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Current ideas about molecular genetic subtypes of ovarian cancer: a personalized approach and a new platform for further research

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Abstract

Highly malignant ovarian cancers are a histopathological diagnosis, but can be multiple diseases at the molecular level. Research aimed at identifying molecular genetic subtypes of ovarian cancer is being conducted to find an answer to the question: can different molecular subgroups influence the choice of treatment? One of the achievements of this direction is the recognition of the dualistic theory of the origin of ovarian carcinomas with their division into High-grade and Low-grade subtypes. However, the data of sequencing of the tumor genome suggest the existence of 6 subtypes of carcinoma, including two LG and four HG subtypes. Patients of subtype C1 are characterized by a high stromal response and have the lowest survival, tumors of C2 and C4 subtypes have a higher rate of intratumoral CD3 + cells, lower stroma gene expression and better survival than C1. The mesenchymal subtype C5 is widely represented by mesenchymal cells, characterized by overexpression of N-cadherins and P-cadherins, low expression of differentiation markers and lower survival than C2 and C4. The use of a consensus algorithm to determine the subtype allows the identification of only a minority of ovarian cancers (approximately 25%). In this regard, the practical significance of this classification still requires additional research, and today it is permissible to talk about the existence of only 2-3 reproducible subtypes. It is thought that it makes sense to randomize tumors into groups with altered expression of angiogenic genes and

with overexpression of immune response genes, as in the angiogenic group there is a comparison of the advantage in survival (prescribing bevacizumab improves it, and in the immune group even increases bevacizumab). Molecular subtypes with poorer survival rates (proliferative and mesenchymal) also benefit most from bevacizumab treatment. The review focuses on some advances in understanding molecular, cellular, and genetic changes related to ovarian cancers with the results achieved so far in describing molecular subtypes of ovarian cancer. The available information is the basis for planning further research.

Key words: ovarian cancer; molecular genetic heterogeneity; molecular subtypes; prognosis; reproducibility.

Relevance. Today, the problem of predicting the course and effectiveness of treatment of malignant tumors is considered simultaneously with the issues of individualization of treatment of cancer patients. The nature of molecular genetic disorders underlies the features of the clinical course, chemosensitivity and prognosis of treatment effectiveness, resulting in the clinical course of the tumor is different even within a homogeneous group of patients [4].

At the heart of this diversity is the biological heterogeneity or heterogeneity of solid tumors, including ovarian cancer (OC). As the disease progresses, the molecular-genetic "portrait" of the tumor changes, there are new "targets" that require a differentiated approach in each case.

To date, several dozen diagnostic and prognostic indicators used in clinical practice are known. Some of them have already received mandatory status, but at this stage there are no absolute signs of sensitivity and resistance of the tumor to drugs, without which the error probability would be quite high in choosing therapy for a particular patient [1]. Molecular markers can have both prognostic value (predicting the course and outcome of the disease) and predictive (predicting the sensitivity and resistance of the tumor to drugs) [14]. Regarding OC, the question of how wide the range of molecular genetic markers and the correlation between their level and effectiveness of treatment remains unresolved, as well as whether it is possible to create an algorithm for tactics and sequence of antitumor therapy based on these parameters.

Molecular genetic classifications of ovarian cancer. In 2007, based on the data of molecular-genetic and clinical-morphological analysis of borderline and invasive ovarian tumors, a molecular-biological classification of OC was created and two fundamentally different pathways of carcinogenesis of serous OC (Kurman RS) were established [12]. In 2013, in the WHO classification, serous carcinomas were first divided into low and high-

grade (low and high grade malignancies). Type-1 (low-grade) tumors, most often arising from borderline tumors, are characterized by different types of mutations (KRAS, BRAF, PTEN and β -catenin) and are relatively genetically stable. Type II (high-grade) tumors are biologically aggressive, probably arising de novo from the coelomic epithelium, show marked genetic instability and mutation in the p53 gene. In 2011, a molecular atlas of high-grade serous ovarian cancer was created: molecular analysis combined data on RNA expression, amplification and deletion of genes and parts of chromosomes, as well as mutations among nearly 500 samples of serous G3 ovarian cancer [6]. It was shown that TP53 mutations occur in almost all cases of HG serous OC (96%), BRCA1 and BRCA2 mutations - in 22% of cases. Seven other genes carrying the mutation have been identified, including NF1, BRCA1, BRCA2, RB1 and CDK12, but they are much less common in 2-6% of cases. The authors concluded that the spectrum of mutations in HG serous ovarian carcinoma is completely different from other histological subtypes of OC. Thus, clear cell carcinomas are accompanied by a number of TP53 mutations, but have recurrent ARID1A and PIK3CA mutations, endometrioid carcinomas have a high frequency of CTNNB1, ARID1A and PIK3CA mutations and a lower frequency of TP53 mutations, and mucinous carcinomas. In addition, 50% of HG carcinomas have a violation of homologous recombination, which may determine the indications for the appointment of PARP inhibitors. The obtained results allowed the authors to justify the appointment of targeted therapy for OC [6].

There are five pathogenetic subtypes of ovarian cancer: LG serous carcinoma (type I), HG serous carcinoma (type II), endometrioid carcinomas (LG and HG), mucinous carcinoma and clear cell adenocarcinoma - account for 98% of cases of OC. These subtypes, in addition to molecular disorders, differ in risk factors, clinical course and response to chemotherapy. It is thought that attempts to divide all ovarian cancers into two large groups (tumors of types I and II) limit the process of understanding the biology of cancer and do not improve treatment outcomes [2].

R.W. study Tothill, aimed at identifying new molecular subtypes of ovarian carcinoma through gene expression profiling, was based on clinical data and survival and led to the identification of six subtypes of serous and endometrioid carcinomas of the ovary, fallopian tubes and peritoneum (C1-C6). According to the study, the vast majority of HG carcinomas are represented by subtypes C1, C2, C4 and C5 serous and endometrioid types, combined into one group due to their molecular similarity, confirmed earlier.

Subtypes C3 and C6 are represented by serous tumors with low malignant potential (LMP) and LG endometrioid carcinomas. Both subtypes (C3 and C6) have very clear gene

expression profiles. Both groups are characterized by low expression of proliferation markers (e.g. MKI67, TOP2A, CCNB1, CDC2, KIF11), which is reflected in the low mitotic index. Subtype C3 (LMP) demonstrates the relative overexpression of genes of mitogen-activating protein kinase pathways (DUSP4, DUSP6, SERPIN5A, MAP3K5, SPRY2), which may be associated with LMP-specific mutations in mitogen-activated protein kinase expression of KRAS and KRAS and (motor proteins). C6 tumors (HG endometrioid carcinomas) are characterized by overexpression of β -catenin / LEF / TCF transcription targets, which are characteristic of endometrioid cancers containing β -catenin mutations [15].

Subtype C5 is a new HG serous subtype defined by genes of mesenchymal origin. The subtype is characterized by overexpression of transcription factors involved in the regulation of development, including homeobox genes (HOXA7, HOXA9, HOXA10, HOXD10, SOX11), as well as a group of cellular motility factors (HMGA2, TOX and TCF7L1). Subtype C5 is rich in WNT / β -catenin and cadherin signaling elements. Immunohistochemical staining for E-cadherin revealed a decrease in protein localization on the membrane in most samples of C5 subtype tumors, compared with its high expression in HG carcinomas of groups C2 and C4 (consistent with the idea of increasing the activity of WNT / β -catenin signaling pathway C5 in cancer). In addition, in C5 subtype tumors, overexpression of genes associated with proliferation and extracellular matrix (COL4A5, COL9A1, CLDN6) and very low expression of immune cell markers (CD45, PTPRC, lymphocyte markers, CD2, CD3) were additionally determined. According to the mesenchymal or dedifferentiated phenotype, C5 tumors had relatively low expression of genes responsible for the synthesis of mucins (MUC1 and MUC16) and kallikreins (KLK6, KLK7, KLK8).

For tumors of the C1 subtype, there was less expression of the cellular component relative to the stromal, which correlated with the severity of desmoplasia, collagen and myofibroblast content, as well as the level of stromal gene expression (ACTA2) compared with C2-6.

The immune signature was overexpressed in subtypes C1 and C2 at very low levels in carcinoma C5, and the immune response genes were significantly overexpressed in carcinoma subtype C2, including markers of T cell activation.

Thus, among the identified subtypes, two subtypes were respectively serous and endometrioid LG carcinomas, the other four - represented by HG carcinomas of serous and endometrioid histotypes. A new subtype of serous HG carcinoma is widely represented by mesenchymal cells, characterized by overexpression of N-cadherins and P-cadherins and low

expression of differentiation markers, including CA125 and MUC1. The subtype of prognostically unfavorable carcinomas is characterized by low expression of stromal genes, correlated with the severity of desmoplasia. Each subtype showed a different degree and pattern of tumor infiltrating immune cells.

Reproducibility of molecular genetic subtypes. Information on the existence of four different molecular subtypes of HG carcinoma was presented in other studies of large cohorts of patients [6, 9, 16, 11], but was questioned because they could not pass an independent test [5], and testified to the existence of only 2-3 reproducible subtypes [17].

In order to assess the reproducibility of published algorithms for classifying subtypes, their reliability and prognostic value, as well as the consolidation of the proposed schemes into a single consensus algorithm, in 2017 a meta-analysis was conducted showing that the use of each of the proposed classifiers does not allow most cases of cancer to any of the four H-Grade subtypes, as each of them identified subtypes that differ significantly in overall survival, but are not resistant to re-installation on independent samples. Modification of existing algorithms with the failure to detect tumors of ambiguous subtype dramatically improves the efficiency of any of these classifiers. As a result, a "consensus" classifier is proposed for 25% of tumors that can be classified with a high degree of confidence. According to experts, the ambiguity of the classification of tumors may arise due to heterogeneous impurities of different subtypes or due to a more homogeneous composition of the indeterminate subtype [7]. And these differences are important from a therapeutic standpoint. According to Lohr et al., 90% of tumors in the TCGA database are polyclonal [13], and the clonal nature of ovarian cancer has been previously confirmed by the results of DNA sequencing of single cells. It remains unclear whether multiple clones in tumors are consistently classified within the same subtype. If the tumor consists of several clones of different subtypes, it is likely that specific therapy for one subtype will lead to recurrence due to survival and continued growth of other clones. Thus, unambiguously classified tumors can be contaminated with small "quantities" of cells of another subtype, which can lead to recurrence after subtype-specific therapy.

Thus, a meta-analysis combining data on 1,774 cases of HG ovarian carcinoma proposed a consensus classifier to identify a minority (approximately 25%) of ovarian carcinomas that are uniquely classified using different gene expression platforms represented by 100-gene signature [7].

Based on the obtained data, the authors conclude that most ovarian cancers (Up to 75%) cannot be subclassified into subtypes, in contrast to the minority of tumors for which it

is assumed that the proposed consensus classifier consolidates and improves the effectiveness of previously proposed classifiers. Its use provides reliable stratification of patients with HG ovarian tumors of a clearly defined subtype, helps to study the role of polyclonality in the case of belonging to the number of tumors that can not be classified into these subtypes.

Based on the meta-analysis, each of the algorithms identified four different subtypes of HGS with specific clinical and pathological characteristics. The division of carcinomas into subtypes is accompanied by a correlation with the age of patients, ability to survive, "purity" of the tumor and lymphocytic infiltration (Table 1) [8]. Associations of subtypes with age and overall viability are presented according to the results of all analyzed data sets (more than 1700 patients) [6, 9, 16, 11], association with "purity" of the tumor and the degree of infiltration of immune cells - according to TCGA. Tumor purity was assessed by TCGA genotyping; lymphocyte infiltration was based on TCGA data [6].

Table data. 1 represent the results of clinical and morphological differences between carcinoma subtypes. The statistically significant nature of the differences in overall survival and parameters of infiltration by immune cells emphasizes the isolated position of the immunoreactive subtype of HG-carcinoma, despite the relative "purity" of tumor samples.

It is expected that general agreement on how to define molecular subgroups will facilitate the use of expression data in the planning of clinical trials. Identification of tumors of a "certain" subtype is an important step towards the identification of promising therapeutic phenotypes [7].

Table 1. Properties of subtypes identified by the consensus classifier

	Immunoreactive	Differentiated	Proliferative	Mesenchymal
Age, years	61	55	64	59
Risk (5-year survival,%)	Low (50%)	High (34%)	High (34%)	Very high (20%)
"Purity" of TCGA samples	71%	87%	91%	62%
Lymphocyte infiltration	~24%	~41%	<5%	<5%
Neutrophil infiltration	~8%	~10%	<5%	<5%

Molecular subtypes, survival and significance of molecular genetic subtypes in clinical practice. In the R.W. study Tothill both recurrence-free and overall survival differed significantly depending on the subtype: patients in groups C3 and C6 had the longest

recurrence-free and overall survival (according to the fact that C3 was predominantly LMP and C6 was predominantly LG) and early stages of endometrioid carcinoma [15].

One-way analysis of only HG subtypes (C1, C2, C4 and C5) revealed statistically significant differences in relapse-free survival ($P = 0.004$, OS, $P = 0.022$): patients with subtype C1 (high stromal response) had the lowest survival of all four HG groups carcinoma. Tumors C2 and C4 with a higher number of intratumoral CD3 + cells and with lower stroma gene expression had better survival than C1 tumors. Mesenchymal subtype C5 showed a tendency to decrease overall survival compared to subtypes C2 and C4. Differences in survival between C1 and C2, C4, C5, the researchers also further confirmed by multivariate analysis.

The characteristics of the C1 subtype remained significantly worse even in the context of known prognostic parameters such as stage, degree of differentiation, residual tumor volume and age [15]. Significant efforts are being made to introduce subtypes into clinical practice, including to predict the effect of angiogenesis inhibitor bevacizumab in ICON7 [8, 10].

The patients included in the study were randomized into four groups: 122 cases of immunoreactive subtype (34%), 96 cases of proliferative subtype (27%), 73 cases of differentiated (20%) and 68 cases of mesenchymal subtype (19%). In a one-way analysis, patients with proliferative subtype tumors benefited most from concomitant chemotherapy and maintenance therapy with bevacizumab for up to 12 months. after primary cytoreduction, which improved the median recurrence-free survival by 10.1 months. In the group of mesenchymal subtypes, bevacizumab gave a statistically insignificant improvement in relapse-free survival by 8.2 months. In the group of patients with the immunoreactive subtype, bevacizumab also led to a modest improvement in relapse-free survival (3.8 months, $P = 0.08$), as in the group with the differentiated subtype (3.7 months; $p = 0.61$). When conducting a multivariate analysis, there was a significant improvement in relapse-free survival in the group with a proliferating subtype ($p = 0.0015$).

The data suggest that molecular subtypes with poorer survival rates (proliferative and mesenchymal) benefit most from treatment involving bevacizumab [10].

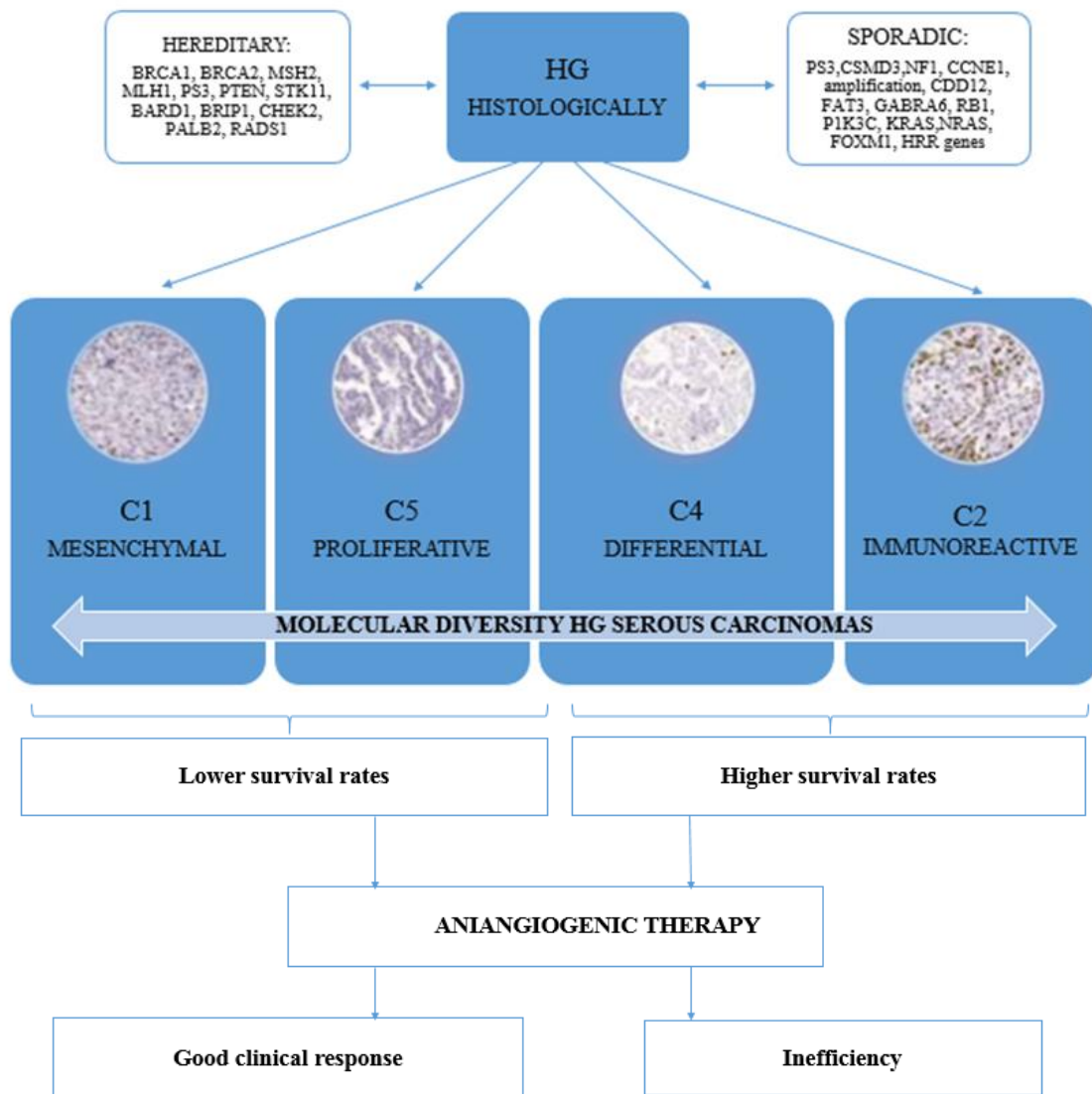


Fig. 1. Molecular subtypes of HGrade ovarian carcinoma

Explanation: 1 molecular damage to major genes associated with HG serous ovarian cancer.

HRR genes: (BRCA1, BRCA2, EMSY, PTEN, RAD51C, ATM, ATR, FANCD2); MMR [3].

2 Classification of ovarian carcinomas into subtypes, according to the level of gene expression and survival rates by R.W. Tothill et al. 2017 (microphotographs show the level of CD3 HR expression by subtypes C1, C2, C4 and C5 during immunohistochemical study) [15].

3 Targeted therapy with inhibitors of neoangiogenesis is used in the case of disseminated OC according to treatment standards, but may be more effective in subgroups of tumors with proangiogenic genomic profile [8]

Analysis using a signature evaluating the expression of 63 genes showed the possibility of randomization of tumors into three main groups: with overexpression of angiogenic genes, with reduced expression of angiogenic genes and with overexpression of immune response genes. It turned out that the latter - "immune" subgroup had an advantage in overall survival compared to the other two subgroups, which allowed to combine them into one. Because angiogenesis-related gene expression was reduced in the immune subgroup, the authors suggested that these patients would benefit less from bevacizumab. Indeed, it was found that in the angiogenic group there was a slight tendency to improve recurrence-free survival with the addition of bevacizumab (17.4 vs. 12.3 months). Of particular interest is that in the immune group (41% of cases), the addition of bevacizumab even led to a deterioration in recurrence-free survival and overall survival compared with patients receiving chemotherapy alone [8].

A deeper understanding of the optimal stratification based on tumor or stromal characteristics and mechanisms of resistance will further benefit from the results of these clinical studies with the possibility of personalized administration of antiangiogenic drugs (Fig. 1) [3, 8, 15].

Conclusion. To date, there are no absolute signs of sensitivity and resistance of OC to drugs. The absence of obvious targets in the treatment of OC is dictated by the lack of clear ideas about the pathogenesis of this disease, however, at the present stage, OC is an extremely heterogeneous disease. The search for markers to predict the course and effectiveness of OC treatment is ongoing and is associated with attempts to systematize / subclassify ovarian carcinomas into individual subtypes. A crucial step in understanding intertumor heterogeneity is the division of ovarian carcinomas into High-grade and Low-grade subtypes. However, the data of sequencing of the tumor genome suggest the existence of several scenarios. The division of carcinoma with the allocation of two subtypes of LG carcinoma (serous and endometrioid), and four subtypes of HG carcinoma is based not only on histopathological and molecular findings, but also on the clinical association with survival rates. Patients of subtype C1 are characterized by a high stromal response and have the lowest survival, tumors of C2 and C4 subtypes have a higher rate of intratumoral CD3 + cells, lower stroma gene expression and better survival than C1. The mesenchymal subtype C5 is a fundamentally new subtype of HG carcinoma, widely represented by mesenchymal cells, characterized by overexpression of N-cadherins and P-cadherins, low expression of differentiation markers, including CA125 and MUC1, while showing a tendency to decrease overall survival in comparison with C2 and C4. The practical significance of this classification still requires additional research, because

despite the fact that similar data are presented in several studies of large cohorts of patients, meta-analytical data suggest that each of them alone can not pass an independent test in other samples, and consolidation of the proposed schemes in a single consensus algorithm allows to identify only a minority of ovarian carcinomas (approximately 25%), which are uniquely classified using gene expression platforms represented by 100-gene signature. Thus, it is currently allowed to talk about the existence of only 2-3 reproducible subtypes. There is evidence that molecular subtypes with poorer survival rates (proliferative and mesenchymal) benefit most from bevacizumab treatment. It makes sense to randomize tumors into two groups: with altered expression of angiogenic genes and with overexpression of immune response genes, as in the angiogenic group the advantage in survival is compared, and the appointment of bevacizumab slightly improves it, and in the immune group increases the addition of bevacizumab.

The review focuses on some advances in understanding molecular, cellular, and genetic changes related to ovarian cancers with the results achieved so far in describing molecular subtypes of ovarian cancer. At the present stage, this knowledge has not yet led to the unambiguous principles of individualization of treatment of patients with OC, but are a springboard for planning further research in this area. The review does not address BRCA-induced ovarian cancer.

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