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Chronic Diseases and Translational Medicine 1 (2015) 14−17

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Perspective

Clinical significance of intratumor heterogeneity for gynecological carcinoma

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Received 26 November 2014

Available online 21 March 2015

Keywords: Intratumor heterogeneity; Diagnose; Tumor marker; Chemotherapy resistance; Follow-up

Heterogeneity is very important to ensure the biodiversity and the existence of nature. Human malignancies consist of cellular sub-populations with various pathological features and biological characteristics.

Intratumor heterogeneity includes differences in the histology, antigenicity, immunity, hormone receptors, metabolism, gene changes, growth rate, and the reactions to drugs.[1] Presently, there are at least two models that describe the heterogeneity of tumors. The first model, intratumor heterogeneity in evolutionary models of tumor progression, refers to the heterogeneity of the phenotypes and proliferation ability of the tumor cell population that originated from one single cell. Malignant transformation of the cells involves multiple steps including the initial mutation, latency, tumor promotion, and progression, which occurs after the mutation of multiple genes. Multiple mutations have been considered to be the basis of the heterogeneity of the tumor cells, while diversities of the genotypes and phenotypes are the basis of tumor heterogeneity. However, studies have also shown that genetic mutation may not necessarily lead to changes of the phenotypes of the cells.[2] The second model is called the 'tumor stem cell model'. Tumor stem cells have the ability to self-renew and undergo multidirectional differentiations which are the basis of heterogeneity.[2,3] Currently, the molecular basis of intratumor heterogeneity has already been confirmed in multiple tumors including breast cancer, malignant glioma, pancreatic cancer, and leukemia.[4] Gynecological tumors also consist of cellular sub-populations with different pathological features and biological characteristics; in other words, intratumor heterogeneity also exists in gynecological tumors.[1,5] While the knowledge about intratumor heterogeneity has progressed greatly with the advance of related studies, the clinical significance of intratumor heterogeneity in the diagnosis, treatment, and follow-up of malignant tumors are still uncertain. In the present study, the clinical significance of intratumor heterogeneity in the diagnosis, treatment, and follow-up of gynecological tumors were reviewed.

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Peer review under responsibility of Chinese Medical Association.

Production and Hosting by Elsevier on behalf of KeAi

http://dx.doi.org/10.1016/j.cdtm.2015.02.005

Detection of intratumor heterogeneity and the significance in the pathological diagnosis of gynecological tumors

Recent advances in the development of new techniques in molecular biology research have created opportunities to improve the diagnosis of tumors, which include immunohistochemical staining, chromosome banding technique, fluorescence in situ hybridization, comparative genomic hybridization (CGH),[6] array CGH, tiling CGH, single nucleotide polymorphism, and next generation sequencing.[7] Especially important are single cell amplification methods[8] and molecular profiling of single circulating tumor cells with diagnostic intention.[9] There are studies that have investigated the intratumor heterogeneity not only at the morphological level of the tumor cells, but also at the level of DNA, RNA, and protein, which will have importance for providing individualized treatments and deciding treatment strategies.

In clinical practice, pathological diagnosis has been accepted as the gold standard for developing treatment strategies for malignant tumors. In addition, correct pathological diagnosis is also of significance for the prognosis of the patients. The morphologies of the tumors are generally very similar when observed under the microscope; however, there are no two tumors with exactly identical gene expression at the molecular level. Hence, conventional pathological diagnosis of tumor could result in several deviations in guiding the treatment selection; specifically in cases of pathological diagnosis on the basis of single biopsy findings with limited available information, treatment selection can be inappropriate.[10] In the studies of tissues obtained from primary breast cancers and their metastases, Rao et al[11] and Yao et al[12] found that there were heterogeneities between the primary breast cancer and their metastatic tumors. Ting et al[5] investigated the polycomb group (PcG) proteins from the tissues of recurrent epithelial ovarian cancers and found significant differences in the expression between the primary cancer tissues and the recurrent cancer tissues. These findings suggest that there is heterogeneity between the recurrent tumors and the primary tumors. Due to the existence of intratumor heterogeneity, multiple specimens from different sites of the tumor are necessary for the pathological diagnosis in clinical practice. Additionally, the size of the specimens should also be considered, and genetic examinations should be performed if necessary to detect the changes of the intratumor heterogeneity, which would guide the choice of treatment strategies for the patients with metastatic tumors or recurrent tumors, provide individualized treatment, and improve the prognosis of the patients.

Furthermore, because of the existence of intratumor heterogeneity, the pathological diagnoses in the future should include the combination of conventional and molecular pathological classifications, which could ensure that clinicians attain accurate diagnosis and individualized treatment for the patients with tumors. The American College of Medical Genetics Cytogenetics Resource Committee discussed the intratumor heterogeneity of the human epidermal growth factor receptor (HER) 2 gene in breast cancer, and standardized the diagnostic criteria of the genetic heterogeneity of the HER1 gene in detail. This helped to choose individualized Trastuzumab treatments for the patients with breast cancer.[13,14]

Significances of intratumor heterogeneity in the clinical treatments of malignant gynecological tumors

Most of the patients with advanced tumors are prone to develop chemotherapy resistance during clinical treatment for malignant gynecological tumors, which results in treatment failure and a poor prognosis. There are multiple mechanisms involved in chemotherapy resistance which are associated with the growth and repair of the tumor cells, intratumor genetic heterogeneity, and heterogeneity in cell growth.

Under normal conditions, the DNA repair mechanism is a very important function to ensure the stability of the cells; however, potent DNA repair function can also rapidly repair the DNA damage in the tumor cells induced by the chemotherapy, and thus induce the resistance to chemotherapy.[15] The intratumor genetic heterogeneity, functional heterogeneity, and growth time heterogeneity of the tumor cells may also induce chemotherapy resistance in the cells.[16,17] Specifically, the effects of the intratumor genetic heterogeneity on molecular targeted therapy can result in chemotherapy resistance.[18,19] In addition, genetic mutations may also occur after the chemotherapy, which could further induce the alterations in the phenotypes followed by chemotherapy resistance of the tumors.[20] Hence, the intratumor genetic heterogeneity and cell growth heterogeneity should be considered when selecting the appropriate individualized treatment strategies to avoid the development of drug resistance.

Recently, sequential chemotherapy was developed based on the Norton-Simon hypothesis. In sequential chemotherapy, non-cross-resistant drugs are used sequentially. In brief, drug A at an appropriate dose is administered for several cycles followed by drug B at an appropriate dose for several cycles. In sequential chemotherapy, a single drug or combination of drugs could be used to target tumor cells with different

chemotherapy sensitivity spectrums to increase the effectiveness of chemotherapy and to reduce the drug-related adverse reactions.[21,22] Theoretically, using sequential chemotherapy could overcome the difficulties caused by the intratumor heterogeneity, and thus it could provide a better treatment strategy. Presently, sequential chemotherapy is being used in the treatment of high-risk trophoblastic tumors, breast cancers, and lung cancers, and it has shown better effectiveness compared with conventional combined chemotherapy.

The National Comprehensive Cancer Network of the United States of America guidelines issued in 2014 recommended a combined chemotherapy of paclitaxel and platinum as a first-line treatment strategy for epithelial ovarian cancer,[23] and 6–8 cycles of chemotherapy for the patients with advanced tumors. However, as there is intertumor heterogeneity among different types of epithelial ovarian cancers, the response of individual types of tumors to chemotherapy can be different. In addition, intratumor heterogeneity also exists within the same type of ovarian carcinoma. Hence, sequential chemotherapy as the first-line treatment strategy is more rational for the treatment of epithelial ovarian cancers.

In a phase II clinical trial, compared the effectiveness and safety of sequential chemotherapy of gemcitabine plus oxaliplatin and carboplatin plus Taxol in 18 patients with stage III/IV ovarian cancer. The study results showed that the complete remission rate was 92.0%, and the toxic reactions induced by the chemotherapy were all acceptable.[24] In the Potamianou et al[25] study, 52 patients with ovarian cancer were treated with sequential chemotherapy of carboplatin plus Taxol or Taxol liposomes plus Adriamycin. The overall chemotherapy reaction rate was 66%, among which the complete remission rate was 44% and the partial remission rate was 22%.The toxic reactions induced by the chemotherapy were also acceptable. Li et al[26] performed a clinical study in 41 patients with epithelial ovarian cancer, and it was found that sequential chemotherapy could improve the progression-free survival (PFS) and reduce the side-effects compared with a conventional chemotherapy group. The findings of these studies suggest that sequential chemotherapy could improve the survival and life quality of the patients with ovarian tumors, especially when molecular targeted therapy is used in combination with chemotherapy. However, as most of the studies that investigated the application of sequential chemotherapy in treating epithelial ovarian cancers were with relatively small sample sizes, more multi-center clinical studies are needed to further identify the best sequential therapy strategy.

Significances of intratumor heterogeneity in the clinical treatment and follow-up of the patients with malignant gynecological tumors

In light of the intratumor heterogeneity, the heterogeneities in the genotypes and phenotypes of the tumor sub-clone could change after chemotherapy. Li et al[27] investigated the tumor biomarkers in the serum after chemotherapy of patients with epithelial ovarian cancers and found that the spectrum of the tumor biomarkers changed during the chemotherapy and in the recurrent tumors; in addition, the changes of the types of biomarkers were associated with the resistance of the tumors and the prognosis of the patients. The study results suggested the existence of intratumor heterogeneity. Moreover, it also suggested that during the treatment and follow-up, the traditional tumor biomarker monitoring strategy should be changed; the biomarkers that were normal before the treatment should also be monitored in addition to the abnormally expressed tumor biomarkers, which might help to identify the abnormal changes occurred in these biomarkers after chemotherapy. This could help to detect the tumor recurrence early and might help to adjust the treatment strategy accordingly.

Ting et al[5] investigated the PcG proteins in the tissues from primary and recurrent epithelial ovarian cancers and found that these proteins were expressed significantly differently between the primary tumors and the recurrent tumors. The expression of B cell-specific Moloney murine leukemia virus integration site 1 and the enhancer of zeste 2 polycomb repressive complex 2 subunit, the two members of the PcG proteins family in the primary and recurrent tumors, could help to predict the differences in the PFS and overall survival of the patients. These findings suggested that there was intratumor heterogeneity in the recurrent and metastatic tumors, which were associated with the prognosis of the patients. In another study, Gerlinger et al.[28] performed exome sequencing, chromosome aberration analysis, copy-number analysis, immunohistochemical staining, mutation function analysis, and messenger RNA expression examinations in primary and metastatic renal cancers. The results showed the molecular base of the intratumor heterogeneity. Moreover, it was identified that the intratumor heterogeneity could affect the response to chemotherapy as well as the clinical outcome of the patients. Gulati S et al[29] performed a further study to systemically investigate the intratumor heterogeneity and the prognostic biomarkers in patients with renal cancer, and the results also showed that the intratumor heterogeneity was associated with the clinical prognosis of the

patients. Hence, more clinical studies focusing on the significance of intratumor heterogeneity in the clinical prognosis of the patients should be performed.

In summary, there is intertumor heterogeneity and intratumor heterogeneity. The major goals of gynecological oncology should include an accurate determination and effective control of tumor heterogeneity and thereby improve the diagnosis, therapeutic strategies, and follow-up of patients and produce an improved quality of life and survival.

During clinical pathological diagnosis, pay attention to the representative biopsy materials and the puncture multi-point sampling. Serum tumor marker expression in patients with epithelial ovarian carcinoma may change after chemotherapy or recurrence, indicating that in addition to the markers that are abnormal before surgery, those markers that are normal should also be monitored during chemotherapy and follow-up. Sequential chemotherapy and individualized treatment strategies are recommended for first-line chemotherapy in patients with epithelial ovarian carcinoma to improve the curative effect and the quality of life, and to decrease the drug resistance and adverse effects.

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Edited by: Yang Pan