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REVIEW

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TUMOR CELL HETEROGENEITY

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The paper deals with the analysis of literary data on the tumor cell heterogeneity. Phenotypic, genetic and epigenetic mechanisms of heterogeneity are considered. The heterogeneity of metastasis is considered too. The importance for the biology of populations of tumor cells and the sensitivity of tumors to therapeutic treatment are discussed.
***Key Words*: primary tumor, metastasis, heterogeneity, malignant phenotype, genetic instability, epigenetic factors.**

INTRODUCTION

Subject or system is called heterogeneous, if it consists of numerous varying units/components, which often cannot be easily sorted or divided. The term "tumor heterogeneity" supposes the existence of numerous differences between cells in tumor, cells of primary tumor and metastases, cells of particular metastases of the same tumor [1]. In 1977, the respected journal "Cancer Research" didn't take in print the article, authors of which, having obtained and characterized 4 different subpopulations of tumor cells (TC) from one random mouse mammary tumor, had stated that these data were the evidence of tumor heterogeneity, and that such heterogeneity was the common phenomenon. The editors have rejected the article and stated that tumor monoclonality is a notorious fact [2]. At the present time it was shown that the majority of tumors possess variability in wide range of morphological and functional characteristics. It was also defined that heterogeneity (pleomorphism) of TC affects phenotypic, genetic and epigenetic features. While tumor's progression, its cells undergo number of various changes [2, 3]. At the same time, series of fundamental questions concerning the causes of tumor heterogeneity, mechanisms of its formation, significance of this phenomenon for evolution of TC population and development of cancer, remain open-ended. TC heterogeneity needs further study and analysis from the position of clinical oncology — for improving of diagnosis and treatment methods. The accumulated information in this field of study is like a bowl, in which incomparable terms are melting and new concepts are maturing.

The aim of this paper is generalization and analysis of data on manifestation forms, causes and mechanisms of formation of TC heterogeneity as well as points of view on this problem, ideas and concepts from the positions of diagnosis and treatment optimization of cancer patients.

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Abbreviations used: TC – tumor cell; GI – genetic instability; Eg – epigenetic.

PHENOTYPIC TC HETEROGENEITY: PHENOMENOLOGY, POSSIBLE CAUSES AND BIOLOGICAL VALUE

There are following forms of heterogeneity of tumors: intertumoral heterogeneity, when different (primarily multiple) tumors in the same organ have different phenotype; intratumoral heterogeneity supposes that every particular tumor consists of phenotypically and functionally heterogeneous TC with unequal behavior [3]. Inasmuch development of primary multiple tumors is quite rare phenomenon, the main corpus of studies on problem are devoted to the intratumoral heterogeneity; in our review we also confine ourselves to inquiry into mentioned phenomenon only.

If we catalogue defined in various researches parameters of tumor heterogeneity, it becomes obvious that intensity of the latter depends on etiology of tumor, its histogenesis, localization in organ. Heterogeneity is typical for both actually TC and components (cellular and noncellular) of tumor microenvironment; in other words, parenchyma, and tumor stroma also may be heterogeneous. According to the publications of the past years, TC of one neoplasm may differ both by the morphological (degree of differentiation, sizes, form, number of nucleus, cytochemical features, karyotype, etc.) and by functional characteristics (morphogenetic reactions, level of proliferation, cell-cell interaction, mobility, invasiveness, inclination to metastasis, sensitivity to inducers of apoptosis, chemotherapeutic agents, and immunotherapy). Heterogeneity of TC is described by composition of cellular membranes; its antigenicity; spectrum of markers of cellular surface, including receptors of growth factors; by activity of signaling pathways, which are controlling proliferation, cellular cycle, DNA reparation, apoptosis, functional response of cells to changes of extracellular environment [2–5].

The parameters of TC growth *in vitro* and tumorigenicity *in vivo* essentially vary (including amount of injected cells, necessary for generating of tumors; latent period and speed of growth of the last) as well as sensitivity to non-specific reactions of antitumor immunity and ability of induction of the host's specific immune response. At the same time, changes of the age and hormonal status of host's organism influence the TC differences [2, 3, 6, 7].

Clonal TC populations are also heterogeneous. It is shown that by the injection of each TC clone in the athymic mice the histologically different tumors may grow. Such heterogeneity is considered to occur mostly in result of phenotypic plasticity and different differentiation of stem TC under the influence of micromedia signals and, probably, some stochastic cell-autonomous mechanisms. Relative impact upon heterogeneity of hereditary and non-hereditary mechanisms is still not clear [8].

Neoplasms or their various segments may also differ by composition of extracellular matrix, cellular and noncellular components of conjunctive tissue (tumor's stroma); by quantity and types of immune system cells infiltrating tumor parenchyma; degree of vascularization (both by blood and lymphatic vessels), by metabolic features of microenvironment [9]. In other words, even in one tumor TC get different signals of microenvironment, which may change phenotype of formerly similar cells [10, 11].

At the same time, it was shown that together with phenotypic differences, which originate as response to changes of environment (TC micromedia), heterogeneity of TC even in the presence of obviously homogenous microenvironment is also possible. For example, genetically homogenous lines of TC may manifest morphological heterogeneity (combination of circular, unable to move epithelioid cells and mobile fibroblast-like cells, which may be detected both *in vitro* and *in vivo*), that is the result of different mutually exclusive and interconvertible activation of G-proteins Rac and Rho [12]. The variability of TC of different primary tumors of the same organ, as well as of each individual tumor, doesn't exhaust all aspects of tumor heterogeneity.

Taking into consideration above-stated information, the following questions are of current importance: a) what is clonal relationship between primary and metastatic tumors: whether cells of metastases are direct derivatives of clones of developed primary tumors or they deviate in the early stages of tumor evolution?; b) what is stage of heterogeneity of metastatic tumors compared to primary, whether they are more or less clonally heterogeneous?

There are 2 main concepts of origin of TC heterogeneity: different subtypes of TC originate from different stem cells (concept of stem cell); different subtypes of TC originate as the result of unmatched genetic and/or epigenetic changes of stem (target) cell (concept of clonal evolution). Each of these concepts is being studied for a long time. Almendro et al. [10] consider that despite concepts of stem cell and clonal evolution (phenotypic plasticity) have lot of similarities, they are mutually exclusive as fundamentally different. However, according to the view of number of researchers, the detected cellular and molecular mechanisms are not mutually exclusive and may act together. When heterogeneity of certain tumors is forming, both concepts (or each of them) may be fair within certain degree. Even if majority of TC in some (or many?) tumors are not able to support proliferation and, thus, may be identified as non-stem, the compartment of stem cell must be phenotypically different and plastic [11].

It is also important to mention that epithelial-mesenchymal transition refers to the complex molecular and cellular programs, which are defying features of heterogeneity and differentiation (intercellular adhesion, apico-basal polarity or its absence, absence of mobility) of epithelial cells as well as acquisition by them the mesenchymal functions (mobility, invasiveness, increase of resistance to apoptosis). The last at large is analyzed in reviews [13–15] which interested reader is being referred to.

In addition, information about synchronic change *in vivo* of some heterogeneous features of TC, which have absolutely different molecular basis, and about coincidence of spectrum of such features at antitumor impacts of different origin, is being accumulated bit by bit. So, simultaneously several features, which are defying resistance of TC to cytotoxic effectors of innate immunity, inherent to malignant (especially metastatic) phenotype of cells of many tumors. This secretion of prostaglandin E_2 (causes the suppression of activity of natural killers, T-lymphocytes, neutrophils) and activation of mechanisms of catabolism H_2O_2/superoxide radicals, in particular, catalases and redox cycle of glutathione (provides protection of products of "oxygen explosion" of macrophages, neutrophils) [16]. At the same time, activation of glutathione system refers to the characteristics, heterogeneously expressed in population of TC in process of formation of drug resistance, in particular, to alkylating agents or cisplatin [17]. The examined examples illustrate the idea that heterogeneity of TC is the reflection of their natural selection by many properties, connected with survival *in vivo*. The acquired in organism resistance of TC to its defense reactions, relative resistance to hypoxia, resistance to radial and drug (including targeted) therapy are connected with different mechanisms, but as phenomena, which are conditioned by selection, have common biological nature. Heterogeneity of tumor is necessary condition of opportunity of such selection.

So, at present time there are no doubts that tumors are not static neoplasms. They start from genetically normal cell and end with forming of population, which consists of billions of TC, which have formed multitude of cellular phenotypes. The presence of many interactive subpopulations (both TC and cells of microenvironment) forms the basis of phenomenon "progression, dissemination and colonization of tumor", when during the time the tumor undergoes heterogeneous changes of its properties. It is obvious that knowledge of features of particular clones of TC is not enough for prediction of tumor's behavior as a whole [18]. It is amazing that despite the apparent heterogeneity of tumors, they often remain relatively stable during the development from localized form to metastases and even to the last stage of disease [19, 20].

MOLECULAR MECHANISMS OF TUMOR HETEROGENEITY

Mechanisms of diversification of TC may be similar (or identical) to the normal diversification in embryonic and postembryonic periods of organism development. As it was stated above, many researchers think that one of the basic causes of heterogeneity of TC population is high changeability. The last one is connected at least with 3 mechanisms: with increase of frequency of true genetic changes, which are fixed in line of cellular generations (genetic/genome instability); with significant increase of probability of origin of TC epigenetic changes, which may result in suppression of expression of one genes and/or intensification of expression of others; with presence of stochastic variability of expression of homological proteins in particular genetically similar cells at the same conditions of environment (Elowitz genetic noise) [21].

Genetic instability (GI) mainly includes 2 types of abnormalities: gene mutations (mutation instability, which is linked with changes in consequence of DNA nucleotides) and rebuildings of chromosomes (chromosomal instability, which originates from their fallacious rearrangements). The structure and number of mutations and changes of chromosomes during the time are changing in TC compared with normal cells [22]. In some human TC were described also other forms of GI, in particular, microsatellite instability, which is characterized by increase or decrease of oligonucleotide repeats, existing in microsatellite sequence of genome [23, 24], as well as kind of GI, for which the increase of frequencies of base pair is typical [25]. In TC and normal tissue are observed differences between microsatellites of the same locus. Destabilization of microsatellite loci, apparently, is not being direct cause of malignization; however, it may be sensitive marker of mutator phenotype, manifestation of GI and one of the features of TC heterogeneity.

GI is characteristic feature of almost all human TC, but in which stage of tumor development it occurs, and what its molecular base in every certain neoplasm is, are the questions, which we are only starting to answer. In basis of GI origin underlie 4 main types of abnormalities — decrease of accuracy of DNA replication and segregation of chromosomes during mitosis; abnormality in systems of reparation of damaged DNA or mistakes of its replication; weakening of cellular cycle control — activation of checkpoints, what results in that cell with impaired DNA or chromosomal changes continues to divide and multiply anomalous population; weakening of induction of apoptosis that results in that cells with genetic abnormalities are not being eliminated from population. All above-described abnormalities one way or another are linked with mutations and inactivation of function of antioncogenes — tumor suppressors. In 1977 Kinzler and Vogelstein have grouped these genes in 2 classes: “caretakers” and “gatekeepers” [26]. Products of genes-gatekeepers are functioning in system of control, which prohibits cell proliferation with different (exactly genetic) abnormalities. Genes-caretakers are coding products, which take part in DNA reparation, stabilizing genome in that way. Some tumor suppressors (*p53, BRCA1, ATM, CHK2* and others) accomplish both functions [27]. At presence of mutation in gene-caretaker there is a high probability of mutation also in gene-caretaker [28]. In 3–31% of sporadic human tumors in genes-caretakers are found one or more mutations. Quite wide spacing of data may be connected with differences in methods, which were used for identification of mutations [29].

In sporadic human tumors chromosomal instability is the basic form of GI; for many hereditary tumors mutations, in particular, in genes of different systems of DNA reparation (DNA breaks — *BRCA1/2,* unpaired bases *MSH2,3,6, MLH1*, *PMS2*, excisional — *XP A-G)* are typical. Today 2 hypothetic models of GI are accepted. First of them — mutational model, which states that GI occurs in pretumor states and stimulates development of tumor by increase of level of spontaneous mutations. The above-mentioned identification of mutations in genes of DNA reparation at hereditary forms of cancer provides strong support of this hypothesis. According to the second model the main role in development of tumor plays induced by oncogenes *(RAS, BCR/ABL)* stressful replication of DNA [29]. Impairment of correct segregation of chromosomes may occur as the result of change of quantity and structure of centrosomes or centers of organization of microtubules. These changes are the result of *RAS* activation and inactivation of tumor suppressors *p53, APC, BRCA1.*

The process of DNA replication in fact puts cell in risk of mutations. Many genes of DNA reparation system and genes, coding enzymes of matrix synthesis of nucleic acids, are called mutator genes (genes-mutators). Impairment of functioning and coordination of expression of genes of nucleotides metabolism brings to mutator phenotype, the same way as impairments of functioning of recombination system, transcription, control of chromatin structure; enzyme systems, which control segregation of chromosomes and number of copies of individual genes; systems, which take part in synthesis of endogenous mutagens [30].

So, finally GI is linked with changes of oncogenes and tumor suppressors. However, as it may be concluded from the researches, conducted on the wide spectrum of human tumors, not many of tumors are mutated, deleted and/or amplified in sporadic neoplasms with high frequency [31–34]. To the most “universal” are referred suppressors *p53, INK4a*, *PTEN, Rb*, genes CKI (cyclin-dependent kinases inhibitors), oncogenes RAS and EGFR (two forms of receptors of epidermal growth factor) [35]. These data support point of view that insignificant population of TC may potentially provide growth of the whole tumor mass, actively supporting heterogeneity of TC inside tumor [4]. The detection of interactions between genetically

heterogeneous TC may become basis for the novel methods of therapeutic intervention.

The human genome is dynamic: according to the calculations, during the day in every cell may be realized over 20 000 damages of DNA and over 10 000 mistakes of replication. Number of proteins, which participate in replicative processes of human cell DNA, is unknown. Researches on the yeasts have showed that support of genetic stability is provided by more than 100 genes [32]. Even if mutagens are not performed in environment, mutations occur spontaneously with approximate speed 10^{-6} mutations on gene during cellular cycle.

During human life every particular gene may undergo approximately 10^{10} different mutations. It results in mutations are being found in the whole genome, including genes, which support genetic instability. From this point of view, problem of malignant tumors consists not in the question, why they occur, but why they, from the one side, occur so rarely, and, from the other side, are saved as relatively stable populations [30, 36].

Epigenetic (Eg) are called hereditary changes in gene expression, which are not linked with qualitative changes in DNA sequences [37, 38]. Genome of eukaryotes is assembled in chromatin-structural complex. Changes of structure of this complex as well as modification of chromatin by non-chromatin proteins may influence the expression of particular genes, be the cause of the activation and/or inhibition of different signaling and metabolic pathways. Genetic and Eg mechanisms are combined and interact in all stages of tumor development. Eg aberrations, in contrast to genetic mutations, are potentially reversible; it is possible to renew their normal state. Eg heterogeneity is considered to be key element in progression of tumor, especially in conditions of unfavorable for the last actions, inasmuch it provides population of TC with necessary for successful selection heterogeneity, complicity and stability. In particular, acquired drug resistance of TC is connected mainly with Eg mechanisms. That is why regulators of the last ones are potential targets for new therapy compositions [39, 40].

Eg heterogeneity realizes through 3 individual mutually reinforcing mechanisms: changes of DNA methylation; posttranslational modifications of core histones; expression of RNA, which are not coding the protein (micro-RNA and small interfering RNA — miRNA). Besides the direct effect on the nuclear processes (such as transcriptional activity) of DNA methylation and modification of histones play also key role in regulation of chromatin structure and expression of genetic information [41, 42], in normal development and support of cellular homeostasis, eliminate activity of repeated elements of DNA, inactivate X-chromosome in women [43].

The level of DNA methylation of normal cells is not inherited from parental gametes, but is eliminated individually in process of embryogenesis. Changes of DNA methylation in TC are characterized by hypermethylation of promoters of individual genes and common hypomethylation of genome compared with normal cell. In all without exception investigated tumors was detected such disbalance. By number of hypermethylated CpG-islands individual tumors may significantly differ. Along with common for several types of tumor hypermethylated sections of DNA, are also detected such sections that are hypermethylated only in one type, i.e. are tumor-specific. Significant heterogeneity of TC by hypermethylation is described: out of 45000 CpG-islands, which exist in human genome, in particular tumors may be from 0 to 4500 hypermethylated (600 in average). Quite typical for TC is hypermethylation of tumor suppressors and genes of DNA reparation system (first of all *p16*^{INC4A,} as well as *Rb, p53, MLH1).* But even in those cases, when hypermethylated segments are not associated with genes, involved in GI, aberrant mutilation 1–10% of CpG-islands in cell may cause phenotypic heterogeneity by the number of signs. During the last years, progress in discovering nature and role of mechanisms, involved in hypermethylation of DNA in cancer genesis, is going fast. Content of genome methylcytosine in TC decreases from 4% in normal tissues to 2–3%, however it is discovered not in all tumors. Precise genome localizations of hypermethylation remain the subject of studies [44]. Despite majority of publications reports that hypomethylation is found in repeated elements, it doesn't provide us with final answer to the question, which stage it originates in and which role plays in carcinogenesis, inasmuch there are differences between various classes of repeated elements. Lots of researchers think that hypomethylation occurs in the early stage of transformation [45], other connect it with later stages of tumor development. Hypomethylation of repeated elements may contribute GI, providing plasticity and advantage of TC growth, and is potential target of therapy [44].

Important Eg mechanism, which regulates structure of chromatin and genes expression, is ***modification of histones***. The main classes of enzymes, participating in these rearrangements, are ferments of chromatin remodeling and histone modifiers. Such posttranslational covalent modifications of histones as acetylation, methylation, phosphorylation, ubiquitination, sumoylation, biotinylation and ADP-ribosylation are mostly characterized. Despite interaction between different modifications of histones is still not defined, many of them contribute the development of different forms of human cancer ("histone oncomodifications") [46]. For example, acetyltransferase of histones Tip60 doesn't influence directly (like tumor suppressors or oncogenes), but makes easier the actions of other proteins, because it is transcriptional co-activator [47, 48]. Its involvement in expression of series of genes, which are regulated by transcription factor NF-κB, was described [49]. The last one is activated by many agonists (proinflammatory cytokines, T- and B-cell mitogens, products of life activity and structural components of bacteria, viral proteins,

double-stranded RNA, heat shock proteins and others), as well as by physical and chemical stresses, including effect of ionizing radiation and chemotherapeutic drugs [50, 51]. Activated factors of NF-κB family regulate transcription of over 400 genes, involved in immunoregulation, inflammatory, regulation of proliferation and apoptosis, growth and dissemination of tumors (in particular, also suppressor gene of metastasis KAI1). The same factor regulates chemo- and radioresistance in different TC. From mentioned list it is clear that variability of effects of NF-κB activation, which is mediated with acetyltransferase Tip60, may cause phenotypic heterogeneity of TC by wide spectrum of features [52]. Acetylation/deacetylation modulates expression of genes, involved in progression of tumor in process of TC selection. For example, hypoxia (one of the factors, which cause heterogeneity of TC) induces expression and activity of histone deacetylase, which in its turn regulates expression of E-cadherin — suppressor protein, which is controlling signaling pathway β-catenin/Cdk/pRb. The loss of expression of E-cadherin breaks processes of adhesion, causes epithelial invasion, which is necessary for the first stage of metastasis [53].

One more important regulator of heterogeneity of tumor is ***miRNA***, which play important role in support of genome integrity, in division of cells, support and differentiation of stem cells (embryonic and mature), in carcinogenesis, migration of TC and metastasis. This list continues growing. On the series of objects of the research is showed that tumor often avoids regulation, mediated with miRNA; repression of miRNA is associated with increasing of tumorigenicity of TC. Each miRNa is able to regulate expression of more quantity of targeted genes. In contrast, one gene may be regulated by many miRNA that may cause cells heterogeneity. Understanding of level of regulation with the help of miRNA may essentially deepen understanding of tumor biology, in particular, problems of TC heterogeneity. MiRNA may be perspective targets of antitumor therapy [54].

Taking into account that epigenetic mechanisms are in the center of many manifestations of phenotypic variability, it is probable that understanding and manipulation with epigenome promises much in prophylaxis and treatment of malignant tumors [3].

Heterogeneity of cells occur naturally and inevitably also from "noise" processes — stochasticity in genes expression, that results in production of different levels of certain proteins in every moment in genetically similar cells. Stochasticity in expression of genes determines essential variations of phenotypes in population. Majority of the researches of this phenomenon was conducted in populations of microorganisms, however, in 2006, at measurement of level of different proteins in genetically similar human cells was noticed 15–30% abmodality in particular cells. "Genetic noises" launch series of useful physiological mechanisms of regulation, cellular differentiation, participate in transfer or blockage of biological signals, coordinating expression of big variety of genes due to inequality of levels of synthesis of appropriate proteins, variability of length of its life and dissemination in cell [55, 56]. Ideal methodology of research of stochastic expression of genes is monitoring of production, degradation and functional status of separate biomolecules in real time in living cells [57]. Methods of mathematical (computed modeling) and experimental observations have showed significance of "noises" for heterogeneity/variability of phenotypes. The last one defines high evolutional potencies of population, high level of adaptation to environmental changes (3-rd type of populations according to Grant) [58], that is typical for population of TC.

For the possibly full analysis of molecular events, which are characterizing the phenomenon of TC heterogeneity, also should be mentioned the following. The various ways of generating the false proteins, which are not involved in processes of proliferation and cells apoptosis, but are important for alteration of 'social behavior' of TC and their selective advantage over normal cells, are described. On the level of translation mistakes may occur cause of incorrect inclusion of amino acid, slippage of translation or absence of modification of tRNA, which causes the mistake of mRNA reading. Such mistakes are found one time on each 1000–10000 of translated codons and are referred to the heterogeneous characteristics of TC [59]. In tumor progression intensification of TC heterogeneity is possible in consequence of posttranslational abnormalities (processing of predecessors, presentation and localization) of proteins, which are significant for preserving and multiplication of tumor population. The example may serve described long ago and observed with different frequency in different TC decrease of expression on cellular membrane of antigens of the main complex of histocompatibility of the I class, which results in "invisibility" of such TC for recognition by effectors of specific cellular immunity [60]. Adaptation of tumors to peculiarities of metabolism in microorganism may be reached by reprogramming of metabolic ways of TC. Expression of some genes, which control key metabolic ways (glycolysis, lipogenesis and synthesis of nucleotides), is radically changing in different stages of tumor progression. Today 3 groups of genes are of particular importance: *GLUT1, G6PD, TKTL1* and *PGI/AMF* (glycolysis); *ACLY, ACC1* and *FAS* (lipogenesis); *RRM2, p53R2* and *TYMS* (nucleotides synthesis), — changing of which may pay important contribution to growth of heterogeneity of TC population [61].

Summing above-stated, it may be noticed that phenotype of cells (including also TC) is finally determined by combination of genetic program, impact of environment and accidental changes [3]. TC populations possess genetic, epigenetic and phenotypic heterogeneity, quantitative and qualitative parameters of which may dynamically change during tumor process. Heterogeneity of tumors is connected with its more aggressive, metastatic behavior and resistance

(primary or acquired) to various antitumor impacts (chemotherapy, radial therapy, and immunotherapy) [62–64].

HETEROGENEITY OF METASTASES

The information on phenotypic and genotypic distinctions between cells of primary tumors and metastases, as well as on heterogeneity of metastatic tumor lesions, is being accumulated. However, data are quite contradictory. In part of researches, in which characteristics of primary tumors and metastases were compared, was found out quite close clonal relationship. In particular, such relationship was found out for primary and metastatic prostate tumors (independently of anatomic localization of metastases), that emphasizes natural monoclonality of these neoplasms. At the same time, heterogeneity both in primary tumors and in metastatic lesions at prostate cancer was discovered by the other researchers. The radical distinctions between primary and metastatic prostate tumors, as well as mammary gland, manifesting itself in loss of alleles, that indicates high degree of genetic divergence, are discovered [65]. The comparison of consequences of primary lobular and metastatic tumors of mammary gland has discovered multiple mutations, inherent in metastases only [66]. It is considered that primary and metastatic tumors may develop as genetically different in cases, when metastatic dissemination occurs in the early stage of tumor progression. It is clear, that the clonal relationship between primary and metastatic populations of TC in malignant neoplasms of different histogenesis needs further study.

The question of clonal heterogeneity within metastases is less studied. The complex network of metastatic micromedia, complex interaction of TC, stroma cells, immune cells, noncellular matrix and dissolving factors are key players of progression and metastasis as well as heterogeneity of metastatic tumors. Again, stromal cells both of tumor and metastases co-evolve with TC, changing their geno- and phenotype with the aim of accommodation to the needs of their permanently changing neoplastic neighbors [67].

Heterogeneity of metastases may be intermetastatic and intrametastatic [68]. Intermetastatic heterogeneity is heterogeneity of various metastatic invasions of the same patient. Individual metastases are rather exception, than rule. It is common for one metastatic involvement to have 20 clonally genetic changes, which are not performed in other metastases of the same patient [69, 70]. Inasmuch they are clonal, these mutations were in stem cell of metastases, exactly in cell, which was seized from the primary tumor and multiplied for formation of metastases. Founder cell for each metastasis is performed in anatomically different regions of primary tumors [69]. Heterogeneity mainly is limited with passenger gene mutations. Passenger mutations are not indifferent for the destiny of tumor. Having accumulated in enough quantity, they may retard or even stop its growth. In majority of researches was showed that in malignant tumors driver genes Mut are performed inside tumor [71]. They contain driver gene mutations (Mut-Driver gene) and are expressed aberrantly in such way that provides advantage of selective growth (Epi-Driver gene). These data are coincided with idea that genetic changes, which are necessary for metastasis, were performed in cell before metastasis itself. The obtained data are also coincided with observation that in patients is observed response to targeted agents in all metastatic involvements, but not only in small part [72]. Intrametastatic heterogeneity is heterogeneity of cells of the particular metastases. Each metastasis grows from one cell (or small group of cells) with set of founder mutations. With growth of metastatic tumor it acquires new mutations with every cell division. Despite initial mutations are able to make metastasis sensitive to antitumor drugs, new mutations provide its drug resistance. Majority of initial metastases recur and terms of origin of new metastases are quite similar. This length by time may be explained by performance of mutations of resistance, which existed inside every metastasis before start of targeted therapy. Calculations show that any metastatic involvement of visualized size has thousands of cells, which already resistant to almost any drug [73–76]. One TC will be resistant to multitude of drugs, which influence different targets. Source of heterogeneity as well as call of resistance to the therapy is plasticity of tumor and immune cells [77]. Complex network of metastatic microenvironment, complex interaction of TC, stroma cells, noncellular matrix and dissolved factors are key players in progression and metastasis as well as heterogeneity of metastatic tumors. So, recurrence of metastasis is just question of time, is enough predictable on the basis of known frequency of mutations and speed of TC growth.

CONCLUSION

Thus, phenotypic, genetic and epigenetic heterogeneity are key elements in progression of tumor, its resistance to “hostile media” and therapeutic impact, inasmuch they provide population of cells with diversity, resistance, opportunity of selection. Heterogeneity of tumor is active dynamic state, which is supported both by cellular and noncellular factors. New variants of cells, interacting between each other, help tumor to resist destructive influences. As any community, tumor is not just sum of components of its subpopulations; it is interacting ecosystem, each feature of which may influence another. Biological features of “early” pre-invasive tumor are not similar to those of the same tumor, when it has reached stage of dissemination. Heterogeneity of tumor has many levels and molecular mechanisms (not finally discovered yet). All they together provide survival and diffusion (dissemination, colonization, and metastasis) of TC population. Every TC in organism of patient is unique by potential opportunity to undergo different changes and one TC,

which avoided effect of therapy, may potentially cause progression of disease.

Problem of clonal heterogeneity remains poorly studied. New approaches (including mathematical modeling) for characteristics of clonal heterogeneity of different types and subtypes of tumors in different stages of its development, as well as in conditions of different treatment impacts, are required. Nevertheless, our knowledge of TC heterogeneity has already found realization in diagnosis and treatment of cancer patients. For example, on the basis of detection of high heterogeneity of cells of gastric cancer and gastroesophageal cancer by expression of HER2/neu were elaborated principles of HER3-testing of these tumors, which differ from the same at human breast cancer.

Implementation of these principles let us essentially increase quality of diagnosis and choose optimal individualized treatment tactics of patients with use of targeted (anti-HER2/neu) drugs [78]. Another example may be recommendations on optimization of regimens of research of TC apoptosis for evaluation of effectiveness of antitumor therapy [79], in particular, patients with human breast cancer [80] or metastatic kidney carcinoma [81], taking into account TC heterogeneity and asynchronicity of their death under the therapeutic effect. Majority of data on heterogeneity is obtained in result of tumor research, cells of which were selected within the framework of organism. However, last researches have showed on the level of particular cell that in culture of cells of mammals after several divisions originates wide spectrum of variability in local density of monolayer, in intracellular contacts, relative localization and quantity of free space on cell, in form and/or polarization of cells as well as their mobility. These parameters in combination constitute population context of individual cells, in which each of them adapts own physiology. Such adaptation may be carried out on the level of genes transcription, translation of particular proteins, regulation of cellular cycle, activity of proliferation, sensitivity of apoptosis, metabolic properties. Listed characteristics define both behavior of individual cell in population and its influence on forming of population context. These complex and non-linear feedback mechanisms on many levels of cellular organization define phenotypic properties of particular cell in population, even when cells are not differentiating [82]. Above-stated summarized important considerations related to intratumor heterogeneity during tumor development and put the problem of cellular changeability and phenotypic heterogeneity not only in the center of today's fundamental oncology, but also in the center of modern cell biology. New discoveries are only at the beginning of an era in which integration of rich data sets that probe the genome, epigenome and transcriptome will help us to unravel the intricate regulatory connections between heterogeneity and cancer. The ability to see heterogeneity processes inside the live cells and understand the causes of TC resistance is no longer a dream.

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