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Bladder Tumor Heterogeneity: The Impact on Clinical Treatment

Key Words

Tumor heterogeneity · Bladder cancer · Personalized medicine · Therapy

Abstract

Bladder cancer relapse and treatment failure in most patients have often been attributed to chemoresistance in tumor cells and metastasis. Emerging evidence indicates that tumor heterogeneity may play an equally important role and extends to virtually all measurable properties of cancer cells. Although the idea of tumor heterogeneity is not new, little attention has been paid to applying it to understand and control bladder cancer progression. With the development of biotechnology, such as Gene sequencing, recent advances in understanding its generation model, original basis, consequent problems, and derived therapies provide great potential for tumor heterogeneity to be considered a new insight in the treatment of bladder cancers.

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Introduction

Bladder cancer is a serious disease endangering human health, the incidence of which ranks ninth in systemic cancer around the world. The major treatment is preferentially given to surgical excision [1]. However, it represents an important economic burden, affects patients' quality of life, and is life threatening when it invades the muscle (nearly one third of bladder cancer is muscle invasive). For instance, the 90-day mortality of elderly patients with MIBC undergoing RC remains substantial. The interrelation between comorbidity, complication rate, and 90-day mortality underlines the need for a comprehensive geriatric assessment of elderly patients with MIBC in whom RC is indicated [2]. Unfortunately, in many ways, bladder cancer remains a neglected disease [3]. It is easy for a patient to relapse after treatment, and most patients die of tumor metastasis and chemoresistance [4].

Recently, emerging evidence indicates that tumor heterogeneity may contribute to this failure by initiating phenotypic diversity and by introducing tumor sampling [5] bias. Although the idea of tumor heterogeneity is not

new, little attention has been paid to applying it to understand and control bladder cancer progression. Determining the molecular events that control this tumor trait would be a major breakthrough in our knowledge of bladder cancer and could lead to more effective diagnostic and therapeutic methods.

Conceptual Advances of Tumor Heterogeneity

The history of tumor heterogeneity research is one of a progressive revolution, commensurating with the increasing sophistication of technology [6]. As early as 1957, Foulds put forward the idea that once a neoplasm is initiated, its characteristics are not fixed for all time. Different characteristics of a tumor progressed independently of one another [7]. Tumors are assumed to originate from a monoclonal composition and are propagated by multiplication of genomically distinct subclones, which may differ with regard 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 [8, 9]. 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 [10–12]. The new generation sequencing technology enables the study and quantification of clonal evolution and intra-tumoral 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 [13, 14]. Much research has found that extensive genetic diversity arises at early stages of tumorigenesis. New genetic variants persist during tumor progression, resulting in ongoing, parallel, and even convergent evolution among different metastases [15, 16].

Generation Model of Tumor Heterogeneity

Tumor heterogeneity extends to virtually all measurable properties of cancer cells, and it represents a major hurdle in bladder cancer therapy. The mechanisms underlying its emergence remain poorly understood. Two models have been presented to explain the process of tumor heterogeneity: the cancer stem cell hypothesis and the clonal evolution model [17].

Cancer Stem Cell (CSC) Hypothesis

Cancer stem cells are capable of indefinite self-renewal and diverse differentiation, leading to the production of all cell types and thereby the generation of tumor heterogeneity [18]. According to this hypothesis, tumor progression is a result of the metastatic spread of cancer stem cells, and cancer recurrence is caused by their resistance to therapy [19]. Cancer stem cells are widely believed to arise from normal stem or progenitor cells, persisting as a small fraction of cells in a tumor [20]. They are long-lived, and more likely than other cells to acquire multiple mutations during malignant transformation [21].

The existence of stem cells in bladder cancer has long been postulated. Using a particular approach, Rani Ojha et al. have identified a subpopulation of cancer stem cell-like side population (SP) cells from bladder cancer cell lines, namely T24 and UM-UC-3. These cells were found to be more tumorigenic than the non-side population (NSP), which formed the bulk of tumor cells [22]. The SP cells isolated from cancer cells have proved to be an attractive alternative for studying CSCs, since these cells have been reported to be enriched with CSCs in several kinds of malignancies [23]. In another study, SP cells representing CSCs of bladder cancer showed an up-regulated expression of MAGE-A3 and a positive coexpression of MAGE-A3 and CD133, which may serve as an immunotherapeutic target for CSCs of bladder cancer [24].

Clonal Evolution Model

The clonal evolution model of carcinogenesis states that over time tumor cells acquire various mutations, and that genetic drift and natural selection for the fittest, most aggressive cells drive tumor progression. According to this idea, tumor initiation takes place once multiple mutations occur in a random single cell, providing it with a selective growth advantage compared with adjacent normal cells. As the tumor progresses, uncontrolled proliferation and genetic instability allow the production of cells with additional mutations and hence new characteristics. 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 [25].

Lately, Jun Wang and colleagues carried out the single-cell exome sequencing of 66 individual tumor cells from a muscle-invasive bladder transitional cell carcinoma (TCC). Analyses of the somatic mutant allele frequency spectrum and clonal structure revealed that the tumor cells were derived from a single ancestral cell, but that

subsequent evolution occurred, leading to two distinct tumor cell subpopulations which provide evidence for the monoclonal origin of TCC [26].

Comparison of Cancer Stem Cell Hypothesis vs. Clonal Evolution Model

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

First, normal stem cells or progenitor cells are the most likely targets of malignant transformation according to the cancer stem cell hypothesis, whereas any random single cell can be the target of malignant transformation in the clonal evolution model.

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

Third, regarding therapeutic resistance, cancer stem cell hypothesis believes that a small pool of cells, the cancer stem cells, are inherently resistant to chemotherapy; whereas in the clonal evolution model, cancer cells have the potential to become resistant to therapies and to cause recurrence [27].

Similarities. There are a few points on which the cancer stem cell hypothesis and the clonal evolution model agree. In each case, tumors originate from a single cell that has acquired multiple mutations and has gained unlimited proliferative potential. In each case, the specific cell of origin, particular genetic alterations involved, and micro-environmental factors can influence the make-up of a tumor and determine its physical characteristics and clinical properties [28]. Tumor heterogeneity could be the product of both the cancer stem cells and cancer cell evolution.

Basis of Tumor Heterogeneity: Genetic Heterogeneity

No matter how tumor heterogeneity is generated, genomic diversity is its common basis. Recent studies have revealed extensive genetic diversity both between and within tumors. In tumors, populations of genetically distinct subclones can intermingle or be spatially separated, and this subclonal architecture varies dynamically throughout the disease course [29]. Genomic instability is a prominent source of genetic diversity within tumors,

generating a diverse cell population that can be subject to selection in a given microenvironmental or therapeutic [30, 31] context. The type of genomic instability operating in a tumor at any point in time is likely to have a significant impact on tumor evolution, not only in terms of which specific pathways are dysfunctional [32] but also in terms of the likelihood of a tumor metastasizing and its response to treatment [33]. Regarding the mechanisms of genomic instability, Alexandrov, L. B. et al. had demonstrated that increased point mutation frequencies can be caused by both exogenous factors [34] and defects in DNA repair pathways, such as mismatch repair and base and nucleotide excision repair [35]. By understanding these mechanisms, we can gain insights into the common pathways of tumor evolution that could support the development of future therapeutic strategies.

‘Every unhappy family is unhappy in its own way’, wrote Tolstoy in *Anna Karenina*. Tolstoy was talking about the uniqueness of human tragedy. And we suggest that this principle also captures the misfortune of bladder cancer. This genetic heterogeneity affects key cancer pathways, driving phenotypic variation, and poses a significant challenge to personalized cancer medicine [36]. As early as 20 years ago, F. Gudat and F. Waldman had shown the heterogeneity of the *erbB-2* gene in bladder cancer cells. Furthermore, they found that the amplification of *erbB-2* showed a strong correlation to bladder tumor grade and stage [37]. In another study, David B. Solit and colleagues [38] found that approximately 60% of bladder cancers harbor potentially actionable genetic alterations that have been validated either as drug targets in other solid tumors or for which selective inhibitors are currently in early clinical testing. Bladder cancers, however, exhibit significant genetic heterogeneity, with most potentially actionable alterations identified in a minority of patients. Recently, the use of genome-wide deep sequencing allowed us to perform extensive work on structural variations, including copy number changes and translocations. Using whole-genome sequencing to identify somatic mutations and chromosomal changes in 14 bladder cancers of different grades and stages, the result shows that *CDKN1A* dysfunction is not simply an alternative mechanism for p53 pathway inactivation, and strong positive associations exist between higher tumor stage/grade and greater clonal diversity, the number of somatic mutations, and the burden of copy number changes [39]. DNA methylation is a significant epigenetic mechanism of gene regulation that can interpret the tumor genetic heterogeneity. Recent studies have demonstrated that hypomethylation of the CpG sites of *ZAR1*

gene was implicated in a high proportion of muscle-invasive bladder cancers [40].

Identifying the genetic diversity of bladder cancer that correlates with tumor heterogeneity set the stage for approaches to the treatment of HGSCs in which aberrant genes or networks are detected and targeted with therapies selected to be effective against these specific aberrations.

The Embodiment of Tumor Heterogeneity: Microenvironmental Heterogeneity

It is now well known that tumor formation involves the coevolution of neoplastic cells together with extracellular matrix, tumor vasculature, and immune cells. Successful outgrowth of tumors and eventual metastasis is determined not solely by genetic alterations in tumor cells but also by the suitable microenvironment [41].

Cancer-associated fibroblasts: Fibroblasts are an abundant mesenchyme-derived cell type that maintains the structural framework in tissues. Cancer-associated fibroblasts (CAFs) can significantly promote tumorigenesis [42]. Compared with normal tissue fibroblasts, CAFs have increased proliferation, enhanced extracellular matrix production, and unique cytokine secretion (for example, stromal cell-derived factor 1, SDF1; vascular endothelial growth factor, VEGF; platelet-derived growth factor, PDGF; and hepatocyte growth factor, HGF) [43]. Heterogeneity may be attributable to unique damage signals to which fibroblasts are exposed or possibly to their origin [44].

Vasculature: Vascular networks are derived through formation of new vessels (angiogenesis), co-option and modification of existing vessels within tissue, or recruitment and differentiation of endothelial precursors from bone marrow (vasculogenesis), all of which contribute to vascular heterogeneity in and among tumors. Vessel formation involves degradation and reincorporation of existing vascular basement membranes that vary in a tissue-specific manner [45]. Distance from vascular beds creates a gradient that has been shown to be crucial for the distribution of drugs to all cells in the tumor [46]. Such variations in the vascular networks can generate distinct microenvironments within the tumor, contribute to inter- and intratumor heterogeneity, and, ultimately, influence clinical outcome.

Immune cells: Immune cell recruitment and localization in the tumor milieu vary widely in and among lesions. Heterogeneity of the tumor immune contexture is

influenced by various factors, including those secreted by CAF, the extent and permeability of the vasculature, and the tumor cells themselves. For instance, tumors with microsatellite instability, such as colorectal cancer subsets, may generate more neoantigens, leading to increased T-cell infiltration [47].

At present, the study of bladder cancer microenvironment heterogeneity is a new hot spot.

Problems Caused by Tumor Heterogeneity

Tumor Structural Heterogeneity

Morphological differences usually exist at organ, tissue, and cell levels. In the microscopic vision, tumor cells differ in size and shape (e.g., cubic, columnar, and circular cells). Tumor structure heterogeneity leads to the complexity of pathological diagnosis [48]. Tumors may have a different sensitivity to radiotherapy and chemotherapy due to tumor structural heterogeneity. Most of the time, the surrounding areas of tumors have more access to blood supply and present more obvious hyperplasia than central regions. However, anti-tumor drugs whose perfusion is also decided by blood would reach there first; thus, in contrast, central cells could be safer during the anti-tumor treatment.

Changes in Molecular Biomarkers

A large number of reports have emerged describing multiple markers in primary bladder cancer. 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 either during the chemotherapy treatment or before and after recurrence. However, dynamic changes in the subclonal architecture of the tumor present great challenges to the interpretation of these predictive or prognostic biomarkers [49]. Chen RJ and colleagues provided evidence that nicotine-treated bladder cancer cells exhibit stronger prosurvival signaling through Stat3 activation, which leads to cell survival in response to antitumor agents. They also found that Stat3C-transfected cells had elevated cell viability compared with T24 cells after treatment with cisplatin or paclitaxel [50]. The Iranian scientists detected the serum level of soluble CD138/Sdc-1 (tumor marker of bladder cancer). They found a significant difference between CD138/Sdc-1 concentrations based on the age groups of patients and implied the increased shedding of CD138/Sdc-1 in moderately differentiated tumors, which indicated that the protein bio-

marker showed a significantly different level in difference grade, age, and stage [51]. 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

As is well known, cancer relapse is the most fearsome aspect of bladder cancer, since it is responsible for the majority of cancer-related deaths. Patients with bladder 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 its 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. 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 are 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 nonlinearly related to the majority of clones 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 [52].

The exploration of therapeutic resistance has largely focused on the tumor cell. However, recent identification of the mechanisms of therapeutic resistance that were conferred largely by alterations, not in the tumor cells, but in their environment, indicates the importance of understanding the tumor cell extrinsic compartments. The immune system can be implicated in both inherent and acquired resistance to targeted therapies [53]. An adaptive, continuous dialog exists between tumor cells and their surroundings, mediated through direct cell contact with stromal components or through secreted signaling factors (cytokines, chemokines, and growth factors). As already discussed, the tumor niche shapes responses to the selective pressure of drug treatment and may affect the emergence of resistance. To envisage a scenario in which targeted therapeutics for multiple compartments are successfully implemented, it is important to review what has

been developed and clinically tested for targeting the tumor stroma [54]. But, more investigations are of great necessity to elucidate this controversy in bladder cancer.

Obstacles in Targeted Therapy

Tumor heterogeneity poses major difficulties for targeted therapy in bladder cancer, since it is not known how many genetic alterations must be corrected in order to achieve tumor eradication. When diagnosing a tumor after surgery, only small regions of tumors are tested in the clinical practice. It might be sufficient to accurately identify stages and subtypes of tumors. However, as the field of cancer treatment is moving toward the development of targeted therapeutic approaches, the question of whether the expression of a therapeutic target in a biopsy is representative of the whole corresponding tumor becomes of primary importance. For example, clonal heterogeneity involving different mutational statuses was documented for KRAS and TP53 genes [55].

The P53 gene that we are familiar with was reported to have more than 2000 point mutations. Similar to many other tumor types, 61% of invasive bladder cancers have p53 mutations, suggesting a role for p53 in bladder cancer progression. More recently, Richard J. Cote has demonstrated that p53 alterations are highly associated with features related to progression of bladder cancer: grade and stage [56]. Moreover, the fact that p53 is the most frequently mutated gene makes p53 a relevant and promising target for therapeutic intervention in bladder cancer. Several preclinical studies in mice demonstrated that restoration of wild-type p53 expression was indeed sufficient for the 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 proapoptotic or pro-senescence response to oncogenic stress [57]. More clinical studies of p53-targeting drugs are of great necessity for the treatment of bladder cancer.

Therapies Regarding Tumor Heterogeneity

Multi-Drug Regimen

Chemoresistance is the central problem in bladder cancer therapy, whereas 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 [58]. Nowadays, the treatment for non-muscle-invasive bladder tumor is still bladder perfusion with single

chemical drugs. Whether multi-drug regimens could improve the clinical outcomes of patients with bladder cancers is still quite controversial.

Inhibiting Tumor Cell Repopulation at Chemotherapy Intervals

In bladder cancer, the bladder perfusions are often administered in multiple intervals of one week, two weeks, and one month. 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. 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 [59]. Therefore, promising strategies for reducing tumor heterogeneity during chemotherapy include modification of dose schedule with the support of combinational use of short-acting tumor-specific cytostatic agents to inhibit tumor cell proliferation.

Adaptive Therapy

Most research efforts in chemotherapy are focused on the discovery of agents and the combination 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. 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 [60].

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 [61]. The adaptive therapy will create new insights in bladder cancer treatment in the future.

Conclusions and Perspectives

Bladder tumor heterogeneity is an important characteristic. An understanding of the nature of bladder tumor heterogeneity has undergone great changes in the past 5 years. Two concepts trying to explain its generation, the cancer stem cell hypothesis and the clone evolution model, share a common basis, namely genetic diversity. Recognition of tumor heterogeneity is essential to any theory of neoplastic development, as well as to experimental design and clinical treatment. Tumor societies are highly adapted for survival. They survive natural and artificial (therapeutic) selection through heterogeneity by producing new variants to 'outflank' it and by utilizing subpopulation interactions to counteract its destructive influence [62].

Despite the substantial clinical significance of the issue of tumor heterogeneity, the subject remains very poorly explored. Therefore, we believe that a more systemic approach is needed to characterize tumor heterogeneity of different types and subtypes of cancers through different stages of tumor development, including disseminated tumor cells and metastases. But, nowadays, we believe that we can improve early diagnosis, prognosis, and therapeutic effects in patients by applying SCS (single-cell sequencing) to bladder tumors with intratumor heterogeneity. SCS can be applied in several capacities based on the existence of intratumor heterogeneity. It can be applied to the detection of rare tumor cells both in lesions and in the peripheral circulation for early diagnosis, evaluation of patient prognosis, and determination of the efficacy of therapeutic approaches by profiling and monitoring primary tumor cells [63].

Tumor heterogeneity also leads to important consequences in personalized medicine in bladder 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, whereas 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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