Récamier (1774–1852), Bernhard von Langenbeck (1810–1887), Rudolf Virchow (1821–1902), Karl Thiersch (1822–1895), Thomas Ramsden Ashworth (bef. 1830–1876), H. C. Engell (1921–2011), Judah Folkman (1933–2008), Lance A. Liotta (born July 12, 1947), Peter C. Nowell (1928–2016), Napoleone Ferrara (born 26 July 1956), Pantel Klaus (born 3 August 1960), new era of CTC research, which is characterized with the most sensitive methods of detection (1998–currently). Red threshold signifies a big step towards understanding a significance of tumor angiogenesis

surgery should take place before the tumor could metastasize through the lymphatic system and affect other parts of the body [4].

Recamier (1829) and later Thiersch (1865) were among the first to report the invasion of malignant cells into the veins and lymphatic vessels of a patient with basal cell carcinoma of the skin (BCC). In 1841, Langenbeck's experimental data obtained by microscopy provided conclusive evidence for the presence of tumor cells in the bloodstream.

In 1858 Virchow's generally accepted theory emerged. It explained metastasis by tumor emboli arrest in the vascular network. Eleven years later, Thomas Ashworth, who performed an autopsy on a patient who had died of metastatic cancer, reported the presence of circulating cells in his subcutaneous vein, similar in morphology to the primary tumor cells. This observation provided further evidence of the ability of individual tumor cells to migrate to blood vessels etc. [5–9].

The work of Engell et al., 1955 on the detection of tumor cells in peripheral blood in cancer patients, is considered to be more "mature" in this scientific direction. This research group found that in 61% of cases of tumor cells in blood samples, their presence was associated with a low degree of tumor differentiation (≤ G3). Because 51% of patients who survived 5 to 9 years after tumor resection had circulating tumor cells, Engell suggested that "these cells must spread into the bloodstream before or during surgery" [10].

The next step in the progress of the theory of circulating tumor cells was the elaboration of experimental models of metastasis in the 70s of the previous century. However, metastases were generally considered at this time as a late event in the development of epithelial tumors. Subsequently, Pantel et al. [11, 12] using the methods of immunohistochemistry, concluded that there is a common phenomenon of early spread of isolated tumor cells for non-small cell lung cancer. In addition, this scientific group has shown that in carcinomas of the breast and gastrointestinal tract, most disseminated tumor cells in the bone marrow of patients are at rest.These scientists also performed an important observation on the detection in histological specimens of tumors of lung cancer patients without metastases. It was found out that a significant proportion of cytokeratin-positive cells were present compared with specimens of patients with metastases.

A rather interesting theory was proposed by Nowell (1976), known as the "model of somatic evolution" [13]. According to this model, metastatically competent disseminated cells are considered as central players in the extremely complex phenomenon of metastasis, where genetic variability and selection of the most aggressive and adapted clones in tumor tissue determines the consistent of the population of malignantly transformed cells and the ability of individual representatives to spread.

Further in a series of pioneering experiments, Folkman et al. [14, 15] identified the critical role of angiogenesis in the metastatic cascade. Folkman himself theorized that tumors need constantly new blood vessels, and thus trigger certain mechanisms of their formation to provide rapidly proliferating cell mass with the necessary oxygen and nutrients. This was the beginning of a scientific field devoted to the study of tumor angiogenesis. However, the scientist's ideas were not approved by the scientific community until in 1980 when Napoleone Ferrara, a scientist at the biotechnology company Genentech, discovered the vascular endothelial growth factor (VEGF), a cytokine that stimulates the formation of new blood vessels.

Folkman's scientific research was continued by Liotta, who proved that there is a balance between factors that stimulate angiogenesis and inhibits this process. They are both secreted by the tumor cells themselves and cells of the tumor microenvironment. By modeling a course of fibrosarcoma in mice daily, the scientist observed a linear correlation between the density of perfusion vessels and a growth of the proportion of tumor embolus cells (four or more). The studies above have shown that vascularization of tumor tissue and the concomitant process of tumor complexes entering the bloodstream are closely related and critical events in the initiation of metastasis [16, 17].

These and many other experimental works and clinical observations have initiated a new era in the study of malignant tumors, associated with the investigation of the origin, phenotypic and functional characteristics, detection methods and clinical significance of CTC.

Epithelial-mesenchymal transition in the life story of the circulating tumor cells

Tumor is a localized mass of malignant cells that contains transformed cancer cells, stromal cells, and cells that infiltrate the tumor. In the process of malignancy, tumor cells acquire a number of common properties: the potential to proliferate in the absence of

exogenic growth factors, the ability to resist pro-apoptotic stimuli, the capacity to stimulate angiogenesis, etc.

Genetic instability of tumor cells is caused by constant errors in chromosome segregation during mitosis, as well as external selective pressure of the microenvironment, including immune surveillance with the need to adapt to it. By acting together, these factors create a basis for the formation of a heterogeneous population of primary tumor cells [18, 19].

Different cells in such heterogeneous population are specialized on the development of one or another tumor property, which determines their fate in the pathological process. For example, an exceptional ability to proliferate locally using autocrine signals guarantees a tumor cell the fate of the cell that creates a mass of the primary tumor, i.e., the destiny of the bulk tumor cell.

A small part of the cells of the heterogeneous population of the primary tumor in a course of their development acquires completely different properties. They lose intercellular contacts, but instead gain the ability to invade the local microenvironment, they intravasate into blood and lymphatic vessels, from which these cells extravasate back into the tissue. Such characteristics are typically unsuitable for local clonal expansion, that is why these cells became 'travelers', or CTCs, due to their feature to initiate foci of tumor growth at a distance from the primary tumor. One of the key events in the life cycle of the CTC is the epithelial-mesenchymal transition (EMT), which was identified by Hanagan and Weinburg as one of cancer features [20].

EMT was discovered in 1982 by Greenberg and Hay [21]. It is the process of a complete loss of the epithelial features and an acquisition of a mesenchymal phenotype by former epithelial cells.

Epithelial cells are characterized by a flat, cubic, cylindrical shape, they are also distinguished by polarity, that is the part of the cell which is located in the area of contact with the basement membrane, differs in a structure from the apical part. The epithelial cells are connected by tight junctions (TJs), gap junctions, adhesive (containing a stretched actin belt) and desmosomal contacts, which cause both linearization of location and tight fit of cells to each other [22].

In contrast to epithelial cells, mesenchymal cells are spindle-shaped and are connected by weak integrin focal contacts with the glycoprotein matrix. At the same time, the mesenchymal morphology of cells guarantees their ability to move. In order to migrate elsewhere, the cell must first get through the surrounding tissues, and then appear in the delocalized areas of the basement membrane. Movement in such conditions is used with the help of specialized structures — invadopodia, which are formed as the result of a membrane's evagination (protrusion) [23].

Despite the detailed description of morphological and functional differences between epithelial and mesenchymal cells, a precise list of phenotypic characteristics that distinguish these cells still remains unformed, and the existing one is contradictory [24].

During EMT activation, epithelial cells lose apico-basal polarity due to spontaneous polymerization of actin fibers and the movement of microtubules. This process requires a rupture of cellular connections (occlusal and adhesive contacts), delocalization of proteins and subsequent reorganization of the cytoskeleton to obtain cellular plasticity [25–27].

Such rearrangements occur when a signal is received from Rho and Rac1 proteins belonging to the GTPase family, which are localized in the posterior and anterior parts of the cell, respectively. Activation of Rac1 mediates cell polarization and lamellipodia formation, while high Rho GTPase concentrations increase the level of actomyosin contractility [28, 29]. Further events are followed by the expression of mesenchymal markers — vimentin, N-cadherin, F-actin, nuclear beta-catenin and others, whereas such markers as cytokeratins 8, 18 and 19, E-cadherins, Mucin-1, occludins, desmoplakinins are thought to be purely epithelial (Fig. 2). Although EMT is subject to a general program, this process is characterized by flexibility and variability, depending on cell type, tissue microenvironment, and the extremely complex multicomponent and closely interconnected set of transcriptional and posttranscriptional, translational and posttranslational signaling pathways, including Hadgehog-, Wnt-, TGF-β-, bone morphogenetic protein (BMP)–, SMAD-depending (and independing), etc. [30]. EMT is a reversible process. The reverse process is called mesenchymal-epithelial transition (MET) [31–33]. Normally, MET is characteristic of pluripotent stem cells [34, 35].

The problem of signaling, which regulates EMT, still remains the least studied. However, today, depending on the biological conditions in which epithelial-mesenchymal transition occurs, there are three functional subtypes of this process. The first is associated

with embryogenesis (gastrulation period), implantation and organ development in Vertebrates. This type of EMT results on theformation of different types of cells, which can be exposed to MET with the derivation of secondary epithelial cells during embryogenesis. Distinctive features of this type of EMT are the absence of fibrosis and cell invasion.

The second type of EMT is associated with wound healing, tissue regeneration and fibrosis of the internal organs. The result of EMT of this type, is formation of fibroblasts and other related cells, which are necessary for tissue repair after wounds and inflammatory injuries. This type of EMT is characterized by the presence of fibrosis, but not invasion. The third type of EMT is inherent in the tumor process, during which the generated cells retain epithelial features simultaneously with the acquisition of mesenchymal characteristics, as well as tumor cells of the mesenchymal type as the consequence. Distinctive features of EMT of this type are invasion and metastasis [36, 37].

The study of the third type of EMT revealed new changes that are induced by this process, but which are not always observed (inhibition of epithelial phenotype gene expression with simultaneous increase in mesenchymal gene expression and resistance to apoptosis and cellular aging), and the fact that EMT is a dynamic process with the existence of intermediate metastable states.

The third type of EMT is associated with oncogenesis. It can be considered as an aberrant variant of this process. In this pathological condition, the ability of mesenchymal tumor cells to avoid activation of anoikis (process of apoptotic cell death that occurs due to insufficient cell-matrix interaction) and immune surveillance are associated with genes activated by EMT [38–40].

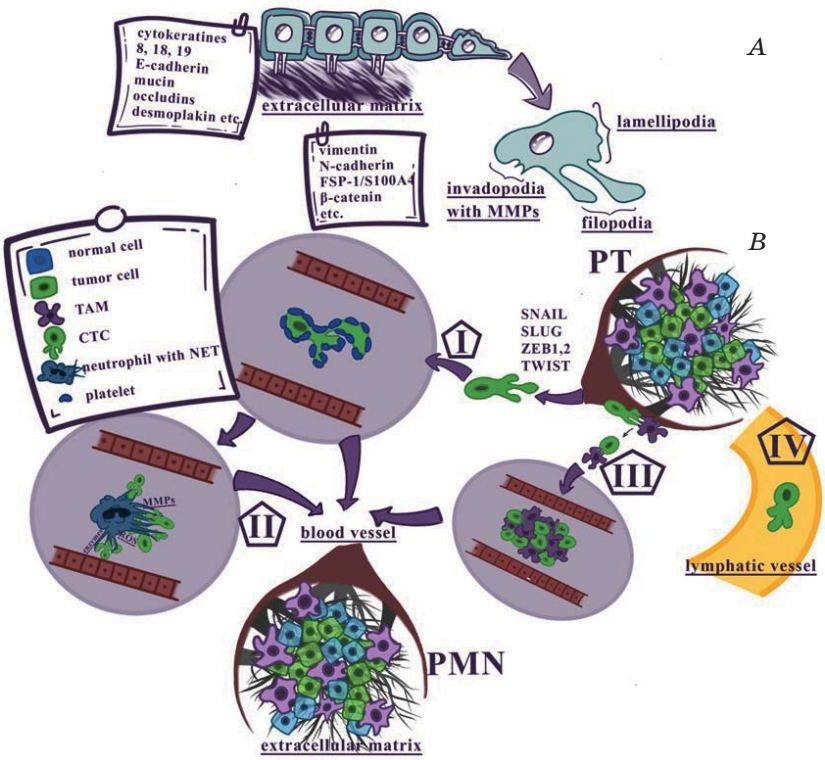

Fig. 2. **The epithelial-mesenchymal transition (EMT) and CTC strategies for the dissemination:**
A — in health condition (embryogenesis, implantation and organ development), epithelial cells lose their phenotypic markers and connections with the cell matrix, and gradually acquire mesenchymal phenotype and form membrane invaginations for the ability to move freely; *B* — incancer, tumor cell high mutagenesis along with signals from the microenvironment create the necessary prerequisites for the EMT and tumor cell dissemination using different strategies: *I* — single CTC moves independently and uses platelets to create protective shield; *II* — Single CTCs can use NET extrusion by activated neutriphils in order to create circulating tumor microemboli (CTM) straight in the blood; III — CTM can be formed within the primary tumor before entering the blood flow. In order to move freely throughout small blood vessels, cells in the microemboli "line up" in the chain without losing adhesive bonds; IV — CTC can also disseminate throug the lymphatic vessels; MMP — matrix metalloproteinases; NET — neutrophil extracellular trap; ROS — reactive oxygen species; PMN — perivascular metastatic niche; PT — primary tumor

The course of EMT during tumor progression is supported by the involvement of cytokines (VEGF, PDGF and TGF-β), which are secreted by cells of the microenvironment, including tumor-associated macrophages (TAM) — resident tumor macrophages polarized to the M2 phenotype. It is now known that TAMs are activators of malignant progression of the primary tumors. They are involved in the activation of metastasis and make a significant contribution to the adhesive integrity of the tumor stroma. A distinctive feature of TAM with this capacity permitting is the high level of expression of the transcription factor ZEB1, which provides activation of tumor metabolic pathways of these phagocytes [41–43].

Transcription factors ZEB1, ZEB2, TWIST1, Snail, Slug, SIX1, E47, ELF5, FOXC2, GRHL2, p53, p63 etc. of the tumor cells are components of signaling pathways involved in the process of their epithelial-mesenchymal transition. After phosphorylation by protein kinases, these transcription factors and the corresponding co-regulators inhibit the expression of epithelial phenotype genes in the tumor cells. This leads to the formation of a mesenchymal phenotype with reduced expression of epithelial adhesion molecules (EpCAM) and cytokeratins (CK), promotes invasion, dedifferentiation and the ability to intravasate into lymphatic and blood vessels [44]. This is how the CTC population appears.

In addition to transcription factors, anothertrans-regulatory elements — miRNA — also participate in complex EMT phenomenon. Moreover, transcription factors interact with miRNA in this sophisticated process, and can create a feedback or feed-forward loop providing cross-gene regulation network [45]. Deep insight into this network may provide a new therapeutic opportunity in cancer treatment.

The primary tumor contains cells that are at different stages of EMT with diverse invasive, metastatic properties and various degrees of differentiation. Tumor cells at varied stages of EMT are localized in different microenvironments and in contact with different stromal cells. In particular, cells with the most pronounced mesenchymal phenotype proliferate near endothelial cells and immune cells with an inflammatory metabolic profile.

Tumor cells at this stage secrete large amounts of chemokines to recruit immune cells and stimulate angiogenesis, thus contributing to the formation of a unique inflammatory and highly vascularized niche. The most effective in the circulation, as well as in the colonization of the distant metastatic niche and the development of metastases, are CTC, which express a mixture of epithelial and mesenchymal phenotypic markers (EMT-CTC) [46].

The life cycle of the CTC requires from them an extreme stress resistance, which is provided by the special genetic, phenotypic and metabolic properties of these cells.

The unique properties of CTC

By definition, CTC are a type of malignant cells that separate from both primary tumor that exists in a body and reaches a few millimeters, and the secondary tumor [47]. CTC enter the blood or lymph through the dense walls of blood vessels, spreading further to other parts of the human body, which become secondary foci of tumor growth [48].

There are different biological phenotypes of CTC: epithelial, mesenchymal, stem cell-like or cells with a mixed phenotype, depending on the stage of EMT [49, 50].

CTC has certain characteristics regardless of the stage of EMT, which distinguish them from stationary tumor cells and allow them to perform their assigned functions of dissemination of the tumor process under the action of numerous stressors:

- ability to separate from tumor tissue;
- ability to resist anoikis activation after separation;
- ability to migrate to blood and lymph vessels;
- ability to maintain viability under hemodynamic stress;
- ability to avoid immune surveillance;
- ability to attach and proliferate in a new tissue microenvironment.

Where do cells with such an exceptional set of properties in the composition of tumor tissue come from? According to the generally accepted hypothesis, CTC originate from stem tumor cells (Cancer Stem Cells, CSC) and differ from them in some features acquired as a result of unique mutations [51, 52].

The circulating tumor cells inherit from the CSC an ability to self-renewal and remain dormant (anabiosis can last from several years to decades), as well as the ability to differentiate into cells with different phenotypes, which enables them to initiate tumor growth in the secondary location. There are several mechanisms for acquiring the following unique properties by CTC:

- genetic or internal mechanisms (high level of mutagenesis with aberrant gene

expression, redistribution of gene expression induced by EMT, "stem-like" properties inherited from CSC);

• influence of the tissue microenvironment of the primary tumor (tumor-associated hypoxia, tumor exosomes, cytokine profile produced by TAM, myeloid-derived suppressor cells (MDSC) and tumor-associated fibroblasts (TAF));

• influence of circulating blood microenvironment (immunosuppressive, promigatory cytokine profile, interaction with platelets, neutrophils, monocytes, natural killer cells (NK), regulatory T cells (Treg), etc.);

• clustering.

Increased mutagenesis and aberrant gene expression in circulating tumor cells result in overexpression of dormancy-related genes, genes associated with increased survival (e. g., survivin gene), and immunosuppressive genes (e. g., PDL-1) [53]. Recently, special attention has been paid to the influence of the circulating blood microenvironment and clustering of CTC.

An important event happens when CTC, by undergoing hemodynamic pressure in the circulating blood, change their metabolism. It is followed by increased generation of reactive oxygen species (ROS) [54, 55]. ROS-dependent signaling enhances the expression of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase), and promotes the transmission of mitogenic signals, which contribute together to the survival of the circulating tumor cells under stress. In addition, ROS have an immunosuppressive effect and promote the differentiation of naive T cells to Treg, and immature myeloid cells — to MDSC [56].

Despite the stress resistance acquired upon mutations and aberrant gene expression, CTC still need to secure movement to the tissue niche and form metastases in the aggressive microenvironment of circulating blood. EMT gives CTC the ability to separate from the primary tumor and to invade surrounding tissue using so-called mesenchymal movement pattern, which involves the destruction of the extracellular matrix. Vasoactive mediators, such as VEGF, produced by microenvironmental cells, in particular TAM, increase vascular permeability, which promotes intravasation of the circulating tumor cells. TAM reconstruct the ECM, which facilitates the movement of CTC to the vessel [57]. Taken together, all this leads to a single CTC entering the bloodstream. Besides, tumor cells that have already passed through EMT but are encounting microenvironmental or xenobiotic stress (e.g. cytotoxic agents), can use mesenchimal-amoeboid transition (MAT) in order to evade the stress. MAT is the most primitive and most efficient movement mechanism, that does not involve destruction of the extracellular matrix and is based on highly deformable cell morphology. This tumor cell migration plasticity allows them to adapt to different environmental conditions and to disseminate to long distance [58, 59]. It is logical to assume that the simultaneous effect on mesenchymal and amoeboid motility may be more effective in preventing the process of metastasis [61].

The cells that have separated from the tumor mass and move alone have a higher migration rate, but they are effectively attacked by the immune surveillance system, experience greater hemodynamic pressure and have an increased risk of anoikis activation [61]. Due to this, less than 0.1% of single CTCs remain viable 1–2.5 h after circulation. The mechanism to avoid such rapid death is the clustering of circulating tumor cells — the formation of dense conglomerates of cells, or tumor microemboli, which include a minimum of 3, maximum 100, usually — 20–30 cells.

Currently, it is supposed that CTC microemboli can be either formed with in the primary tumor before entering the blood flow or within the circularly system [62]. At the same time, the rather large size of the cluster set up before entering the circulatory system does not prevent it from moving freely throughout small blood vessels due to the ability of cells in the microemboli to "line up" in the chain without losing adhesive bonds (Fig. 2). Intercellular bonds in circulating tumor microemboli (CTM) are formed by placoglobin (γ-catenin, which is a member of the Armadillo family of proteins and a paralog of β-catenin, an intracellular component of adhesive and desmosomal contacts), which also provides additional stress resistance of clusters during their transit into the blood [63, 64].

The composition of the microemboli is heterogeneous at the genetic, transcriptomic, proteomic and metabolic levels and contains not only tumor cells but also tumor stroma cells, such as TAM [65]. The latter make a significant contribution to the adhesive integrity of the structure. The formation of CTM is also possible in the circulatory system. Circulating neutrophils become important partners of the tumor cells in this structure. It concerns those neutrophils which are activated before NET extrusion or NETosis — the release of a network

containing mitochondrial or nuclear DNA, and the components like antimicrobial peptides, reactive oxygen species, enzymes, etc. into the extracellular space [66–68].

In spite of the large number of works concerning the study of the role of stromal cells in CTM, both the list of functions and phenotype of these cells have been insufficiently studied [69]. A crutial role in the structure of CTM is played by platelets that surround them in the form of the so-called "shield". Thrombocytes provide protection against the recognition of tumor cells by immunocompetent cells, prevent their lysis by NK cells.

When CTM moves, it is later "stuck" in the capillaries, which serve as a kind of "catcher" of malignant cells, platelets lead to a state of hypercoagulation and the formation of vascular thrombi, which are a hallmark of hematogenous spread of malignant tumors. In addition, the release of platelet granules, which contain vasoactive, antiapoptic and other biologically active mediators, is a way of apoptotic resistance of CTM cells, promotes their survival and increases metastatic potential many times [70, 71].

The movement of single CTC in the circulating system can be identified with the movement of cars during a rally, where the winner is the one who overcomes the path "from point A to point B", which is not always associated with a high level of safety. Coordinated CTM migration is reminiscent of car traffic provided that the prescribed traffic rules are followed and a mandatory stop at the red light — it is slower but secured by coordinated community action and provides more guarantees of reaching the final destination (Fig. 3).

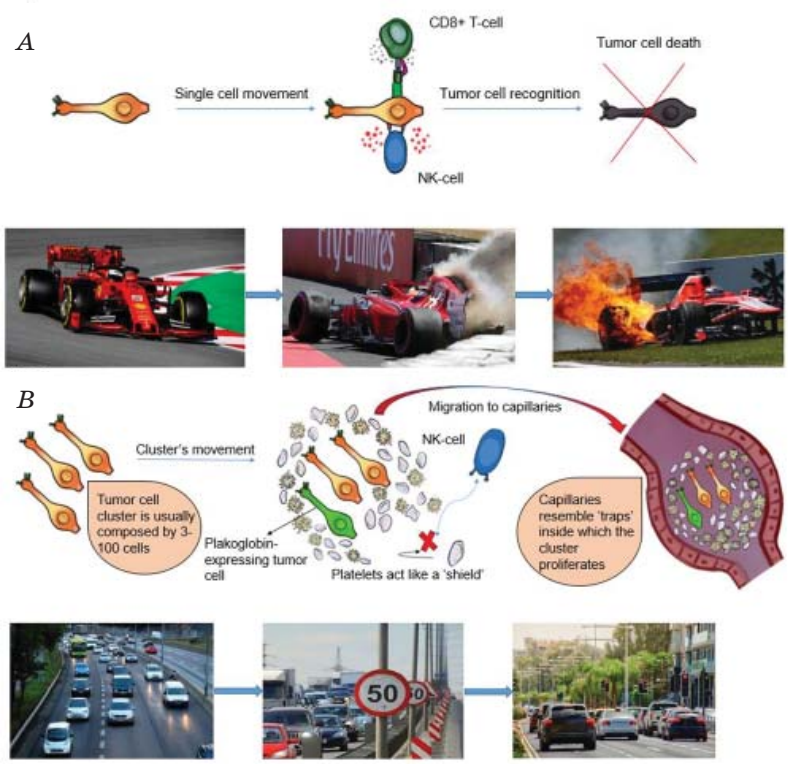

Fig. 3. **Interpretation of the movement of single circulating tumor cell vs tumor microemboli:**
A — The circulating tumor cell, which was recently shed from a primary tumor and actively moves through the blood flow has lack of survival mechanisms and thus successfully recognizes and attacks by the main cells of immune antitumor surveillance (CD8+T-cells and NK cells). This way the majority of single-moving CTC risk to be either subjected to the mechanical damage or anoikis in the blood circulation.The same events are happening when audience watches live streaming of Formula 1. It is often questionable whether a F1 car can survive a rally stage, because driving at the high speed enhances probability of the car crash. *B* — the circulating tumor microemboli have high potency to survival during the transit in the blood stream due to molecular and cellular mechanisms. Being coated with thrombocytes, CTM are endowed with an ability to avoid direct attack of NK-cells and continue their migration to the capillaries. But this unimpeded access has the other side, since small vessels own a bandwidth limit. This results in CTM being stuck inside the capillary. The circulating tumor microemboli movement resembles the car traffic at a steady speed where stopping at a red light is the basic law of survival according to main principle — 'run silent, run deep'

The formation of microemboli gives CTC the properties of "stemness" — maintaining a differentiated phenotype, the ability to divide and self-renewal through overexpression of transcription factors Hadgehog, Wnt, TGF-β and chemokine CXCL12. Binding sites for transcription factors associated with "stemness" and proliferation are specifically hypomethylated in CTC clusters, including binding sites for OCT4, NANOG, SOX2, and SIN3A [72, 73].

The initiation of metastasis, which is marked by the appearance of CTC in the circulation, is associated with a significant increase in the number of NK — important effector cells of antitumor surveillance. CTM clasterization significantly contributes to their resistance to NK cytotoxicity. CTC themselves and partner cells of the microenvironment in CTM produce a significant amount of immunosuppressive mediators, such as IL-10, prostaglandin E2, etc., which inhibit the expression of activator immunoglobulin NK receptors responsible for their antitumor activation [69, 74].

CTM formation is accompanied by overexpression of CD44, a non-kinase transmembrane glycoprotein. CD44 binds to hyaluronan within a tissue metastatic niche and activates numerous signaling pathways associated with activation of proliferation, survival of circulating tumor cells, and alteration of their cytoskeleton which enhances invasive movement [66]. This allows the CTC to acquire the status of disseminated tumor cells (DTC) and to initiate the next stage of their life cycle — the formation of a metastatic tumor foci.

Disseminated tumor cells

A small population of tumor cells, including CTC and disseminated tumor cells (DTC), which persists in the patient in a state of complete morphological remission after treatment, causes the so-called minimal residual disease (MRD) [75]. DTC are responsible for the post-extravasation stage and can be found in distal organs and all the tissues, including bone marrow [76].

DTC are a heterogeneous population of cells that share both epithelial and mesenchymal characteristics. DTC can be found singly or as clusters formed of 10–20 cells [77]. The peripheral blood sample, due to the fact that the DTC is an extremely rare component, contains at most 1 disseminated tumor cell per 1 ml [78]. The main morphological characteristics of the DTC are a large nucleus with granulation or stippling, strong or uneven staining for cytokeratin and cytokeratin filaments [79].

Circulating throughout the body, DTC are able both to enter and exit target organs without initiating tumor growth [80]. In order to form micrometastases ranging in size from 0.2 to 2 mm in tissues and organs remote from the primary location, DTC must adapt to the microenvironment of the metastatic niche [81, 82].

The microenvironment of the tumor consists of cellular and extracellular components. In the growth zone of the primary formation, the fraction of tumor cells is about half mass of the tumor, the rest are non-malignant cells of the microenvironment. In areas of metastasis, the proportion of tumor cells is incomparably little in relation to the number of non-malignant tissue elements, so the DTC face an extremely difficult task to adapt to such a potentially aggressive microenvironment. Given this, 80% of DTC are able to survive extravasation, 3% can reach the stage of micrometastases, and less than 0.02% are able to form secondary foci of metastasis [83].

DTC have a potential to reach any organ or tissue. However, recurrence of tumor growth usually develops in a limited number of tissues and organs. Moreover, different types of tumors are characterized by metastasis only in certain organs and tissues, which are probably suitable for the formation of the most favorable metastatic niche.

The perivascular metastatic niche (PMN) microenvironment, where DTC from the circulations arrive, is evolutionarily adapted to sustain the viability of stem tissue elements of the adult organism for a long time [84]. Evolutionarily formed physiological features of PMN are successfully used by DTC. These cells stay in a long latent period with subsequent reactivation of tumor growth. DTC form a direct link with stromal cells, which are a valuable reservoir of potentially promethastatic soluble mediators [85, 86].

The largest number of studies in this regard concerns DTC detected in the bone marrow and the role of their microenvironment for the persistence, latent phase and further reactivation of these cells [87–89]. In addition to the bone marrow, investigations of tissue metastatic niche often involve the brain and lungs. The long-term survival of DTC in the bone-marrow depends on the formation of gap junctions with osteogenic niche cells that is osteoblasts. The

one of the functions of osteoblasts is to serveas a calcium reservoir for the future activation of DTC from dormancy [90, 91].

In the brain, DTC occupy the tissue niche of pericytes or Rouget cells: they disrupt the blood–brain barrier (BBB) and migrate along the surface of brain capillaries in the space between pericytes. Brain DTC resemble pericytes in their morphology [92, 93]. In the lungs, airway smooth muscle cells become forced DTC partners in the colonization of this metastatic niche [94, 95].

In all instances, the extracellular matrix molecules play a key role in the successful homing of DTC in the metastatic niche [96]. DTC of various origins are characterized by overexpression of certain adhesion molecules, which determine their affinity for cells in the metastatic niche, that overexpress the corresponding ligands. For example, airway myocytes overexpress collagen III, the receptor for which (CD167a), in turn, is overexpressed in tumor cells characterized by pulmonary metastases [97].

It should be noted that an important factor in successful DTC homing is MET. DTCs that complete MET with coordinated re-expression of E-cadherin may occupy distant organs, form small preangiogenic metastases, or larger vascularized metastases [98–100].

After successful homing in a metastatic tissue niche, DTC enter a state of metastatic dormancy in which they can remain for a very long period of time in order to survive and relapse, sometimes decades later. Metastatic dormancy precedes the development of a secondary tumor. Its main features are growth arrest and resistance of malignant cells to therapy [81, 101].

Maintenance of metastatic dormancy is ensured by three important components: angiogenic dormancy, immune-mediated dormancy and intrinsic latency mechanisms of the DTC. Angiogenic mechanisms are largely regulated by hypoxia in the DTC microenvironment. The hypoxic environment of the metastatic niche causes overexpression of HIF1α and activation of the tumor angiogenesis [102].

Immune-mediated mechanisms include the possibility of the DTC to avoid immune surveillance (recognition and killing by cytoxic CD8 + T cells and natural killers) by mechanisms that are still at the research stage [103]. The internal mechanism of DTC latency is their “arrest” in the G0 phase with the full stop of proliferation. This process occurs without enhancing cell death [104].

The question of how these three programs, which form the preconditions for the development of tumor recurrence, relate over time and which of them is of paramount importance and, therefore, should become the main target for the development of approaches for the prevention and treatment of cancer recurrence, remains open. A key feature of DTC in metastatic dormancy is their ability to maintain a high degree of epigenetic and transcriptional plasticity and to reactivate various programs in order to stop growth and survive.

Successful homing, dormancy, and lack of proliferation make DTC insensitive to chemotherapeutic factors. Additional resistance of DTC to cytotoxic agents is provided by integrin signaling, which is ensured by close contacts with the corresponding extracellular matrix ligands in PMN. All of this allows the DTC to survive for a long time at dormancy state in a colonized metastatic niche.

Later, the life story of DTC passes to the stage of an exit from the state of dormacy and activation of tumor growth. The triggers for the transition of dormant DTC to the phase of tumor growth activation is still unknown. Some assumptions are made about the existence of internal and external DTC reactivation triggers. One of the hypothetical internal triggers for the DTC to exit metastatic dormancy is their overexpression of CD36 — a fatty acid translocase and one of the macrophage scavenger receptors capable of binding collagen and thrombospondin [105]. Cumulative absorption of fatty acids involving CD36 provides a substrate for β-oxidation of lipids, a highly efficient method of generating ATP required DTC to exit from dormancy [106].

An important external trigger for DTC reactivation is tissue inflammation, which in turn can be triggered by a variety of events and factors. Inflammation is known to be accompanied by recruiting into the area of its development the circulating myeloid cells (neutrophils, monocytes, etc.), which causes the remodelling of the ECM and the activation of dependent signaling of DTC in a dormant state.

These signaling cascades cause the activation of DTC proliferation and their metabolic activation. This is accompanied by the transformation of the state of immune balance in the metastatic niche (when the cytotoxic activity of antitumor immune effectors inhibits the growth of the tumor nodule) to the so-called immune editing —

changing the metabolic profile of immune cells from antitumor to pro-tumoral. Immune editing results in the formation of a tumor microenvironment, which promotes not only the growth of the tumor nodule, but also provides further drug resistance of tumor cells. For instance, smoking or bacterial infection in the lungs with lipopolysaccharide exposure causes the recruitment of neutrophils with activation of the NET extrusion. Neutrophil elastase, matrix metalloproteases and other enzymes that are part of NET mediate the ECM remodeling followed by the activation of laminin III and α3β1 integrin-dependent DTC signaling pathways, which increased a proliferative activity of these cells [77, 107].

The study of numerous unexplored issues in the life cycle of CTC and further DTC requires the development of adequate model systems that will reproduce the entire metastatic cascade *in vivo* in the patient's with the maximum proximity.

Preclinical models in the study of CTC

Preclinical models for the study of CTC can be divided into two categories: isolation and cultivation of CTC *ex vivo*, followed by the investigations of their biology and drug susceptibility testing; modeling the behavior of CTC in the metastatic cascade using murine models [108, 109].

Ex vivo technologies are a good alternative to animal models, which are time- and labor-consuming, as well as quite costly. This type of clinical study was introduced to study the biology of the metastatic cascade in colon cancer, as well as breast, prostate and lung cancer. However, this methodology has also a number of disadvantages and limitations [68, 110].

The CTC are present in small amounts in the peripheral blood — approximately 1 CTC per 7.5 ml of blood sample volume in patients with metastatic cancer [111]. According to other estimates, the frequency of CTC ranges from 1 to 10 cells among 5 billion erythrocytes, 10 million leukocytes, 200–500 platelets in 1 ml of peripheral blood, which is equivalent to finding a needle in a haystack [112–114]. The difficulty in isolating CTC is due to their significant heterogeneity and non-identified phenotypic profile. There is also a lack of information on the most valid markers for cells with the highest metastatic potential, the detection and study of which is diagnostically and prognostically significant [116, 117].

A wide range of methodological approaches is used to identify CTC. Enrichment strategies can be based on both biological properties (expression of membrane markers) and physical characteristics (size, density, electric charge) and are usually combined with identification methods based on the detection of membrane markers (e.g., immunofluorescence, immunohistochemistry, FISH, etc.) or expression of specific genes, which are identified by real-time PCR or quantitative PCR.

Isolation of CTC can be based on their both positive selection among normal blood cells and negative selection with leukocyte depletion. However, the use of *ex vivo* technologies does not allow imitating the entire metastatic cascade, the components of which are CTC. These methods are more suitable for studying the CTC molecular biological characteristics and screening of targeted drugs [118–120].

The murine models, which are used to study CTC in the metastatic cascade, are divided into several types or groups. The first group includes models of spontaneous metastasis [121, 122]. These models are based on orthotopic transplantation of tumor cells (e.g., transplantation of prostate cancer cells into the prostate gland of male mice) followed by monitoring of disease progression over time. The unquestionable advantage of this methodological approach is the ability to model all stages of the tumor process from the growth of the primary tumor to the development of metastases.

The second group combines animal models based on intravenous injection of tumor cells [123–125]. The advantage of these models is their shorter duration, because there is no stage of development of the primary tumor. Despite the fact that tumor cells introduced in this way are potentially able to spread to all tissues and organs, in reality they form a foci of tumor growth where they first enter the capillary bed. Therefore, the disadvantages of this approach are the lack of ability to predictively model metastasis in a particular organ. In addition, the absence of the primary tumor stage makes the reproduction of the pathophysiology of the process incomplete.

Both described groups of methods for modeling the metastatic process use syngeneic tumor cells. The third group consists of genetic engineering models (GEMM), based on the manipulation of the expression of proto-oncogenes or oncosuppressor genes, and includes transgenic models and models based on gene knockout [126, 127]. These models allow us to

investigate the role of genes associated with the formation of CTC and their ability to acquire DTC status and initiate the recurrence of the tumor process. However, genetic manipulations in such models affect all cells, such as a target organ or tissue, and thus do not replicate the natural course of events during the sporadic development of cancer that occurs due to the accumulation of genetic changes in a single cell.

The most eye-catching from the point of view of interpretation of pathophysiology of metastatic cancer are the models based on xenotransplantation of tumor cells, i.e., on introduction of human tumor cells to immunodeficient (immunocompromised) mice (PDX) [128–130]. Such models of metastatic tumor growth are created by orthotopic or subcutaneous transplantation of fresh surgically removed samples of human tumor tissue, rather than cultured tumor cells. This is the only type of model that allows personalized study of the metastatic cascade.

In spite of the undoubted advantages of this methodological approach, it has a number of disadvantages as well. Firstly, the creation of immunodeficiency in animals deprives the opportunity to study the participation of the immune system in the studied processes by experimenter and thus excludes from the study an important element of the pathophysiology of the metastatic cascade. Furthermore, such models do not allow the investigations of immunotherapeutic approaches in the treatment of metastatic disease.

Secondly, there is a significant variability in the engraftment of xenogeneic grafts, which depends on the type of tumor, the degree of its differentiation, etc. Thirdly, it is not always possible to obtain fresh tissue material for transplantation. Finally, in these models, achieving a detectable amount of CTC in the circulation sometimes takes a considerable period of time. Despite such significant limitations, xenotransplantation technology is widely used to study the biology of the metastatic cascade in breast cancer, melanoma, lung and prostate cancer, etc. The use of xenotransplantation of the enriched population of CTC obtained from peripheral blood samples from patients is an approach to improving this technology [131, 132]. However, there is also an obstacle to this improvement. The point is that for many cancers, isolation of CTC from the blood is a significant problem because of their extremely small number.

Nowadays, the solution to the problem of modeling the metastatic cascade for the study of the biology of CTC and DTC is a unification of all these methodological approaches and the combined efforts of specialists in various fields of biology and medicine.

Applications of the CTC in clinical strategies

Assessment and prediction of the disease course are of paramount tasks in oncology, as they allow stratify patients in order to develop an optimal treatment protocol and prevention of recurrence and metastasis. This problem requires the use of non-invasive methods, as invasive procedures, such as fine-needle aspiration biopsy, are traumatic, not representative of the disease as a whole and pose a risk of dissemination of the malignant neoplasm.

The appearance of liquid biopsy has opened up new perspectives for diagnosing and predicting recurrence and metastasis of cancer. Liquid biopsy — the analysis of tumors using biomarkers circulating in fluids such as blood — attracts considerable attention of specialists, despite the fact that this methodological approach has not entered the arsenal of standard tools in clinical oncology yet. The method of liquid biopsy analyzes various components of the tumor, which are released into the blood and other biological fluids, such as circulating nucleic acids (circulating tumor DNA (ctDNA), cell-free RNA (cfRNA)), tumor exosomes and CTC [133].

cfRNA includes mRNA and miRNA [134, 135]. Sequencing of cfRNA, which is found in the serum and plasma of cancer patients, especially miRNA, allows to obtain unique genetic information about the tumor. For example, circulating levels of miR-375 are a diagnostic marker for several types of cancer, including liver cancer, colorectal cancer and lung cancer [136].

ctDNA includes nuclear and mitochondrial DNA. Nucleic acids are released into the biological fluids including blood as a result of apoptosis or necrosis of tumor cells. High levels of free nucleic acids and the presence of specific mutations can be used as reliable biomarkers to determine the development and progression of the disease. In different types of tumors, ctDNA is characterized by specific changes in integrity and methylation, some changes in microsatellites and mutations. The main part of cell-free DNA (cfDNA), which is found in blood plasma, is the DNA of normal cells. ctDNA is only a small fraction of it. However, in terms of quantitative indicators,

this biomarker exceeds the indicators of CTC, which has a clear advantage.

Like CTC, ctDNA can characterize the stage of a tumor and even its metastatic potential, leading to a lengthy debate about the comparative characteristics of these two types of liquid biopsy. Nevertheless, the detection and analysis of CTC in liquid biopsy samples still has a number of advantages. In particular, the quantitative characteristics of ctDNA are insufficiently diagnostically and prognostically relevant, because the level of this circulating nucleic acid increases, for example, during pregnancy or during the physiological activation of hematopoiesis [137]. In addition, the ctDNA study does not make it possible to assess the sensitivity of the tumor to drugs, immunotherapy, etc.

The quantitative indicators of CTC in the blood correlate with the overall survival and disease free survival in many types of tumors [138]. For some tumors (stomach, lung, breast cancers, etc.), the number of CTC increases with an enlargement of the tumor size and with the development of distant metastases [139]. Diagnostic and prognostic significance of liquid biopsies with the determination of CTC is enhanced by its complementary proteomic analysis of isolated cells.

Determining the phenotypic characteristics of CTC allows to assess their metastatic potential and the risk of disease recurrence. For example, the presence of HER2-positive CTC in breast cancer biopsy specimens indicates a high risk of distant metastases and reduced overall survival [140]. The most promising phenotypic characteristics of the CTC in terms of their diagnostic and prognostic significance are markers of different stages of EMT and signs of "stemness" of these cells. In particular, the presence of transcription factors associated with EMT, such as Twist, Snail, ZEB-1, etc., as well as the detection of the so-called hybrid phenotype of CTC (cytokeratin + / vimentin + / CD45-) characterizes them as EMT-CTC and is a marker of their high metastatic potential [141]. Newly recognized markers of cell stemness and EMT along with well documented phenotypic characteristics might comprise a good set for cancer theranostics [142].

The expression of EMT-CTC markers of CTC increases the prognostic value of liquid biopsy with their detection [143] and predicts the risk of metastasis. The expression of certain phenotypic markers of CTC allows characterize the sensitivity of the tumor to chemo- and immunotherapeutic agents as well. For example, the expression level of PD-L1 is a prognostic marker of response to immunotherapy with PDl/PD-L1 pathway inhibitors [144, 145].

Despite significant advances in the development of liquid biopsy techniques in CTC analysis to predict metastasis and recurrence of oncopathology, there are some reports of the ineffectiveness of this methodological approach for some cancers. For instance, Chen et al. report the lack of prognostic value in assessing the quantitative and phenotypic characteristics of CTC for predicting the course and metastasis of hepatocellular carcinoma [146]. Yet, one of the reasons for the negative result may be the imperfect pre-analytical procedures used by this study group, as Sun et al. report that the quantitative and phenotypic characteristics of CTC isolated from the tumor efferent vessels and postpulmonary peripheral vessels differ significantly. According to the authors of this publication, the quantitative characteristics of CTC in the hepatic vein and peripheral vessels can be used to predict the development of metastases of hepatocellular carcinoma in the liver and lungs, respectively [147].

It is possible that higher prognostic value can be achieved by a combination of different types of liquid biopsy. Furthermore, there are reports of new candidates for the role of biomarkers of metastasis in liquid biopsy specimens. Lin et al. and Lei et al. detected circulating tumor endothelial cells (CTEC) in liquid biopsy specimens, which the authors of those publications believe are the result of an endothelial-mesenchymal transition (Endo-MT) process. Both research groups emphasize that aneuploid CD31+ CTEC, along with CD31- CTC, constitute a unique pair of circulating cell biomarkers for predicting tumor metastasis [148].

From the point of view of the clinical perspective, new treatment strategies focused on the mechanisms of formation and residence of CTC deserve some attention. For example, an anticoagulant therapy used in cancer patients and animal models of tumor growth may reduce the risk of tumor metastasis by minimizing CTM arrest in the PMN of the metastatic niche. Due to the fact that platelets promote the circulation and homing of CTM, experimental studies show the effectiveness of therapeutic strategies aimed at inhibiting the interactions of these structures. In addition, animal models have shown the effectiveness of genetically modified and functionalized platelets to control MRD [53].

A new step towards the development of a methodology for the clinical use of CTC

biology is the development of a photoacoustic method for detecting the minimum number of these cells in patients with melanoma with the possibility of their further elimination using laser pulses [149].

Immunotherapeutic approaches are promising from the point of view of development of treatment modalities targeting CTC. As it was mentioned earlier, the cells of the immune system in the microenvironment of the primary tumor contribute to the formation of CTC and their EMT, as well as they are a part of CTM and contribute to their viability both in the circulatory system and in the metastatic niche. An additional argument in favor of the development of targeted immunotherapy to combat CTC is the fact that they are able to avoid immune surveillance. Tumor growth models have shown the effectiveness of several immunotherapeutic approaches against CTC. The first is the use of PD-1/PD-L1 signaling cascade inhibitors. Blockade of these signaling pathways makes CTC sensitive to cytotoxic effects of tumor-specific CD8 + T lymphocytes [150]. Still, it should be noted that not all tumor cells, including CTC, are characterized by overexpression of PD-L1, so the impact on this checkpoint in this case may not be effective enough. An attractive candidate for the role of a target for immunotherapy targeting CTC is CD47 is one of the so called “do not eat me” signals, which is overexpressed on the surface of majority tumor cells and prevents their phagocytosis by macrophages [151]. Lian et al. ombined in their study the blockade of both ligands, which resulted in a profound inhibition of metastasis of breast cancer in mice [152].

The next immunotherapeutic approach involves the use of monoclonal antibodies to specific and functionally important CTC membrane receptors. For example, antibodies against CD44 inhibit the adhesion of CTC and CTM in the metastatic niche to the ECM [153], whereas antibodies against EpCAM perform the functions of opsonins and make CTC sensitive to both macrophage phagocytosis and antibody-dependent cellular cytotoxicity (ADCC) mediated by natural killers [154]. The use of leukocytes functionalized with E-selectin or TRAIL prevents the adhesion of CTC and CTM to vascular endothelium in PMN and is also considered as a promising immunotherapeutic approach for the prevention of metastasis, directed against CTC [155].

These and other innovative approaches give a hope that the theranostic potential of CTC is much greater than what we know today.

Conclusions

Summing up recent advances on the biology of CTC and the prospects for their use in cancer theranostics, it should be noted that significant progress in this area of science, even more convincingly proves the attractiveness of these cells as targets for cancer therapy, including cancer relapse and its metastasis from the standpoint of precision and personalized medicine.

Endowed with numerous mutations and aberrant gene expression, migration plasticity and properties of stem cells, the capacity to avoid immune surveillance, having an immunosuppressive effect and maintaining long-term latency without losing all these properties, CTC embody all the pathological changes that distinguish malignant cell from normal cell. For this reason, probably, the traditional antiproliferative therapy concedes individualized therapy targeted at these cells, which is believed is the future of oncology. For the purpose of embodiment an individualized therapy in reality, it is still necessary to solve a very difficult task — to accept a challenge entitled “catch me if you can” ran by CTC.

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ЦИРКУЛЮВАЛЬНІ ПУХЛИННІ КЛІТИНИ: НА ЧОМУ МИ ЗУПИНИЛИСЯ?

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Метастазування та рецидив раку є основними причинами смертності у хворих на онкологічну патологію. Пухлинні клітини, які відокремлюються від первинної або вторинної пухлини і поширюються у кров, називають циркулювальними пухлинними клітинами (ЦПК). Вони характеризують мінімальну залишкову хворобу і є ключовими рушіями поширення пухлини у прилеглі тканини та віддалені органи. Використання ЦПК для діагностики і лікування онкологічної патології у клінічній практиці потребує глибокого розуміння їхньої біології, а також ролі в уникненні пухлиною імунного нагляду, стійкості до хіміо- та імунотерапії та феномену метастатичного спокою.

ЦИРКУЛИРУЮЩИЕ ОПУХОЛЕВЫЕ КЛЕТКИ: НА ЧЕМ МЫ ОСТАНОВИЛИСЬ?

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Метастазирование и рецидив рака являются основными причинами смертности у больных с онкологической патологией. Опухолевые клетки, которые отделяются от первичной или вторичной опухоли и распространяются в кровь, называются циркулирующими опухолевыми клетками (ЦОК). Эти клетки характеризуют минимальную остаточную болезнь и являются ключевыми двигателями распространения опухоли в окружающие ткани и отдаленные органы. Использование ЦОК для диагностики и лечения онкологической патологии в клинической практике требует глубокого понимания их биологии, а также роли в ускользании опухоли от иммунного ответа, устойчивости к химио- и иммунотерапии и феномена метастатического покоя.

Мета. Огляд сучасних знань щодо біології ЦПК, а також перспектив їх використання для діагностики та спрямованого лікування метастатичної хвороби.

Методи. Комплексний огляд літератури із застосуванням баз даних MEDLINE, Biological Abstracts та EMBASE.

Результати. В огляді узагальнено і проаналізовано історичні відомості та сучасні дані щодо біології CTC, основних етапів їхнього життєвого циклу, ролі у метастатичному каскаді, клінічних перспектив їх використання як маркерів для діагностики та прогнозування перебігу захворювання, а також мішеней для лікування раку.

Висновки. Значний прогрес у галузі біології ЦПК та їх застосування у тераностиці раку переконливо доводить деєвість цих клітин як мішеней для прогнозу і лікування метастатичної хвороби. Ефективному використанню рідкої біопсії з кількісною та фенотиповою характеристикою ЦПК перешкоджає недосконаліть методології взяття біологічного матеріалу і відсутність надійних маркерів для оцінювання метастатичного потенціалу ЦПК різного походження. Різноманіття механізмів міграції та інвазії пухлинних клітин потребує розроблення комплексних терапевтичних підходів для антиметастатичної терапії щодо ЦПК. Зусилля, орієнтовані на вирішення цих основних питань, можуть сприяти розробленню нових ефективних стратегій лікування раку.

Ключові слова: циркулювальні пухлинні клітини, циркулювальні пухлинні мікроемболи, метастазування, епітелійно-мезенхімний перехід, мінімальна залишкова хвороба.

Цель. Обзор современных знаний по биологии ЦОК, а также перспектив их использования для диагностики и направленного лечения метастатической болезни.

Методы. Комплексный обзор литературы с использованием баз данных MEDLINE, Biological Abstracts и EMBASE.

Результаты. В обзоре обобщены и проанализированы исторические сведения и современные данные, касающиеся биологии ЦОК, основных этапов их жизненного цикла, роли в метастатическом каскаде, клинических перспектив их использования в качестве маркеров для диагностики и прогнозирования течения заболевания, а также мишеней для лечения рака.

Выводы. Значительный прогресс в области биологии ЦОК и их использования в тераностике рака убедительно доказывает действенность этих клеток в качестве мишеней для прогноза и лечения метастатического заболевания. Эффективному использованию жидкой биопсии с количественной и фенотипической характеристикой ЦОК препятствуют несовершенство методологии взятия биологического материала и отсутствие надежных маркеров для оценки метастатического потенциала ЦОК различного происхождения. Многообразие механизмов миграции и инвазии опухолевых клеток требует разработки комплексных терапевтических подходов для антиметастатической терапии, нацеленной на ЦОК. Усилия, направленные на решение этих основных вопросов, могут способствовать разработке новых эффективных стратегий лечения рака.

Ключевые слова: циркулирующие опухолевые клетки; циркулирующие опухолевые микроэмболы; метастазирование; эпителиально-мезенхимальный переход; минимальная остаточная болезнь.