Observational Study

Medicine

OPEN

Research progress in intratumoral heterogeneity and clinical significance of ovarian cancer

Tengda Pu, MM[a], Chengyuan Zhang, Bachelor[a], Bingfeng Su, Bachelor[a], Li Li, Bachelor[a], Jingjing Fu, Bachelor[a,*]

Abstract

Intratumoral heterogeneity has been a hot topic of cancer research in recent years, which has become a part of resolving cancer metastasis, recurrence and drug resistance. Intratumoral heterogeneity shows that cells undergo different division and proliferation during the process of tumor development, and their genomic cells exist in the process of tumor development. Protein and epigenetic changes can lead to differences in proliferation, migration and invasion, sensitivity and pharmacological prognosis of tumor cells, promote sustainable development and development of cancer cells, produce greater adaptability, and lead to metastasis, recurrence and drug resistance of malignant tumors. In recent years, the molecular mechanism and clinical application of intratumoral heterogeneity have captivated widespread attention from researchers. In the era of precision medicine, oncologists attempt to improve the clinical efficacy of targeted tumor therapy via intratumoral heterogeneity. In this article, recent advances in the study of intratumoral heterogeneity, molecular mechanism of intratumoral heterogeneity, systematic evolution and quantification and clinical significance of tumor heterogeneity were reviewed.

Abbreviation: EOC = epithelial ovarian cancer, HGSOC = high-grade serous ovarian carcinoma, PcG = polycomb group.

Keywords: individualized treatment, intratumoral heterogeneity, ovarian cancer, precision medicine

1. Introduction

Ovarian cancer has a strong and direct clonal evolution branch, which is a highly-heterogeneous tumor.[1] The degree of intratumoral heterogeneity is negatively correlated with clinical prognosis of cancer patients. Intratumoral heterogeneity has been proven to lead to initial efficacy and subsequent resistance to chemotherapy, as well as the failure of most targeted drug interventions.[2] Clarifying the degree of intratumoral heterogeneity will improve patient stratification and enhance clinical outcomes, which is more conducive to exploring the guiding factors of intratumoral heterogeneity. Inhibition therapy for these driving factors is a highly promising approach, which utilizes intratumoral genetic heterogeneity to improve clinical efficacy of cancer targeted therapy.

Ethical approval was not necessary, there is no human or animals in this study.

2. Research status

Ovarian cancer is a life-threatening malignant tumor in female population. According to the statistics of Global Cancer Statistics 2020,[3] there are approximately 295,000 new cases of ovarian cancer worldwide, including roughly 180,000 deaths. Among ovarian tumors, supradermal tumors are the most common, accounting for 50% to 70%. Age, family history, ovulation cycle and mutation gene (BRCA 1/2) are the important risk factors. It is difficult to distinguish ovarian cancer from the digestive system and urinary system tumors due to nonspecific the symptoms during the early stage. Therefore, routine diagnosis and treatment procedures, such as gynecological examination, CA125 serum level and transvaginal ultrasound, fail to diagnose early ovarian cancer. Upon diagnosis, more than half of ovarian cancer patients are classified in advanced stage complicated with metastasis, and the 5-year survival rate is merely 20% to 25%.[4] For patients who can be diagnosed in the early stage, the 5-year survival rate probably reaches up to 90%. Metastasis and chemotherapy resistance are major challenges in the treatment of ovarian cancer. Consequently, it is necessary to unravel the exact mechanism underlying the incidence, development, metastasis and drug resistance of ovarian cancer, thereby screening effective targets for ovarian cancer.

Tumors can grow dynamically under the interaction between tumor cells and surrounding microenvironment, which gradually forms heterogeneity during this process. Some scholars suggest that genetic instability is the basis of the heterogeneity of tumor development. In the early 1950s, the concept of "genetic heterogeneity" of cancer was proposed.[5] External stimuli and endogenous chromosome aberration can promote the progression of cancer, leading to genetic and phenotypic variations of tumor cells. Peter Nowell et al[6] demonstrated that cancer

The authors have no funding and conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

[a]Department of Gynecolgy, Hainan Cancer Hospital, Haikou City, China.

**Correspondence: Jingjing Fu, Department of Gynecolgy, Hainan Cancer Hospital, No.9, Changbin West 4th Street, Xiuying District, Haikou City, Hainan Provence, 570100, China (e-mail: fujingjing2028@163.com).*

How to cite this article: Pu T, Zhang C, Su B, Li L, Fu J. Research progress in intratumoral heterogeneity and clinical significance of ovarian cancer. Medicine 2024;103:4(e36074).

Received: 22 July 2023 / Received in final form: 19 October 2023 / Accepted: 20 October 2023

http://dx.doi.org/10.1097/MD.0000000000036074

cells exhibit different sensitivities to cancer treatment due to different molecular characteristics, leading to drug resistance. Oligomers are formed by clonal evolution. In solid tumors, the branching evolution model is adopted. All types of subcutaneous tumors originate from the identical origin. Some hematological tumors develop in a linear pattern through persistent variations, which is beneficial to survival. These limitations complicates the genome map represented by a single tumor biopsy sample, and poses a huge challenge to the development of disease biomarkers and the formulation of individual therapeutic regimens.

3. Molecular mechanism of intratumoral heterogeneity in ovarian cancer

Genome sequencing reveals that the changes in somatic mutation and copy number lead to the diversity of tumor phenotype.[7] The gene mutation of 160 tumors in 3 tumor regions has been analyzed in patients with secondary ovarian cancer. Pathological examination shows that the degree of nuclear specificity is increased from one region to another region C. Gene sequencing results suggest that grass 2 is amplified in regions B and C, with a copy number (CN) = 32(B) and 26(C), while grass 2 is slightly amplified in region C (CN = 6). KRAS amplification is detected in region C(CN = 4) rather in regions A and B. Somatic mutation and the changes of copy number can be observed in 3 different regions, indicating that subcutaneous tissues formed by tumor is an obstacle to targeted therapy. For high-grade ovarian cancer, TP53 mutation is the most common feature. However, recessive TP53 mutation probably occurs in tumors and forms a subtype without expressing p53. Some studies[8] have found that clinical prognosis of patients with this type of cancer is worse than that of their counterparts with TP53 mutant subtypes. To further clarify the deletion of p53 in ovarian tumors, Panici et al[9] selected 6 main lesions, 2 recurrent pelvic lesions and 4 recurrent extrapelvic lesions from the same patient for incomplete sequencing. A total of 42 out of 102 gene mutations can be detected in all samples. Only 7 mutant genes with CN = 18 are detected in all samples. Researchers have also screened the genes which are the most significantly associated with the recurrence of ovarian cancer, such as gpneb and tfd1, etc.

In special types of ovarian cancer, typical time differences (the difference between initial concentration and metastasis) and spatial heterogeneity can be found. Ovarian cell carcinoma is a rare ovarian tumor with long incubation period and high recurrence rate. After gene sequencing of primary blood and comprehensive recurrence concentration, changes can be detected in the expression levels of TP53, tet2, med12, and other genes, indicating that these genes are correlated with the disease progression and recurrence.[10] In a study of clear cell cancer and ovarian cancer, 4 regions of each sample are selected from 6 patients to represent the whole genome.[11] More than 7000 single cell mutations and 112 copy number mutations are identified. Full sequencing, single cell sequencing and T cell receptor sequencing have been adopted in more and more studies, which not only reveals the clonal evolution of different regions of tumors, but also clarifies the molecular mechanism underlying intratumoral heterogeneity, emphasizing that gene mutations and copy number variations might serve as potential therapeutic targets.

4. Origin theory and existing problems of intratumoral heterogeneity

Rapid proliferation of single cells and subsequent improvement of aneuploid cell survival rate can be achieved through tumor mutation. There is also an explanation for "cancer stem cell model" that persistent rapid proliferation of stem cells with self-renewal and differentiation potential can lead to intratumoral heterogeneity.[12] With the development of high-speed genomics, protein genomics and epigenetics, it has been found that tumors can originate from multiple cells, including random cells and stem cells with proliferation advantages, or both.[12] Therefore, tumors are influenced by post-transcription and external environment, such as hypoxia and acidosis, followed by heterogeneity. Therefore, heterogeneity is the inheritance of these 2 factors. Mutual genetic information is stored in the genome, spreads and accumulates along with cell proliferation, and responds to external environment and selection pressure through changes in gene expression. In 1980s, cancer treatment is mainly delivered based on cytotoxic drugs. The discovery of intratumoral heterogeneity hinders the screening of novel chemotherapy drugs. Cancer has been regarded as a disease with gene mutation.[13] The identification of cancer-related gene mutations urges "targeted therapy," primarily small molecular inhibitors or monoclonal antibody drugs, to replace broad-spectrum cytotoxic drugs. For instant, trastuzumab can target HER2 receptor in breast cancer. However, therapeutic effect of most targeted drugs is not satisfactory, and most drugs are ineffective or only effective in a short period of time. Although epithelial tumors with high expression of HER2 receptor, trastuzumab exerts no therapeutic effect, indicating that the same mutation lacks of functional preservation among different tumors. Due to intratumoral heterogeneity, the average overall survival in cancer patient who have been treated with FDA-approved targeted drugs is estimated to be 2 months from 2002 to 2012. Therefore, the therapeutic targets found in a single tumor sample cannot represent the current molecular state of heterogeneous tumors. Identifying the causes of the formation of various heterogeneous subcomponents of tumors, the molecular phenotype and gene map characteristics related to tumor metastasis, recurrence and prognosis remains to be urgently achieved. Giannini A. et al[14] have reviewed recent progress in use of PARP inhibitors in newly-diagnosed and recurrent ovarian cancer and illustrated that PARP inhibitors provide benefits in newly-diagnosed advanced ovarian cancer, even in the absence of BRCA mutation. PAOLA-1 study supports the adoption of olaparib plus bevacizumab in patients with homologous recombination deficiency, whereas partial patients develop resistance to PARP inhibitors. Consequently, novel combinations are investigated to identify individualized treatment strategies to overcome this resistance.

5. Systematic evolution and quantification of intratumoral heterogeneity of ovarian cancer

With the development of multi-regional cancer sequencing technology, targeted therapy has been gradually applied in clinical settings, but drug resistance in the treatment process has increasingly highlighted the importance of disease evolution research, which indicates that further research is needed to predict disease development.[15] Constructing a "phylogenetic tree" based on multi-regional sequencing results can better represent the tumor development process of patients. During the process of tumor development, the evolution of tumor cells does not follow the sequential or linear pattern. However, it regards each new mutation as a branch and eventually forms a tree structure. Samples were obtained from a 17-year-old patient with stage II ovarian cancer, including 7 cancers and 3 metastases. Using complete exon sequencing data, a "phylogenetic tree" is constructed, forming 2 primary tumor clusters (P1 and P2) and one metastatic tumor cluster (M). Through analysis and comparison, P1 and P2 clusters have been differentiated in the initial stage of tumor development. The genetic structure of M cluster is similar to that of P1 cluster, with less genetic mutation and copy number variation. According to the "phylogenetic tree," besides subcutaneous evolution, microenvironment tumors can also promote tumor growth and metastasis by secreting specific inducers.

Tubular lesions are considered as precursors of ovarian cancer. To clarify the sequence of ovarian cancer, samples were collected in 9 different regions of 4 patients for the next-generation sequencing to analyze the single nucleotide variation and establish a "phylogenetic tree" of ovarian cancer and abdominal metastasis, and they found that after quantifying the distance between mutant branches and roots, transformation and differentiation occur in the initial stage, resembling the mutation of primary tumors, suggesting that ovarian cancer probably originates from tubular lesions.[16] To determine the association between intratumoral heterogeneity and clinical prognosis, a minimum event distance algorithm was designed to compare the copy number in tumors and quantitatively analyze the fiber formation. Gene sequencing is performed in 135 patients receiving 14 types of platinum-based treatment. All samples are divided into the upper and lower EC groups according to the median value of 0.74. After comparison, each patient exhibits a high degree of heterogeneity. EC value is correlated with clinical prognosis of patients. The survival in the upper EC group is shorter than that in the lower EC group (12.7 months vs 6 months; progression-free survival time: 10.1 vs 23.0 months). To sum up, the "phylogenetic tree" can accurately describe the evolutionary trajectory of tumors. Combined with the quantification of "phylogenetic tree," it can be utilized as a prognostic factor for ovarian cancer.

6. Research progress on intratumoral heterogeneity of ovarian cancer

Ovarian cancer is one of the 3 major gynecological tumors with the highest mortality rate. Epithelial ovarian cancer (EOC) accounts for 85% to 90%, and clinical prognosis is poor. More than 75% EOC patients are classified as advanced stage upon diagnosis. The tumor spreads outward in the pelvic cavity and exists in many parts of the abdominal cavity. The 5-year survival rate in stage I patients is 90%, which is significantly declined to 4% in stage IV individuals. High-grade serous ovarian carcinoma (HGSOC) is a common EOC, accounting for more than 2 thirds of all categories of ovarian cancers.[17] HGSOC is manifested with high heterogeneity and genomic instability, rapid progression, high malignancy and strong invasive capability. Upon admission, a majority of patients are diagnosed with advanced HGSOC.[18]

7. Molecular mechanism of intratumoral heterogeneity in ovarian cancer

Microsatellite polymorphism and single nucleotide polymorphisms of samples were collected from 22 HGSOC patients. Significant genetic heterogeneity exists among samples from different parts of the same patient. Based on TCGA database, the genetic heterogeneity of HGSOC has been extensively studied. Systematic biomolecular analyses including genomics and epigenomics were performed in 489 HGSOC patients and found that the mutation rate of TP53 gene reaches up to 96%.[18] The mutation rate of BRCA1/2 gene is 22%, and approximately 2% to 6% for the remaining 7 genes. In addition, under the influence of mutation and promoter methylation, mutated DNA repair genes, such as BRCA somatic mutation and deletion of homologous recombination gene (HR), such as RAD51C, lead to more than 50% homologous recombination defects in HGSOC patients.[19]

The frequency of cdk2d-wdfy2 in HGSOC patients is 20%, which is the most common recombination event in HGSOC.[7] Cdk2d-wdfy2 fusion gene can be used as a specific molecular marker of cell subsets in heterogeneous HGSOC, which is of significance to reveal the pathogenesis of HGSOC, screen early clinical cases and design individual treatment regimens.

The micro-sequence of primary lesion/disseminated intra-abdominal lesion/lymphatic metastasis/recurrent papillary adenocarcinoma. At the protein level, the expression levels of polycomb group (PcG) proteins are up-regulated in these 4 diseases, and different expression profiles of PcG proteins lead to functional heterogeneity of EOC cells.[20] EOC cells with high PcG protein expression are superior to those with low PcG protein expression in terms of cell proliferation, migration, invasion, chemotherapy resistance and independent survival. Such heterogeneous expression is correlated with clinical prognosis of patients with ovarian cancer. High expression of PcG proteins can be used as an independent factor for poor prognosis. PcG protein can be utilized as a molecular marker of intratumoral heterogeneity in the progression of EOC. CNTNAP1 gene mutation activates the PI3K/Akt/Akt/Akt/mTOR signaling pathway through signal translation. Abnormal expression of related genes in ovarian cancer cell lines SKOV3 and A2780 is heterogeneous. Intravascular signals transmitted by low-invasive cellular components and PI3K/Akt/mTOR components may become the therapeutic targets of malignant tumors.

8. Clinical application of quantification of intratumoral heterogeneity in ovarian cancer

The most useful clinical significance of intratumoral heterogeneity is targeted molecular therapy. However, its function significantly varies at different tumor sites. EOC targeted molecular therapy also faces these challenges. Therefore, in patients with ovarian cancer, the evaluation of intratumoral heterogeneity is as important as the development of traditional tumors.[21] Patients with low EC are more conducive to targeted molecular therapy. However, in human blood circulation, quantitative methods provided by tumor cells are relatively low, which negatively affects the overall heterogeneity. Deep sequence of circulating tumor DNA has been adopted to detect EGFR mutation in chemotherapy-resistant tumor cells, but it should also be considered that circulating environment may not accurately represent tumor microenvironment and its surrounding matrix.

Another approach is to design a universal "phylogenetic tree" from which patients can predict intratumor heterogeneity. Although the number of EOC patients used in evolutionary research is insufficient, the "phylogenetic tree" of EOC has been found to have a short trunk and multiple branches, which represents the amplification of the first clone and high instability of the genome. Therefore, alternative algorithms should be chosen to objectively correlate more evolutionary maps in HGSOC patients to observe the similarity of branching patterns and identify gene mutation information that drives branching events. The branching time on the "phylogenetic tree" is also of clinical significance, which is helpful to achieve higher efficacy in targeted therapy. In-depth understanding of EOC phylogenetic tree also realizes scanning of 309 branch events, distinguish which mutations are curable and validate which mutations do not represent the disease. Pinpointing key mutation events to the trunk of the phylogenetic tree can minimize the differences of clinical efficacy and drug resistance, thus improving the consistency in clinical efficacy for cancer.

The long-term goal is to change the heterogeneity of EOC to obtain better chemical properties, which depends on the key factors to identify and inhibit intratumoral heterogeneity. Intratumoral heterogeneity does not only exist in gene mutation, it also occurs in transcription efficiency, epigenetic changes, immune response and tumor microenvironment, protein expression and mitotic variation. In lung cancer patients, APOBEC-damine mutation caused by RNA modification is the early driving event of heterogeneous lung cancer. Infiltrating lymphocytes can be detected in all EOC tumor sites, suggesting that the therapeutic target protein unaffected by genetic diversity might be the optimal target protein for tumor

therapy. D'Oria O. et al[22] have indicated that the available diagnostic programs are insufficient for gynecological cancer. Consolidating existing programs and exploring novel options are essential. The team also illustrated that tailored diagnosis and therapy for all gynecological cancers play a significant role in enhancing patient outcomes, lowering costs and shortening the duration of follow-up.

9. Conclusions

Taken together, ovarian cancer has a strong ability of direct clone evolution (branching). The degree of intratumoral heterogeneity is negatively correlated with the prognosis of ovarian cancer patients. Such intratumoral heterogeneity can provoke drug resistance to initial treatment and subsequent chemotherapy, leading to the failure of multiple target drugs. Clarifying the degree of intratumoral heterogeneity contributes to improving the stratification and disease expectation and exploring the guiding factors of intratumoral heterogeneity. Inhibition therapy of these drivers is a potential approach to enhance clinical efficacy by using genetic heterogeneity. Intratumoral heterogeneity plays an important role in the occurrence and development of ovarian cancer. More and more studies reveal the correlation between intratumoral heterogeneity and drug resistance. Besides, quantification of intratumoral heterogeneity is related to poor prognosis of ovarian cancer. Consequently, unraveling the pathogenesis of intratumoral heterogeneity contributes to screening the major factors of ovarian cancer development. Integrating genomics tools to describe the disease development trajectory is a promising procedure to predict disease progression and guide clinical treatment. To deliver precision treatment, extensive emphasis should be placed upon the changes of key driving genes and the variations of tumor heterogeneity, analyzing the interaction between heterogeneity and tumor microenvironment and provide evidence for novel targeted therapy.

Author contributions

Conceptualization: Tengda Pu, Chengyuan Zhang, Bingfeng Su, Li Li, Jingjing Fu.
Data curation: Tengda Pu, Chengyuan Zhang, Bingfeng Su, Li Li, Jingjing Fu.
Formal analysis: Chengyuan Zhang.
Investigation: Tengda Pu, Bingfeng Su, Li Li, Jingjing Fu.
Methodology: Tengda Pu, Chengyuan Zhang, Bingfeng Su, Li Li, Jingjing Fu.
Project administration: Tengda Pu, Chengyuan Zhang, Bingfeng Su, Li Li, Jingjing Fu.
Resources: Tengda Pu, Chengyuan Zhang, Bingfeng Su, Li Li, Jingjing Fu.
Software: Tengda Pu, Chengyuan Zhang, Bingfeng Su, Li Li, Jingjing Fu.
Supervision: Tengda Pu, Chengyuan Zhang, Bingfeng Su, Li Li, Jingjing Fu.
Validation: Tengda Pu, Chengyuan Zhang, Bingfeng Su, Li Li, Jingjing Fu.
Visualization: Tengda Pu, Chengyuan Zhang, Bingfeng Su, Li Li, Jingjing Fu.
Writing – original draft: Tengda Pu, Chengyuan Zhang, Bingfeng Su, Li Li, Jingjing Fu.
Writing – review & editing: Tengda Pu, Chengyuan Zhang, Bingfeng Su, Li Li, Jingjing Fu.

References

[1] Reid BM, Permuth JB, Sellers TA. Epidemiology of ovarian cancer: a review. Cancer Biol Med. 2017;14:9–32.
[2] Khalique L, Ayhan A, Weale ME, et al. Genetic intra-tumour heterogeneity in epithelial ovarian cancer and its implications for molecular diagnosis of tumours. J Pathol. 2007;211:286–95.
[3] Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209–49.
[4] Kurman RJ, Shih I-M. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer: shifting the paradigm. Hum Pathol. 2011;42:918–31.
[5] Vang R, Shih I-M, Kurman RJ. Ovarian low-grade and high-grade serous carcinoma: pathogenesis, clinicopathologic and molecular biologic features, and diagnostic problems. Adv Anat Pathol. 2009;16:267–82.
[6] Nowell PC. The clonal nature of neoplasia. Cancer Cells. 1989;1:29–30.
[7] Hoogstraat M, de Pagter MS, Cirkel GA, et al. Genomic and transcriptomic plasticity in treatment-naïve ovarian cancer. Genome Res. 2014;24:200–11.
[8] Network TCGAR. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474:609–15.
[9] Panici PB, Marchetti C, Salerno L, et al. Dualistic classification of epithelial ovarian cancer: surgical and survival outcomes in a large retrospective series. Ann Surg Oncol. 2014;21:3036–41.
[10] Gurung A, Hung T, Morin J, et al. Molecular abnormalities in ovarian carcinoma: clinical, morphological and therapeutic correlates. Histopathology. 2013;62:59–70.
[11] Kobayashi H, Kajiwara H, Kanayama S, et al. Molecular pathogenesis of endometriosis-associated clear cell carcinoma of the ovary (review). Oncol Rep. 2009;22:233–40.
[12] Kurman RJ, Shih I-M. The origin and pathogenesis of epithelial ovarian cancer - a proposed unifying theory. Am J Surg Pathol. 2010;34:433–43.
[13] Tothill RW, Tinker AV, George J, et al. Novel molecular subtypes of serous and endometrioid ovarian cancer linked to clinical outcome. Clin Cancer Res. 2008;14:5198–208.
[14] Giannini A, Di Dio C, Di Donato V, et al. PARP inhibitors in newly diagnosed and recurrent ovarian cancer. Am J Clin Oncol. 2023;46:414–9.
[15] Geyer JT, López-García MA, Sánchez-Estevez C, et al. Pathogenetic pathways in ovarian endometrioid adenocarcinoma: a molecular study of 29 cases. Am J Surg Pathol. 2009;33:1157–63.
[16] Swanton C. Intratumor heterogeneity: evolution through space and time. Cancer Res. 2012;72:4875–82.
[17] Bashashati A, Ha G, Tone A, et al. Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling. J Pathol. 2013;231:21–34.
[18] Schwarz RF, Ng CKY, Cooke SL, et al. Spatial and temporal heterogeneity in high-grade serous ovarian cancer: a phylogenetic analysis. PLoS Med. 2015;12:e1001789.
[19] Lee J-Y, Yoon J-K, Kim B, et al. Tumor evolution and intratumor heterogeneity of an epithelial ovarian cancer investigated using next-generation sequencing. BMC Cancer. 2015;15:85.
[20] Pasini D, Di Croce L. Emerging roles for polycomb proteins in cancer. Curr Opin Genet Dev. 2016;36:50–8.
[21] Sagi A, Yeela S, Liron B, et al. Intratumoral heterogeneity in the self-renewal and tumorigenic differentiation of ovarian cancer. Stem Cells. 2012;30:415–24.
[22] Golia D'Augè T, Giannini A, Bogani G, et al. Prevention, screening, treatment and follow-up of gynecological cancers: state of art and future perspectives. Clin Exp Obstet Gynecol. 2023;50:160.