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REVIEW

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TUMOR MICROENVIRONMENT AND METABOLIC FACTORS: CONTRIBUTION TO GASTRIC CANCER

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Malignancy may be characterized as a state formed in the setting of specific tumor-host relationships at the molecular and cellular microenvironment levels. R.E. Kavetsky and his collaborators distinctly outlined the concept of tumor-host interaction. Tumor is a complicated biological system closely connected with the organism, where it arises and develops. Tumor cells are in the environment of different factors that form tumor microenvironment playing an active role in the disease progression. There are two types of tumor microenvironment: the metabolic microenvironment mediated by factors of tumor microphysiology (blood flow, vascular permeability, oxygenation, extracellular pH, interstitial fluid pressure, etc.) and the cellular-molecular microenvironment comprising interactions between tumor cells and non-tumor cells and the factors of the stromal compartment. Factors of tumor microphysiology can modify the interaction between tumor cells and surrounding non-tumor cells and molecular components and they form the tumor profile that influences the pressure of tumor on the host. The review presents the data concerning the role of metabolic microenvironment of tumor cells from the point of tumor-host interaction in order to employ these parameters to working out the methods of diagnosis and prognosis of disease outcome in patients with gastric cancer. Special attention has been paid to hypoxia as a key factor of metabolic microenvironment that positively affects tumor progression, stimulating its aggressiveness, metastasis and resistance to therapy and is regarded as a factor of unfavorable prognosis. It was shown that there is possible clinical relevance of tumor classification based on the level of tumor oxygenation that may be advantageous for selection of patients for individualized therapy that may give the hope for enhancement of treatment efficacy.

***Key Words*: tumor-host interaction, tumor microenvironment, gastric cancer, hypoxia, clinical relevance.**

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"Cancer cells without a favourable environment have no chance to survive"
Prof. Lido Calorini

Problem of oncological disease continues to be one of the most serious at present. During many years, efforts of researchers have been concentrated exclusively on the investigation of tumor cells with firm faith that transcription of their biology would help to explain the nature of tumor growth undervaluating that tumor develops in host organism with complicated mutual relations.

Today it is clearly shown that in the process of initiation and development of malignant tumors that include aggressiveness of neoplasia and ability to metastasis important role belongs to the microenvironment.

Tumor is not autonomous, it is in close interaction with the organism and, what is very dramatically, the effects of tumors on their host are very often more essential than the reaction of the host to tumor development. A. Bogomolets arrived at a negation of tumor autonomy, in the sense of tumor cells being independent of the host, whereby he recognized that metabolic disturbances and the reaction of the body are of utmost importance for the origin and development of pathological processes [1]. It should be emphasized here that he addressed the problems of the etiology and pathogenesis of malignant growth from the point of view that the organism should be considered as being one unity and can itself be recognized as forming one unit with its outside environment. At the same time, he supported the idea that metabolic disturbances and the reaction of the organism are very important in the genesis and development of pathological processes.

R.E. Kavetsky and his collaborators [1] distinctly outlined the concept of "tumor–host" interaction. Having summarized vast amounts of experimental and clinical data, R.E. Kavetsky stated that at first, tumor growth as such is a host reaction to the variety of physical, chemical and biological factors of the environment. Secondly, an autonomy or independence of tumor growth is out of the question due to the mutual relationships between the neoplasm and the host from an early stage of tumor formation.

The molecular aspects of tumor–host interaction became the focus of intense research [2] considering malignant tumor to be a "product" of pathological misbalance in the tissue–cell assembly. Malignancy may be characterized as a state formed in the setting of specific tumor–host relationships at the molecular and cellular microenvironment levels when the "host" participates in the induction, selection and expansion of neoplastic cells, influences tumor growth and modulates its peculiarities, and in turn receives potent impacts generated by the developing tumor resulting in the change of function of many systems of the organism. The locally activated "host" microenviron-

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Abbreviation used: HIF – hypoxia-inducible factor; VEGF – vascular endothelial growth factor.

ment (cellular and non-cellular components) modifies the proliferative and invasive behavior of tumor cells resulting generally in an increased aggressiveness of the primary tumor and malignant tumor progression. Tumor and host are considered as a unified whole where tumor is only a part that has to be annihilated.

McAllister and Weinberg [3], while summarizing the recent advances in tumor–host relationships declared that many aspects of tumor biology can only be explained by a detailed understanding of both local and systemic interactions.

Different physiological-biochemical reactions that promote the vitality of tumoral as well as normal cells form the basis of the metabolic mechanisms of tumor–host interaction. This aspect of the tumor's relationship with the host is based on the microenvironment of tumor cells, which, as it is clear from numerous investigations, is the dominant factor in the "tumor–host" dialogue.

Tumors are more than insular masses of proliferating cancer cells. Instead, they are complex tissues composed of multiple distinct cell types that participate in heterotypic interactions with each other. That is a complicated biological system closely connected with the organism, where tumor is initiated and develops. Conception of the tumor microenvironment or more specifically, the molecular-cellular microenvironment of tumor cells has been developed.

Biology of tumors can no longer be understood simply by enumerating the traits of the cancer cells but instead must encompass the contributions of the "tumor microenvironment" to tumorigenesis [4]. The interactions between cancer cells and their micro- and macroenvironment create a context that promotes tumor growth and protects it from immune attack. The functional association of cancer cells with their surrounding tissues forms a new 'organ' that changes as malignancy progresses. Thus, targeting tumor environment is necessary for a helpful and successful treatment of cancer.

Tumor cells are in environment of different factors that form tumor microenvironment, namely cellular-molecular microenvironment that represents sophisticated "network" of different components, their multiple functions and activities. That is the complex of interactions between cells (tumor, stromal and immune cells), the acellular matrix and soluble factors within malignant tumors and which are known to be a key player in modulation of the metabolism, tumor growth, progression and metastasis to distant sites, the development of acquired treatment resistance, and finally for poor patients prognosis [5].

METABOLIC FACTORS OF TUMOR MICROENVIRONMENT

Two types of microenvironment for tumor cells are commonly referred to and these should be distinguished from one another since they are different in their respective nature: the cellular-molecular microenvironment comprising interaction between tumor cells and non-tumor cells and factors of the stromal compartment. This micromilieu is relevant for "dialogue between tumor cells and the host tissue" (i.e. stroma). Moreover, the metabolic microenvironment is mediated by factors of tumor microphysiology (oxygenation, extracellular pH, blood flow, vascular permeability, interstitial pressure, etc.). Factors of tumor microphysiology can modify the interaction between tumor cells and surrounding non-tumor cells and molecular components and moreover, they form the tumor profile and influence the pressure of tumor on host and mediate the tumor–host interactions [6].

At present four types of tumor microenvironment are under consideration: pathophysiological, metabolic (metabolic micromilieu), stromal, and immunological (active elements).

Much attention of scientists is concentrated on tumor microphysiology, in particular on metabolic microenvironment of tumor cells and its significance for tumor growth as well as its impact on anticancer therapy. Among the factors of the metabolic microenvironment, the poor oxygenation, i.e., hypoxia, is the dominant parameter representing a common feature of solid tumors which is recognized as a main factor in tumor aggressiveness and progression, being as a predictor of poor prognosis in a variety of solid human tumors. Whereas normal cells modulate anabolic and catabolic pathways in response to changes in nutrient availability, cancer cells exhibit unregulated growth even under nutrient scarcity and can survive in poorly vascularized areas despite the low oxygen tension [7]. Low oxygen tension or hypoxia is a determining factor in the course of many different processes in animals, including those when tissue expansion and cellular metabolism result in high oxygen demands that exceed its supply. This is mainly happening when cells actively proliferate and the proliferating mass becomes distant from the blood vessels, such as in growing tumors [8].

Owing to abnormal structure and function, tumor vessels are unable to deliver adequate levels of nutrients and oxygen to tumors and to remove acidic waste products out of tumors. The regions far from blood vessels become chronically hypoxic. Blood flow in tumor vessels is temporally heterogeneous. Even the presence of blood flow does not guarantee the delivery of oxygen in solid tumors. Some of the perfused tumor vessels carry almost no oxygen and there is no clear relationship between blood flow rate and oxygen tension of individual tumor vessels [9].

Hypoxia underlies many of the processes associated with cancer progression including tumor cell survival and proliferation, genetic instability, immune responses, angiogenesis, invasion and metastasis and metabolic adaptive responses. Four possible levels of intervention have been described for hypoxia as a driving force in malignant progression: at transcriptome level, leading to hypoxia-induced transient changes in gene expression coordinated by a special set of hypoxia-responsive transcription factors such as hypoxia-inducible factors (HIFs), that are overexpressed during hypoxia and involved in metastasis formation; at proteome-metab-

olome level, via adaptive gene expression [10]; at genome/epigenome level, hypoxia can induce genome and epigenome instability driving malignant progression; at the cell population level, severe hypoxia can promote tumor aggressiveness by clonal selection and clonal expansion according to phenotype fitness [11].

Cellular response to hypoxia depends on HIFs, which are stabilized under low oxygen conditions. HIFs represent a highly evolutionarily conserved family of dimeric transcription factors that are central to mediating the cellular response to hypoxia by regulating the expression of a diverse array of targets. In a hypoxic context, various inducible HIF-α subunits are able to form dimers with constant beta subunits and bind the hypoxia response elements in the genome, acting as transcription factors. Typically, the HIF pathway has been shown to enhance vascular endothelial growth factor (VEGF) expression, which would be responsible for angiogenesis and, therefore, re-oxygenation of the hypoxic sites. The regulation of angiogenesis by hypoxia is an important component of homeostatic mechanisms that link vascular oxygen supply to metabolic demand. VEGF is one of the most important proangiogenic factors secreted by tumor cells [12]. Hypoxia and HIF activation are associated with treatment failure, resistance and poor clinical outcomes.

However, physiological hypoxic conditions occur during early embryonic development; and in adult organisms, many cells such as bone marrow stem cells are located within hypoxic niches. Embryonic stem cells inhibit a severe hypoxic environment, which dictates their glycolytic metabolism, whereas differentiated cells shift toward the more efficient aerobic respiration for their metabolic demands. Thus, certain processes take place in hypoxia, and recent studies highlight the relevance of hypoxia in stem cell cancer physiology [13].

The physiology of tumors is uniquely different to that of normal tissues. Significant regions of tumors often grow in hypoxic conditions owing to the lack of a functional vasculature, and cancer cells can survive in these areas despite the low oxygen tension. The adaptation to hypoxia requires both biochemical and genetic responses that culminate in a metabolic rearrangement to counter-balance the decrease in energy supply from mitochondrial respiration [14]. Metabolic reprogramming serves to generate biosynthetic precursors, thus facilitating the survival of rapidly proliferating malignant cells. Metabolic changes in response to hypoxia are elicited through both direct mechanisms, such as the reduction in ATP generation by oxidative phosphorylation or inhibition of fatty-acid desaturation, and indirect mechanisms including changes in isozyme expression through hypoxia-responsive transcription factor activity [15].

In the hypoxic microenvironment of cancer cells, metabolism shifts from oxidative phosphorylation to anaerobic glycolysis. Under aerobic conditions, normal cells process glucose, first to pyruvate via glycolysis in the cytosol and thereafter to carbon dioxide in the mitochondria; under anaerobic conditions, glycolysis is favored and relatively little pyruvate is dispatched to the oxygen-consuming mitochondria. Malignant transformation of cells leads to enhanced glucose uptake and the conversion of a larger fraction of pyruvate into lactate, even under normoxic conditions [16]. The ability to switch from a predominantly oxidative metabolism toward nonoxidative glucose fermentation with production of lactate even when oxygen is plentiful is a key characteristic of cancer cells, and affects not only tumor cell growth but also tumor cell migration. This metabolic switch, known as the "Warburg phenomenon" is the process that was dubbed as "aerobic glycolysis". That is a unique phenomenon occurring in almost all malignant neoplasia, with activity being seen to increase with tumor progression and consisting in an increase in glycolysis maintained in conditions of high oxygen tension. HIF-1α-dependent glycolytic genes are readily induced in tumor cells at mild hypoxia, low enough to induce HIF1α activity but high enough to support the oxidative phosphorylation. Therefore, to maintain their energetic needs, cancer cells show an increased glucose uptake through an enhanced expression of HIF-1α-dependent glucose transporters; this is useful for diagnostic purposes, as in the case of monitoring uptake of glucose analogue tracer 18-fluorodeoxyglucose (FdG) by positron-emission tomography [17].

It is worth to notice that glycolysis typically boosted in cancer cell as part of the "Warburg effect" regulates YAP/TAZ activity. YAP and TAZ are highly related transcriptional regulators pervasively activated in human malignancies and able to reprogram cancer cells into cancer stem cells and incite tumor initiation, progression and metastasis. They are essential to trigger numerous cell-autonomous responses, such as sustained proliferation, cell plasticity, therapy resistance and metastasis and recognized as a hub of the network of signals exchanged within the tumor microenvironment that provides a fresh perspective on the molecular principles of tumor self-organization, promising to unveil numerous new vulnerabilities [18].

A dramatically enhanced glycolysis under hypoxia results in the formation of a large lactate content that accumulates in tumor tissue due to defected local perfusion. Among the soluble factors present in the tumor microenvironment lactate is of particular importance.

The sequence of the enzymatic reactions that take place during glycolysis was described in 1940 by Embden, Meyerhof, and Parnas, and, since then, "lactate has regularly been vilified as a useless and frequently toxic end product of anaerobic glycolysis" [19]. It has emerged long ago that lactate is not a waste metabolic byproduct at all but rather a bioenergetic substrate. More recently lactate has been regarded as regulatory molecule with signaling properties that directs the metabolic reprogramming of tumor cells and modulates the integration of metabolism [20]. In particular, lactate activates HIF-1 and triggers tumor angiogenesis and

tumor growth; an activity that was found to be under the specific upstream control of the lactate transporter monocarboxylate transporter 1 expressed in tumor cells. Having immunosuppressive role, lactate affects several immune cell functions such as T-cell proliferation, cytokine production, cytotoxic activity of $CD8^+$ T cells, etc. [21]. Recently, Zhang et al. [22] have shown that $CD8^+$ T cells enhance signaling of peroxisome proliferator-activated receptor alpha and fatty acid catabolism under the acidity, nutrient deprivation, and hypoxia condition to partially preserve effector functions. Such metabolic reprogramming of T-cells using a peroxisome proliferator-activated receptor alpha agonist improves tumor growth control, which is enhanced in combination with PD-1 blockade [22]. Lactate was considered as a "comprehensive" marker of various malignant traits including metabolic activity, hypoxia, acidosis, microcirculation and other factors [23]. The evidence for lactate as a major player in the coordination of whole-body metabolism has since grown rapidly. Lactate has been described to modulate enzymes catalytic properties to affect hormonal release and responsiveness, and to control body homeostasis. Moreover, these properties are directly related to the genesis and the sustainability of pathological conditions such as diabetes and cancer. Lactate should be considered as a regulatory molecule that directs the metabolic reprogramming of tumor cells thereby serving as an additional selective pressure and modulates the integration of metabolism [20, 24]. It was postulated that lactate is a cell-signaling molecule, "lactormone", that can upregulate gene and protein expression [25]. Lactate content in the primary tumor was assumed to be a basis for a new tumor metabolic classification that may improve the efficacy of cancer patient treatment [26, 27]. The possible role of lactate as a predictive biomarker of overall survival in cancer patients arises from numerous studies that lactate intratumoral levels are inversely correlated with overall and disease-free survival [28–30].

As a consequence of aerobic glycolysis by involving an enormous amount of glucose in metabolism the tumor accumulates an excess of lactic acid which leads to sharp self-acidification of the extracellular microenvironment while intracellular environment remains alkaline by means of reversed pH gradient across the cell membrane between intra (pHi) and extracellular (pHe) compartments as compared with normal cells [31]. The most notable pHi regulatory system of tumor cells is the Na^+/H^+ exchanger, known to play a key role *in vivo* in tumor development, in particular when highly glycolytic cells produce large amounts of lactate. Acidic pH of the tumor microenvironment represents a niche engineering strategy that promotes local invasion, whereas alkalinized intracellular pH is as adaptive feature that promotes cell survival thereby maintaining cell proliferation. The outer acidic pH gradient of cancer cells originates as a response to the metabolic adaptation to hypoxic tumor milieu [32, 33].

The hypoxia and acidosis-induced phenotype promote DNA instability and lead to selection of tumor cells bearing supplementary genetic defects that prompts their plasticity and progress. Extracellular acidity drives mutagenesis and invasiveness through the pH-dependent activation of cathepsins and promotion of upregulation of matrix metalloproteinase-2 and -9, VEGF, interleukin 8, suppression of tumoricidal activity of cytotoxic lymphocytes and natural killer cells, clonal selection of cells with high invasive capacity associated with a poorer prognosis [34, 35]. Co-existence of hypoxia and acidosis complicates the interpretation of the role of pH in tumor progression. Moreover, simultaneous high-resolution mapping of tissue hypoxia and pH revealed that there is a lack of spatial correlation among these parameters. These findings have significant implications since both hypoxia and pH are important determinants of tumor growth, metabolism, and response to a variety of therapies.

According to the concept of Kavetsky [6], two linked processes form the basis of carcinogenesis, namely local formation of cancerous tissue with subsequent transformation into a genuine malignant tumor that growth by means of infiltration, and more general alteration in the organism which under definite condition provide an opportunity for the primary tumor to spread in the organism through metastasis.

The occurrence of metastasis in solid tumors signals the breakdown of normal tissue homeostasis and dramatic rearrangement of tumor–stromal interactions. It has been increasingly recognized that hypoxia is a powerful driving force for such transitions in tumor progression [36]. For this complex event to occur, the cells must invade their surrounding tissue, enter the bloodstream and colonize another location, where secondary tumors called metastases form. The capacity of tumor cells to escape from the primary tumors is determined by the deregulation of cell migration during cancer progression [16]. Hypoxia can modify the tumor microenvironment in a way beneficial for selecting cells that are able to survive and proliferate during periods of oxygen and nutrient deprivation, thereby encouraging metastasis. Metastasis is a multistep process that can be influenced by both the immediate microenvironment (cell–cell or cell–matrix interactions) and the extended tumor microenvironment (for example vascularization) involving reciprocal interplay between cancer cells and host stroma. Hypoxia increases tumor invasion and metastasis by activating relevant gene expressions through HIFs-1 which activate the transcription of over 100 genes involved in a range of processes, including angiogenesis, erythropoiesis, metabolism, apoptosis, and proliferation [37].

Hypoxia in the primary tumor may influence the metastatic seeding at distant organs even before tumor cell dissemination. In some types of cancer, the primary tumor may release systemic suppressor factors that render micrometastases dormant,

as revealed clinically by explosive metastatic growth soon after resection of the primary growth [38]. Clinical evidence concerning the dormancy of tumor has become available due to investigation, which focused on events of cancer recurrence after primary surgery [39]. Cancer cell dormancy is a relevant problem because patients with cancer can develop recurrent metastatic disease with latency periods that range from years even to decades. This pause can be explained by cancer cell dormancy, a stage in cancer progression in which residual disease is present but remains asymptomatic. Kaplan *et al.* [40] led to the insight that primary tumors can influence and prepare the microenvironment of secondary organs for future metastatic growth, even before tumor cells arrive at these sites, by forming in distant organs so-called premetastatic niches by hematopoietic bone marrow derived cells under the influence of soluble factors released from primary tumors [41] capable of inducing a fertile microenvironment that favors the seeding and proliferation of metastatic cells at unique sites and that is stipulated by acidic pH of the tumor microenvironment. Among these factors, chemokine receptor CXCR4 is considered as a homing protein that participates in the migration of tumor cells into the new sites to prolong the growth and form metastases. CXCR4 plays a key role in metastatic homing of tumor cells to organs expressing high level of its ligand SDF-1α [42]. CXCR4/high SDF-1α expression is significantly higher in patients with advanced stages of the disease with the worst prognosis [43]. In the tumor microenvironment under hypoxic condition there is possible mechanism by which cells of a growing tumor are reprogrammed to express the CXCR4 receptor thereby enhancing the metastatic potential of the tumor cells [44].

Recently the link has been shown between hypoxia and CXCR4 expression in breast cancer suggesting "that hypoxic condition selects the tumor cells which go on to proliferate and metastasize by activating the expression of CXCR4 in these cells" [45]. Hypoxia may increase metastatic homing by inducing CXCR4 expression in renal cell carcinoma [46], thyroid cancer [47], and colorectal cancer [48].

The extensive data characterizing the tumor pathophysiology, especially tumor microenvironment allow to start to understand the various interrelationships between tumor and host that mediate the tumor progression and host response to neoplasia. It may be suggested that metabolic microenvironment of tumor cells (hypoxia, low extracellular pH, low perfusion, etc.) is a key factor determining the character and direction of tumor-host interactions.

A better understanding of the relationships between hypoxia-associated signaling pathways, metabolic peculiarities that positively influence tumor progression may elucidate not only how the aggressive tumor phenotype is formed but also may assist in the development of new approaches for the treatment of cancer patients.

CONTRIBUTION OF HYPOXIA AS A FACTOR OF TUMOR MICROENVIRONMENT IN GASTRIC CANCER: CLINICAL STUDY

Our investigations have been devoted to the role of metabolic microenvironment of tumor cells from the point of "tumor–host" interaction in order to employ these parameters to working out the methods of diagnosis and prognosis of disease outcome of patients with gastric cancer. Special attention has been paid to such key factor of tumor microenvironment as hypoxia because clinically it is very relevant that tumor hypoxia may help to select patients for individualized therapy that gives the hope for enhancement of treatment efficacy [49].

It was determined that gastric cancer is characterized by three levels of hypoxia: severe, moderate and week (satisfactory oxygenation) [50] that has not only prognostic significance, but may be as additional criterion for choice of treatment methods. Clinically, tumor hypoxia is independent of tumor size, stage, histological diagnosis, and tumor grade. Overall survival of patients with tumors characterized by severe and moderate hypoxia was shorter as compared with patients with satisfactory oxygenated tumors. Moreover, the inefficacy of surgical treatment alone in patients with hypoxic tumors was shown. Both neoadjuvant and adjuvant chemotherapy used for patients with satisfactory oxygenated tumors were found to be more effective. Thereby it may be confirmed that hypoxia being a factor of microenvironment decreases the efficacy of neoadjuvant as well as adjuvant chemotherapy that reflects the negative influence on neoplasia response to cytostatic chemotherapy [51].

The hypoxia in primary tumor corrects the well-known fact of negative impact of regional metastasis on disease outcome. It was revealed that the risk of death increased by more than twice for patients with gastric cancer with N_0 category and when primary tumor was characterized by severe hypoxia that indicates, probably, the necessity to use adjuvant chemotherapy in patients with hypoxic tumor even with "negative" lymph nodes, i.e. without diagnosed regional metastases. High level of tumor hypoxia may be a possible marker for an unfavorable prognosis on patients with lymph node-negative disease, having prognostic significance and may be used as an additional criterion for choice of treatment methods [52].

Hypoxic status that has been detected in adjacent histological uninvolved gastric mucosa in gastric cancer patients (before the morphological alterations in mucosa) may be assessed as neoplastic transformation. Observed metabolic rearrangement in gastric mucosa may be influenced by tumor, which affects normal tissues confirming the existence of active tumor–host interaction. Overall survival of patients with gastric mucosa characterized as hypoxic was significantly poorer as compared with patients with gastric mucosa characterized as oxygenated [53]. Therefore, hypoxia level in primary tumor may be also proposed as an independent prognostic factor of treat-

ment efficacy as well as clinical outcome in patients with gastric cancer.

The inverse correlation was found between hypoxia and lactate levels in gastric cancer tissue when the tumor tissue was hypoxic. But the important observation should be noted that the interrelation between hypoxia and glycolysis is very complicated. It was established that when favorable disease prognosis could be expected due to a mild hypoxia level in primary tumor, i.e. gastric cancer tissue is considered to be satisfactory oxygenated, its high lactate content caused a two-fold higher risk of unfavorable disease outcome that emphasize the clinical relevance of evaluating both hypoxia and lactate levels in human tumors, in particular in gastric cancer for the appropriate management of patients [6]. It was determined that high lactate content correlated with malignant progression only at an early stage of disease that may be explained by extended areas of necrosis in tumor at more advanced stage while lactate is a product of vitality cells setting in well perfused regions [54, 55].

MINIMAL RESIDUAL DISEASE

It is known that tumor cells spread from their primary site into the bone marrow and can exist in bone marrow within long-time period before the formation of metastases in distant sites. It implies that bone marrow might be a reservoir for disseminated tumor cells from where they may recirculate into circulating system and then colonize in other distant organs.

Tumor cells in bone marrow of patients with gastric cancer were found in 90% of cases where severe hypoxia was evident in primary tumor, whereas under moderate hypoxia tumor cells were found in 20% of cases. There is a significant correlation between frequency of disseminated tumor cells in bone marrow and the level of tumor hypoxia. In patients with category M_0, the probability of appearance of tumor cells in bone marrow was increased by a factor of 11.8 when tumors were characterized by severe and moderate hypoxia [56]. These data indicate a possible positive impact of hypoxia-associated signaling pathways on the escape of tumor cells from the primary tumor and their settling in the bone marrow.

The suggestion concerning CXCR4 participation in the homing may be partly confirmed by our finding that CXCR4-positive cells in tumor were detected in 80% of patients with the presence of disseminated tumor cells in bone marrow and in patients with M_0 — in 63%. The last finding may be discussed in the context of predisposition of bone marrow to be a new site for tumor cells by CXCR4 expression. Presence of $CXCR4^+$ cells in bone marrow was associated with overexpression of homing-protein CXCR4 in tumor that are regulated by primary tumor hypoxia of gastric cancer patients: the probability of the appearance of high number of CXCR4-positive cells in hypoxic tumor is increased by a factor of 5.

The obtained results have allowed suggesting that the expression of CXCR4 plays an important role in the migration of tumor cells from primary tumor and homing in the distant organs that is regulated by the hypoxia [36]. We have determined also that in the patients with bone marrow as well with tumors characterized by CXCR4-negative status, the overall survival was longer [57] and overall survival of patients with category M_0 with disseminated tumor cells in bone marrow was shorter than in patients with category M_0 and without disseminated tumor cells in bone marrow.

The detection of disseminated tumor cells in bone marrow at primary diagnosis of cancer predicts an unfavorable prognosis and may be considered as obligatory procedure before the decision concerning further treatment, in particular of patients with category M_0 [58].

Understanding of the new aspects of molecular mechanisms and signaling pathways controlling tumor cell migration is critical for development of therapeutic strategies for cancer patients. Clinical trials using these approaches will require careful assessment of the tumor microenvironment using imaging or other techniques in order to incorporate hypoxia assessment as a part of a standard of cure. This approach will serve well to be one step closer to individualized cancer medicine and improved patient outcome.

The mechanisms that can control the long-term persistence of disseminated tumor cells in bone marrow before the relapse or metastasis remain unclear. The answer to this question could help to develop the methods to control the tumor dormancy. Farrar *et al.* [59] demonstrated that CD8-positive, but not CD4-positive, T cells are required for the maintenance of dormancy in BCL1 Ig-immunized BALB/c mice. The authors suggested that CD8-positive T cells via endogenous production of interferon-γ in collaboration with humoral immunity can induce and maintain the tumor dormancy. Mahnke *et al.* [60] have suggested that the bone marrow microenvironment has special features for the maintenance of tumor dormancy and immunological T-cell memory. Recent publications presented data confirming this suggestion [61, 62]. In this context, there is very important question about the participation of memory T cells occupied bone marrow in the control of tumor dormancy.

We have shown that the number of lymphocytes in tumor of gastric cancer patients is in correlation with hypoxia level. Low density of $CD8^+$ T cells infiltration is associated with severe and moderate hypoxia in tumor tissue and with increased incidence of disseminated tumor cells in bone marrow. But we have failed to find any association between the presence of $CD8^-$ and $CD45RO^-$ T lymphocytes in bone marrow and hypoxia in primary tumor as well as with disseminated tumor cells in bone marrow. It may be suggested that the entry of the tumor cells into bone marrow is not linked with the presence and activity of CD8 and CD45RO T cells, but most likely CD8 and CD45RO T cells determine the subsequent behavior of disseminated tumor cells. At the same time the association between presence of CD8 and

CD45RO T lymphocytes and survival was shown: high density of $CD8^+$ and $CD45RO^+$ T cells in primary tumor or high density of $CD8^+$ and $CD45RO^+$ T cells in bone marrow are the most favorable for patients. These data may allow to consider intratumoral CD8 and CD45RO T lymphocytes as a "keeping factor" that prevents the exit of tumor cells from primary node, dissemination in the circulation and hit into the distant organs, in particular bone marrow, and allow to assume that tumor cells in bone marrow may be existed in a dormant state through the control by T cells in particular by CD8-positive T cells [63, 64].

The reaction of the organism to the development of pathological processes has been also observed by our investigations. It was shown that concentration of phospholipids in blood serum as well as hemoglobin concentration are affected by the level of primary tumor hypoxia.

PHOSPHOLIPID LEVEL IN BLOOD SERUM OF PATIENTS WITH GASTRIC CANCER

It is well known that phospholipids play an important role in biological events of progression of cancer. Significant decrease in the phospholipid level in serum of patients with acute leukemia, malignant lymphomas, renal cell carcinoma, digestive tract tumors at the time of diagnosis displayed a good correlation between stage of disease in patients responding and non-responding to therapy. It was shown that statistically significant change of lysophosphatidylcholine level is the most sensitive indicator in the monitoring of treatment and forecasting resistance to therapy as well as the possibility of evaluating advancement of disease [65].

In our investigation it was determined that level of lysophosphatidylcholine in blood serum of patients with gastric cancer depends on hypoxia in primary tumor and moreover the probability of appearance of tumor cells in bone marrow is increased by a factor of 6.0 under such conditions. It is suggested that changes of lysophosphatidylcholine concentrations in blood serum of patients with gastric cancer may be as relevant biomarker of tumor progression for monitoring of disease outcome and may be of great importance to predict the recurrence especially under individualized therapy [66, 67].

CANCER-RELATED PRETREATMENT ANEMIA

An assumption concerning the destructive impact of a tumor on its host, leading to anemia and general weakness and sometimes even to serious constitutional consequences was made by Coats and Sutherland [68]. Numerous investigations have been devoted to the pretreatment anemia in patients with malignancy that has come to be as a relatively common condition with the actual incidence largely dependent on the type and extent of the malignancy especially among those with more advanced disease, and it is referred to as "anemia of chronic disease", that is the second most prevalent after anemia caused by iron deficiency [69]. It is the most common paraneoplastic syndrome, particularly its hypoproliferative forms that seems also to impair tumor responsiveness to radiotherapy and surgery [70]. The severity of cancer-related anemia has been associated with more aggressive tumors [71] and more aggressive tumors are hypoxic that have the worst prognosis and the most likely metastasis associated with shorter survival time in cancer patients with lung carcinoma, uterine cervical carcinoma, head and neck carcinoma, prostate carcinoma, lymphoma, and multiple myeloma [72]. However, it was not clear whether a direct correlation between anemia and tumor oxygenation exists. Vaupel et al. have shown deterioration in tumor oxygenation status in patients with hemoglobin concentration below 12 g/dl and that anemia (found in patients at diagnosis) considerably contributes to the development of hypoxia, especially in low-flow tumor areas. In tumors of moderately or severely anemic patients, hypoxic areas are found more frequently than in non-anemic patients. A pivotal part in worsening tumor oxygenation in cancer patients is played by anemia arising as a result of cancer cell products [73].

In our recent investigations we have shown an association between pre-operative anemia in gastric cancer patients and level of hypoxia in primary tumor: a probability that pre-operative anemic patients have hypoxic tumors is increased by a factor of 3.0 but for patients with obesity such probability is increased by a factor of 7.13. The results obtained have shown that preoperative anemia is related to tumor aggressiveness due to a link between preoperative hemoglobin concentration and level of tumor hypoxia, level of VEGF expression as well as vascularization in primary tumor. Therefore, pre-operative anemia may be a sign of a destructive impact of a tumor on its host and at an increased risk of failure independent of the treatment modality and may help to pave the way for more effective treatments. Up today, pretreatment hemoglobin level in patients with malignancy is a determining factor in the prognosis of treatment outcome.

However, there remains an urgent need for the identification and evaluation of new factors and markers to assist in early diagnosis and disease prognosis to guide clinicians in providing treatment appropriately.

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