**Summary.** Most patients with ovarian cancers relapse, and treatment failure has often been attributed to chemoresistance in tumor cells. Emerging evidence indicates that tumor heterogeneity may play an equally important role. Although the idea of tumor heterogeneity is not new, little attention has been focused on applying it to understand and control ovarian cancer progression. Recent advances in understanding its generation model, original basis, consequent problems, and derived therapies provide great potential for tumor heterogeneity to be a new insight in treatment of ovarian cancers.

**Key words:** Tumor heterogeneity, Chemoresistance, Ovarian cancer, Personalized medicine, Therapy.

**Introduction**

Ovarian cancer is the leading cause of death from gynecological malignancy (Siegel et al., 2014). The overall response rate associated with platinum and paclitaxel has reached 70%. Nevertheless, most patients relapse, and treatment failure has often been attributed to chemo-resistance. Recently, emerging evidences indicate that tumor heterogeneity may contribute to this failure by initiating phenotypic diversity and by introducing tumor sampling bias (Yap et al., 2012). Although the idea of tumor heterogeneity is not new, little attention has been focused on applying it to understand and control ovarian cancer progression. Determining the molecular events that control this tumor trait would be a major breakthrough in our knowledge of ovarian cancer and could lead to more effective diagnostic and therapeutic methods.

**Conceptual advances of tumor heterogeneity**

As early as 1957, Foulds put forward the idea that, after a neoplasm is initiated, its characteristics are not fixed for all time. Different characteristics of a tumor undergo progression independently of one another (Foulds, 1957). Tumors are assumed to originate from a monoclonal composition and are propagated by multiplication of genomically distinct subclones, which may differ with respect to morphology, karyotype, metastatic capacity, sensitivity to cytotoxic drugs, expression of cell surface antigens and hormone receptors, immunogenicity, sensitivity to the immune reaction of the host, and other properties (Woodruff, 1983; Heppner, 1984). As such, the divergence of clonal cell populations confers a heterogeneous genomic tapestry with implications for clinical endpoints, including the acquisition of metastatic potential and chemotherapeutic resistance (Baldus et al., 2010; Ding et al., 2010; Walter et al., 2012). Next generation sequencing technology enables the study and quantification of clonal evolution and intratumoral diversity in cancer, including inference from distributions of digital allelic representation of mutations in single samples, along with serial and regional comparisons of mutation profiles (Shah et al., 2009, 2012; Nik-Zainal et al., 2012a,b). Much research has found that extensive genetic diversity arises at early
thought of as cancer stem cells that undergo an aberrant of differentiation. Therefore, tumorigenic cells can be anomalous differentiation of tumorigenic cells. Both normal stem cells and tumorigenic cells exhibit various degrees of differentiation. Therefore, tumorigenic cells can be thought of as cancer stem cells that undergo an aberrant and poorly regulated process of organogenesis analogous to what normal stem cells do (Reya et al., 2001).

The existence of stem cells in ovarian cancer has long been postulated. Cancer cells with CD117+ phenotype isolated from human ovarian serous adenocarcinoma tissues or ascites could reproduce the original tumor heterogeneity and be serially generated (Bapat et al., 2005; Luo et al., 2011). Zhang et al observed that a subpopulation of primary human ovarian cancer cells with a high expression of CD44 and CD117 were highly tumorigenic and capable of reestablishing their original tumor hierarchy when injected into mice (Zhang et al., 2008). Gao et al identified a subpopulation enriched for ovarian cancer stem cells defined by CD24 phenotype in a series of cancer cell clones. Their results demonstrated that a CD24+ subpopulation could efficiently form tumors containing an array of cell types similar to those found in the original carcinoma samples when injected into immunocompromised mice, while CD24− cancer cells could not (Gao et al., 2010). In another study, Baba et al reported that CD133+ cells from ovarian cancer cell lines exhibited enhanced resistance to platinum-based therapy and formed more aggressive tumors in mice compared to CD133− cells (Baba et al., 2009).

**Clonal evolution model**

The clonal evolution model of carcinogenesis states that tumor cells over time acquire various mutations, and that genetic drift and natural selection for the fittest, most aggressive cells drive tumor progression (Campbell and Polyak, 2007). According to this idea, tumor initiation takes place once multiple mutations occur in a random single cell, providing it with a selective growth advantage over adjacent normal cells (Campbell and Polyak, 2007). As the tumor progresses, uncontrolled proliferation and genetic instability allow the production of cells with additional mutations and hence new characteristics (Campbell and Polyak, 2007). Because these mutations have differing and heritable effects on the fitness of tumor cells, mutant clones might expand or contract in the neoplasm, resulting in tumor heterogeneity.

In 1976, Peter Nowell proposed the clonal evolution model of cancer, and applied the evolutionary model to understand tumor growth and treatment failure as well as the phenomenon of increased tumor aggressiveness that occurs during the natural history of advanced solid tumors (Nowell, 1976). Tumors arise from a single mutated cell, and subsequent additional alterations give rise to multiple subpopulations within the original neoplasm. Natural selection occurs in the neoplasm, because genetic and epigenetic mutations generate heritable variation, and some mutations confer a selective advantage or disadvantage on the cell (Merlo et al., 2006). Those fitness advantages will be amplified in tissues with repeated wounding, in which repeated cycles of cell death and proliferation enable a mutant
clone with a survival or reproductive advantage to expand (Merlo et al., 2006). During this process, cancer cells have the potential to become invasive, causing metastasis, or become resistant to therapy, resulting in relapse.

Three decades of research have broadly supported Nowell’s description of cancer as an evolutionary system. Studies of ovarian cancers have shown the patterns of genetic alterations between premalignant lesions, primary tumors, metastases, and recurrence, agree with what is expected from clonal evolution.

Khaique and colleagues analyzed the genetic alterations of multiple areas of tumor tissue taken from 16 primary epithelial ovarian cancers, and suggested a monoclonal origin of tumors and subsequent clonal divergence demonstrated by mixed populations of genetically distinct cells within the tumor (Khaique et al., 2007). In another study, Castellarin et al performed whole exome sequencing on tumor cells harvested from ascites at three time points (primary, first recurrence, and second recurrence) for three high-grade serous ovarian carcinoma (HGSC) patients receiving standard treatment, and found that the vast majority of somatic variants found in recurrent tumors were present in primary tumors. The authors reached the conclusion that recurrent HGSC arose from multiple clones present in the primary tumor (Castellarin et al., 2013).

Comparisons of cancer stem cell hypothesis vs. clonal evolution model

The cancer stem cell hypothesis and the clonal evolution model have significant differences in the following aspects.

First, regarding the origin of tumors, both models agree that tumors originate from a single cell that over time has acquired multiple mutations and has gained unlimited proliferative potential. However, normal stem cells or progenitor cells are the most likely targets of malignant transformation according to the cancer stem cell hypothesis, while any random single cell can be the target of malignant transformation in the clonal evolution model.

Second, these two models explain tumor heterogeneity in different mechanisms. A program of aberrant differentiation of cancer stem cells is the cause of tumor heterogeneity in the cancer stem cell hypothesis, while genetic instability and natural selection primarily produce multiple subpopulations within a single tumor in the clonal evolution model.

Third, according to cancer stem cell hypothesis, tumor progression is a result of the metastatic spread of cancer stem cells. In the clonal evolution model, any tumor cells having acquired selective advantageous mutations (often the fittest and more aggressive ones) will drive the tumor progression.

Fourth, concerning therapeutic resistance, cancer stem cell hypothesis believes that a small pool of cells, the cancer stem cells, are inherently resistant to chemotherapy, while in the clonal evolution model, any cancer cells have the potential to become resistant to therapies and cause recurrence.

Despite the above differences, the cancer stem cell hypothesis and the clonal evolution model are not mutually exclusive, and may compatibly coexist during tumor development and progression. For example, cancer stem cells produce multiple malignant cells with different genetic background; meanwhile, new cancer stem cells may appear due to genetic mutations. Tumor heterogeneity could be the product of both the cancer stem cells and cancer cell evolution.

Basis of tumor heterogeneity: genomic diversity

No matter how tumor heterogeneity is generated, genomic diversity is their common basis. High-grade serous ovarian carcinoma is a genetically unstable disease, showing highly rearranged karyotypes with numerical and structural chromosome aberrations (Bayani et al., 2002). This genetic instability has great potential for generating significant genetic diversity (Ahmed et al., 2010; Cooke et al., 2010).

Through exome sequencing, copy number analysis, targeted amplicon deep sequencing and gene expression profiling on 31 spatially and temporally separated HGSCs tumor specimens, including ovarian masses, distant metastases and fallopian tube lesions, Bashashati et al. found widespread intratumoral variation in mutation, copy number and gene expression profiles, with key driver alterations in genes present in only a subset of samples, and concluded that HGSCs exhibited highly individual evolutionary trajectories and diverse genomic tapestries prior to therapy, exposing an essential biological characteristic to inform future design of personalized therapeutic solutions and investigation of drug-resistance mechanisms (Bashashati et al., 2013).

The Cancer Genome Atlas project analyzed mRNA expression, miRNA expression, promoter methylation, and DNA copy number in 489 HGSCs and the DNA sequences of exons from coding genes in 316 HGSCs. Their results showed that HGSC was characterized by TP53 mutations in almost all tumors; low prevalence but statistically recurrent somatic mutations in 9 additional genes; 113 significant focal DNA copy number aberrations; and promoter methylation events involving 168 genes. Their analyses delineated 4 ovarian cancer transcriptional subtypes, 3 miRNA subtypes, 4 promoter methylation subtypes, and a transcriptional signature associated with survival duration, which sheds new light on the impact on survival of tumors with BRCA1/2 and CCNE1 aberrations (Cancer Genome Atlas Research Network, 2011).

Identifying the genetic diversity of ovarian carcinomas that correlate with tumor heterogeneity set the stage for approaches to treatment of HGSCs in which aberrant genes or networks are detected and targeted with therapies selected to be effective against these specific aberrations.
Problems caused by tumor heterogeneity

Tumor sampling bias

Laparoscopic examination is often applied in ovarian cancer to establish a diagnosis and to assess the extent of disease. A biopsy of all suspicious-looking areas is crucial to surgical staging before laparotomy. The tumor subclones that may ultimately influence therapeutic outcome may evade detection because of their absence or presence at low frequency at pathological diagnosis or because of their regional separation from the tumor biopsy site.

Tumor heterogeneity is dynamic during neoplastic progression. Subclones in one tumor may change and compete with each other for dominance during the disease course and through the treatment. A therapeutic decision in oncologic practice is often made with reference to the primary lesion, diagnosed months or even years previously. Such approaches are likely to ignore the tumor sampling bias, because tumor heterogeneity exists between the primary and metastatic/recurrent lesions, between different metastatic/recurrent sites, or even within the primary tumors.

Changes in molecular biomarkers

A large number of reports have emerged describing multiple markers in primary ovarian tumors. Many of the established biomarkers have been used to predict tumor development, progression, recurrence, and therapeutic effects. It is postulated that the profile of biomarkers does not change during the chemotherapy treatment or before and after recurrence. However, dynamic changes in subclonal architecture of the tumor present great challenges to interpretation of these predictive or prognostic biomarkers (Keats et al., 2012). Dong et al detected the serum level of three tumor markers of epithelial ovarian cancer, CA125, CA199, and CP2, at different time points in the same individual. Their results indicated that the profile of tumor markers showed significant differences in number and type before and after chemotherapy, as well as at presentation and at relapse (Dong et al., 2009). Conceivably, the changing dynamics of tumor subclonal architecture may result in previously subdominant clones, perhaps either absent or barely detectable in the primary, gaining preeminence, as well as the alteration of tumor molecular profile correspondingly. Tumor heterogeneity confounds the validation of single biomarkers, and simultaneous determination of multiple tumor markers would be a better alternative to evaluate treatment effects.

Chemoresistance and cancer relapse

Patients with ovarian cancers typically experience disease relapse within two years after the initial treatment. Further treatment can extend survival, but relapse eventually occurs again. Treatment failure has often been attributed to chemoresistance. How does chemoresistance occur? What are the potential mechanisms? Much evidence suggests that tumor heterogeneity may play an important role in the evolution of chemotherapy resistance.

Two potential models have been proposed (Ding et al., 2012). First, genetically heterogeneous clones may preexist within the tumor mass prior to treatment. Subpopulations with selective advantageous mutations are allowed for survival and expansion, and previously dominant sensitive clones were cleared by chemotherapy and replaced by a resistant clone. Second, resistance evolves progressively by mutations under the selective pressure of chemotherapy. The first model requires the presence of significant genetic heterogeneity within a tumor prior to treatment, resulting in a relapsed genome that is non-linearly related to the majority clone at presentation. The second model predicts that the genome at relapse evolves from a presentation genome, with sequential accumulation of mutations conferring increased drug resistance.

Cooke and his colleagues investigated genome evolution and genetic heterogeneity in the acquisition of drug resistance in high-grade serous ovarian carcinoma. An extensive and non-linear genetic divergence between treatment-sensitive and treatment-resistant clones cultured from the same individual was observed. Their observations indicated that profound intra-tumor genetic heterogeneity existed, and the extent and type of the differences between the early and late cell lines were only very distantly related and had evolved in parallel rather than as a direct progression. Genetically divergent subclones that were intrinsically resistant to platinum-bases treatment might therefore exist within a tumor at the time of presentation and before treatment begins (Cooke et al., 2010).

Although some studies have proven that intrinsically resistant subclones may have already existed within a tumor at presentation before the treatment, we cannot exclude the possibility that resistance could be developed under the selective pressure of chemotherapy since mutations are occurring continuously. The data of Ding et al demonstrated that acute myeloid leukemia cells routinely acquired a small number of additional mutations at relapse, and suggested that some of these mutations might contribute to clonal selection and chemotherapy resistance. The genome in an individual patient with acute myeloid leukemia is clearly a moving target, and eradication of the founding clone and all of its subclones would be required to achieve cures (Ding et al., 2012). It is reasonable that these two models are compatibly coexisting in the evolution of resistance.

Augmentation of tumor heterogeneity

Since chemotherapy may select for resistance, tumor architecture would consequently be changed by cancer
therapies. Is the tumor heterogeneity enhanced or attenuated by therapy? Up to now, whether therapeutics may in some cases contribute to enhance tumor diversity and adaptation is still quite controversial.

McAlpine et al compared multiple synchronous tumor samples in primary and recurrent ovarian cancers by in vitro chemoresistance assay, and found that recurrent lesions exhibited greater heterogeneity and more frequent extreme drug resistance (McAlpine et al., 2008). On the contrary, Cooke et al observed more genomic complexity at presentation than at relapse (Cooke et al., 2010). Therefore, we can only draw the conclusion that in some cases therapeutics may contribute to an enhanced tumor diversity and adaptation. More investigations are of great necessity to elucidate this controversy.

Obstacles in targeted therapy

Tumor heterogeneity poses major difficulties for targeted therapy in ovarian cancer, since it is not known how many genetic alterations must be corrected in order to achieve tumor eradication. It has been found that driver mutations in TP53 are ubiquitous in HGSC, and all other key driver alterations in genes present in only a subset of samples (Ahmed et al., 2010; Bashashati et al., 2013). The TP53 tumor suppressor gene encodes a DNA-binding transcription factor that induces cell growth arrest, senescence and cell death by apoptosis upon cellular stress (Vousden and Prives, 2009). The key role of p53 in the cellular response to oncogenic stress, and the fact that p53 is the most frequently mutated gene, make p53 a relevant and promising target for therapeutic intervention in ovarian cancer. From a therapeutic standpoint, we are concerned about the effect of restoration of wild type p53, and whether p53 reactivation will lead to tumor elimination even if the tumor carries multiple other alterations in critical cancer genes. Several preclinical studies in mice demonstrated that restoration of wild type p53 expression was indeed sufficient for elimination of tumors even in the presence of other tumor-associated genetic alterations, which presumably is due to the critical role of p53 in the cellular pro-apoptotic or pro-senescent response to oncogenic stress (Martins et al., 2006; Ventura et al., 2007; Xue et al., 2007). Furthermore, various approaches have led to the identification of small molecules that can rescue mutant p53, among which APR-246 has been tested so far in a phase I/II clinical trial with promising results (Bykov and Wiman, 2014). More clinical studies of p53-targeting drugs are of great necessity for treatment of ovarian cancer.

Therapies regarding tumor heterogeneity

Multi-drug regimen

Chemoresistance is the central problem in ovarian cancer therapy, and tumor heterogeneity plays a role in resistance generation. Several possible approaches have been proposed that might address the problem of therapeutic resistance, among which only the multi-drug therapy has been explored experimentally and/or clinically (Chabner and Roberts, 2005). Whether multi-drug regimens could improve the clinical outcomes of patients with ovarian cancers is still quite controversial.

Currently, the combination of platinum plus paclitaxel is considered the standard first-line treatment in ovarian cancer. Despite high response rates and prolonged survival achieved, over 80% of patients with advanced diseases relapse and eventually die. In an attempt to maximize tumor cytoreduction with first-line treatment, sequential doublet and triplet combinations have been tried. Potamianous et al evaluated the activity and tolerance of two sequential doublets (paclitaxel-carboplatin and liposomal doxorubicin/carboplatin) administered as first-line treatment in women with advanced ovarian cancer, and the regimen was demonstrated to be feasible and active (Potamianous et al., 2005). Other sequential regimens, such as gemcitabine-carboplatin followed by paclitaxel-carboplatin also appeared to be feasible in chemotherapy-naïve ovarian cancer. Maenpaa et al found the overall response rate following completion of 4 cycles of gemcitabine/carboplatin and 4 cycles of paclitaxel/carboplatin reached 92%, with median progression-free survival 12.8 months (Maenpaa et al., 2006). On the contrary, the study of Bookman et al found that compared with standard paclitaxel and carboplatin, the addition of a third cytotoxic agent provided no benefit in progression-free survival or overall survival after optimal or suboptimal cytoreduction (Bookman et al., 2009). Their work actually killed any further attempts to overcome resistance by sequential doublets or 3-drug regimens. The authors concluded that the development of new interventions beyond surgery and conventional platinum-based chemotherapy would be required to additionally improve outcomes of women with advanced ovarian carcinomas.

Inhibiting tumor cell repopulation at chemotherapy intervals

In ovarian cancer, chemotherapeutics are often administered in multiple doses with intervals of about three weeks. Although this allows the recovery of normal tissues between treatments, surviving cancer cells also proliferate during the intervals, and this process of repopulation is an important cause of treatment failure (Kim and Tannock, 2005). Repopulation in the treatment intervals will increase the number of tumor cells and change the tumor cell architecture, resulting in exacerbation of tumor complexity. Therefore, the method of chemotherapeutic drug administration might affect the heterogeneous status of a neoplasm.

The interval between courses of chemotherapy is
**Tumor heterogeneity in ovarian cancer**

determined by the requirement that the bone marrow repopulate the white cells and platelets in the blood before the next treatment cycle, thereby minimizing the chance of infection or bleeding. With the support of growth factors such as granulocyte-colony stimulating factor (G-CSF), the repopulation of bone marrow can be accelerated, so that courses of chemotherapy could be given at 2-week instead of 3-week intervals (Kim and Tannock, 2005). Compared with standard 3- or 4-week schedules, 2-week schedules with growth factors have shown prolonged survival in non-Hodgkin’s lymphoma and breast cancer (Citron et al., 2003; Pfreundschuh et al., 2004).

Katsumata et al compared a conventional regimen of paclitaxel and carboplatin with a dose-dense weekly regimen in women with advanced ovarian cancer, and found that median progression-free survival and overall survival at 3 years were longer in the dose-dense treatment group than in the conventional treatment group. They reached the conclusion that dose-dense weekly paclitaxel plus carboplatin improved survival analysis, providing a new treatment option in women with epithelial ovarian cancer (Katsumata et al., 2009).

In addition, many molecular-targeted agents have been developed to inhibit the signaling pathways for stimulating proliferation. A logical strategy would be using a short-acting cytostatic agent between courses of chemotherapy to inhibit the repopulation of tumor cells and to stop it before the next cycle, so that tumor cells can resume proliferation and be maximally sensitive to cytotoxic drugs (Kim and Tannock, 2005). Hormonal agents provide an ideal tumor-specific strategy because the bone marrow is not affected.

Therefore, promising strategies for reducing tumor heterogeneity during chemotherapy include modification of dose-schedule with the support of growth factor to shorten the treatment interval, and combinational use of short-acting tumor-specific cytostatic agents to inhibit tumor cell proliferation.

**Adaptive therapy**

Currently, systemic administration of cytotoxic drugs is the primary treatment for patients with advanced ovarian cancer. Whereas many effective therapies are available, the amplitude and durability of tumor response to chemotherapy is limited by tumor cell resistance. Drug resistance arises as a result of temporal and spatial heterogeneity in cancers that typically contain both multiple subpopulations and multiple micro-environmental subregions.

Most research efforts in chemotherapy are focused on the discovery of agents and combinations of agents, doses, and dose schedules that maximally kill tumor cells while minimizing the toxicity to the host. However, cancers are highly dynamic and adaptive systems that can evolve phenotypic strategies to overcome proliferation barriers in their environment. The standard cancer therapy is typically imposed in a rigid fashion, with drug type, doses, and intervals fixed by protocol and altered only in the event of excessive patient toxicity. Thus, although a tumor is a dynamic system that evolves during treatment, therapeutic strategies tend to remain relatively static.

Recently, a new conceptual model of cancer treatment has been proposed, which is adaptive therapy. A general principle of adaptive therapy is that cancer treatment should be as dynamic as the tumor populations that are being treated. Specifically, therapeutic strategies should evolve in response to and in anticipation of tumor adaptation through continuous adjustment of drugs, dose, and timing (Gatenby et al., 2009).

By using a mathematical model and computer simulations, Gatenby et al found that in the absence of therapy, the fitter, chemosensitive cells actually suppressed the growth of the less fit but resistant population. Therapies designed to kill the maximum number of cancer cells produced an environment in which the resistant cells survived and were unopposed by the fitter, chemosensitive populations. Alternatively, if therapy was limited to allow a significant number of chemosensitive cells to survive, they would, in turn, suppress the growth of the resistant population (Gatenby et al., 2009).

Furthermore, in vivo experiment models were established by inoculation of an ovarian cancer cell line (OVCAR-3) into combined immunodeficient mice and administration of carboplatin adjusted continuously to maintain a stable tumor volume. Their results showed that adaptive therapy could achieve a substantially longer survival than standard high dose density strategies by maintaining a tumor volume that was either stable or slowly increasing for a prolonged period of time (Gatenby et al., 2009).

Their experiments represented only a simplistic test of the model, because the therapy variables were limited to the dose and timing of a single drug and assessed tumor response only by changes in size. Nevertheless, their results did confirm that a prolonged survival could be achieved through application of adaptive therapy to maintain a stable tumor volume, creating new insights in ovarian cancer treatment in the future.

**Conclusions**

Tumor heterogeneity is a common feature of ovarian cancer. Two concepts trying to explain its generation, the cancer stem cell hypothesis and the clone evolution model, share a common basis, genetic diversity. Tumor heterogeneity leads to important consequences in personalized medicine in ovarian cancer, such as tumor sampling bias, dynamic biomarker profile, and chemoresistance. Treatments attempting to attenuate tumor heterogeneity, such as multi-drug regimen and inhibition of tumor cell repopulation at treatment intervals, focus on maximally killing tumor cells, while adaptive therapy is designed to maintain a certain tumor burden that is stable for a prolonged period of time.
through continuous adjustment of drugs, dose, and timing.

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References


Durrett R., Foo J., Leder K., Mayberry J. and Michor F. (2011). Intra-
tumor heterogeneity in evolutionary models of tumor progression. Genetics 188, 461-477.


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