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REVIEW

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Advances in tumor subclone formation and mechanisms of growth and invasion

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Abstract

Tumor subclones refer to distinct cell populations within the same tumor that possess different genetic characteristics. They play a crucial role in understanding tumor heterogeneity, evolution, and therapeutic resistance. The formation of tumor subclones is driven by several key mechanisms, including the inherent genetic instability of tumor cells, which facilitates the accumulation of novel mutations; selective pressures from the tumor microenvironment and therapeutic interventions, which promote the expansion of certain subclones; and epigenetic modifications, such as DNA methylation and histone modifications, which alter gene expression patterns. Major methodologies for studying tumor subclones include single-cell sequencing, liquid biopsy, and spatial transcriptomics, which provide insights into clonal architecture and dynamic evolution. Beyond their direct involvement in tumor growth and invasion, subclones significantly contribute to tumor heterogeneity, immune evasion, and treatment resistance. Thus, an in-depth investigation of tumor subclones not only aids in guiding personalized precision therapy, overcoming drug resistance, and identifying novel therapeutic targets, but also enhances our ability to predict recurrence and metastasis risks while elucidating the mechanisms underlying tumor heterogeneity. The integration of artificial intelligence, big data analytics, and multi-omics technologies is expected to further advance research in tumor subclones, paving the way for novel strategies in cancer diagnosis and treatment. This review aims to provide a comprehensive overview of tumor subclone formation mechanisms, evolutionary models, analytical methods, and clinical implications, offering insights into precision oncology and future translational research.

Keywords Tumor subclones, Clonal evolution, Genomic instability, Intratumoral heterogeneity, Phylogenetic analysis, Multi-omics integration, Big data in oncology

Background

Tumor subclones refer to genetically distinct cell subpopulations within a single tumor that originate from a common ancestral cell. These subclones exhibit unique genomic or epigenomic characteristics, leading to variations in biological behavior and treatment responses.

During cellular proliferation, individual cancer cells gradually accumulate mutations, some of which confer survival advantages. These mutations drive the acquisition of hallmark traits of cancer, including rapid proliferation, immune evasion, and drug resistance [1].

In the early stages of tumorigenesis, the accumulation of multiple driver mutations, such as those in KRAS, TP53, and SMAD4, is required to establish an ancestral cancer cell, which undergoes clonal expansion, giving rise to the initial cancer clone-the foundational population from which tumor subclones emerge [2]. Subsequent

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evolutionary forces, including selection, mutation, genetic drift, and spatial separation, further drive the expansion of cells harboring advantageous mutations, ultimately leading to the formation of multiple subclonal populations [3]. This evolutionary process results in the coexistence of diverse subclones within the tumor, each characterized by distinct mutational profiles that may influence treatment sensitivity and interactions with the tumor microenvironment (Fig. 1).

Recent advancements in high-throughput sequencing technologies, including single-cell sequencing, spatial transcriptomics, and liquid biopsy, have significantly enhanced our ability to dissect the subclonal architecture of tumors. These technologies provide critical insights into intratumoral heterogeneity(ITH), enabling researchers to reconstruct clonal evolution, track subclonal dynamics, and identify key driver mutations. The integration of multi-omics approaches and artificial intelligence-based data analysis has further refined our understanding of tumor evolution, offering new perspectives for precision oncology.

Formation mechanisms and characteristics of tumor subclones

Mechanisms of tumor subclones formation

The fundamental mechanisms underlying the formation of tumor subclones can be summarized into three basic processes: genetic mutation, genetic drift, and natural selection. Genetic mutations, including single-nucleotide variants (SNVs) and copy number variations (CNVs), provide the initial genetic variations necessary for subclonal formation, typically driven by genomic instability [4]. Genetic drift, on the other hand, involves random fluctuations in allele frequencies within tumor subclones, resulting in the continuous accumulation of neutral ("passenger") mutations. Although these mutations usually have minimal impact on cancer cell phenotype [5], they significantly increase intratumoral genetic diversity. This reservoir of genetic diversity may rapidly confer adaptive advantages when the tumor environment or selection pressures change, facilitating the emergence and expansion of more adaptive subclones [6, 7].

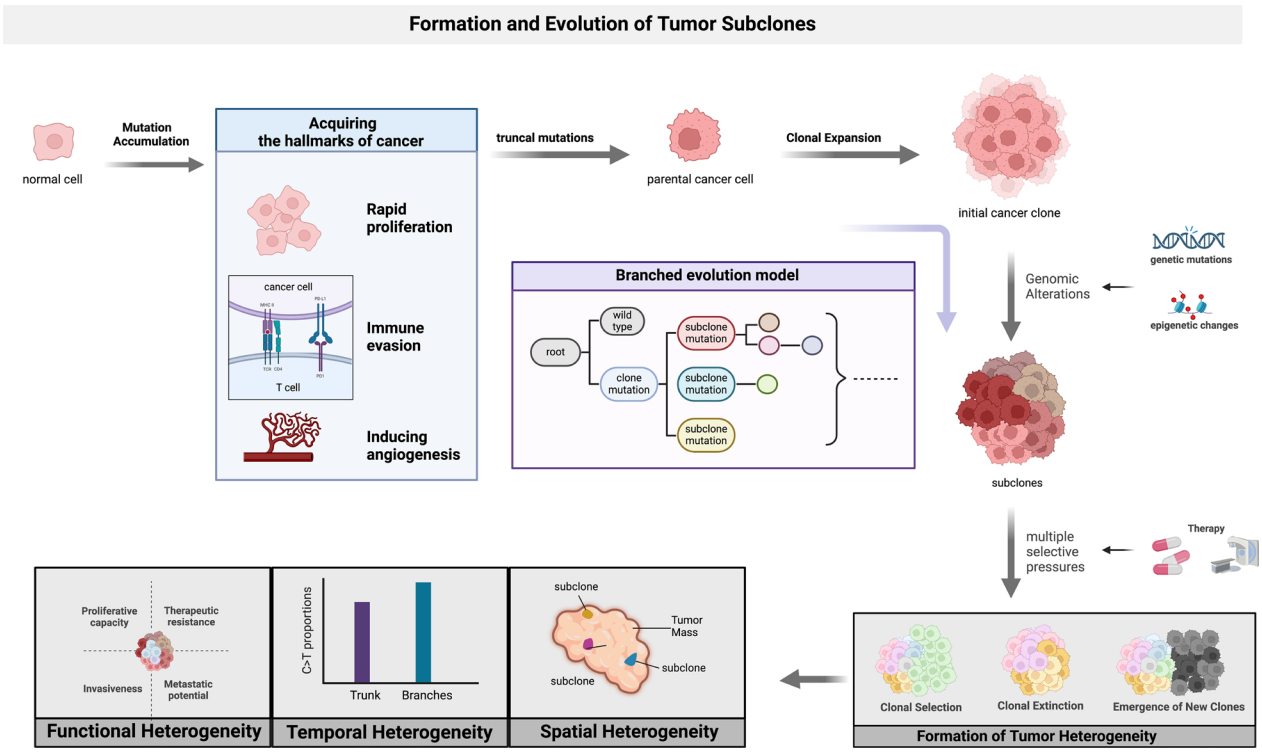

Fig. 1 Formation and Evolution of Tumor Subclones. This figure illustrates the formation and evolution of tumor subclones and their contribution to tumor heterogeneity. Normal cells acquire mutations and gradually develop hallmark cancer traits such as rapid proliferation, immune evasion, and angiogenesis. Truncal mutations lead to the emergence of a parental cancer cell, which undergoes clonal expansion to form the initial tumor clone. Subsequently, subclonal diversification is driven by additional genetic and epigenetic alterations, spatial constraints, and selective pressures such as therapy and immune surveillance. These processes follow a branched evolutionary model, resulting in clonal selection, extinction, and the emergence of new clones. The dynamic interplay of these factors generates functional, temporal, and spatial heterogeneity, which complicates therapeutic strategies and accelerates tumor progression

Natural selection, beyond the variation introduced by genetic mutations and drift, is a key driver of subclonal evolution in cancer. Positive selection predominates over negative selection during tumor progression [8]. Selective pressures-such as therapy or changes in the tumor microenvironment-favor subclones carrying advantageous driver mutations, enabling their expansion. In pancreatic cancer, subclones harboring CNTN5 or MEP1 A mutations are thought to be better adapted to hypoxia-induced metabolic and oxidative stress, promoting their selective growth [9, 10]. Therapeutic stress can induce widespread cell death, yet some tumor cells activate compensatory stress-response pathways that allow survival and clonal outgrowth [11, 12]. These treatment-resistant subclones were characterized by key driver mutations, including CDK6 amplification, FGFR2 and MYC amplification, and RUNX1 deletion [13]. In chronic lymphocytic leukemia, treatment drives the expansion of subclones with SF3B1 and TP53 mutations, leading to drug resistance and disease progression [14]. Collectively, these examples illustrate how diverse selective forces shape subclonal dynamics and contribute to the emergence of intratumoral heterogeneity.

In addition to genetic mechanisms, epigenetic alterations also play a critical role in subclonal formation and adaptive evolution by modulating gene expression. For example, in breast cancer cells, DNA methylation can silence the tumor suppressor gene BRCA1, promoting the emergence of drug-resistant subclones [15]. Similarly, the MCM2-2 A mutation disrupts parental histone binding and reduces repressive histone marks such as H3 K27 me3, thereby activating genes involved in proliferation and epithelial-to-mesenchymal transition (EMT) [16]. These epigenetic changes enhance the proliferative, survival, and migratory capacities of emerging subclones.

As additional sources of diversity, cancer stem cells contribute to subclonal expansion through their capacity for self-renewal and long-term tumor propagation, particularly following therapy [17]. Rare spontaneous fusion events between cancer cells may also generate hybrids with highly heterogeneous or polyploid genomes [18, 19]. In breast cancer, such hybrid cells have been shown to exacerbate genomic instability and, through ploidy reduction and genomic recombination, give rise to novel subclones with enhanced phenotypic plasticity and adaptive potential [20, 21].

Clonal evolution models

Clonal evolution in tumors is commonly described by two models: the linear and the branched evolution models. The linear model, which depicts the sequential accumulation of mutations along a single lineage, often reflects limitations in sequencing resolution. In contrast, most tumors exhibit features consistent with branched evolution. Distinct mutational profiles across tumor regions support this model and underscore the therapeutic challenges posed by subclonal diversity [22]. For example, in B-cell lymphoma patients receiving CD20-targeted therapy, CD20-positive subclones re-emerged at later time points, accompanied by spatial heterogeneity in CD20 expression [23]. These findings highlight the clonal complexity of tumors under treatment and suggest that targeting a single antigen may be insufficient to eliminate all subclonal populations.

Spatial, temporal, and functional heterogeneity of tumor subclones

The differential microenvironment across distinct tumor regions leads to spatially heterogeneous distributions of subclones. Geographical stratification of clonal structures is a well-documented phenomenon in renal, pancreatic, colorectal, and prostate cancers, where subclones harboring driver mutations tend to expand within specific localized regions rather than being uniformly dispersed throughout the tumor [24–26]. The genomic diversity observed across different tumor regions highlights the need for treatment strategies that target multiple subclonal populations, rather than focusing solely on the dominant clone. Failure to address this heterogeneity may lead to the persistence of therapy-resistant subpopulations, ultimately driving tumor progression. Interestingly, tumors exhibiting significant intratumoral heterogeneity are often associated with larger tumor size. This phenomenon may be explained by the ability of distinct subclones to adapt to different regional microenvironments, thereby preventing a single dominant clone from undergoing complete clonal sweeps [25]. This adaptive advantage facilitates continued tumor growth and progression.

Over time, the composition and characteristics of tumor subclones can undergo dynamic changes, reflecting the evolving selective pressures and genomic alterations during tumor progression. Preferred temporal sequences of somatic mutation accumulation have been identified in colorectal, pancreatic, and hematological malignancies [27, 28]. This pattern suggests that certain tumors follow a distinct "mutational evolutionary trajectory," where some driver mutations tend to arise early in tumorigenesis, while others emerge at later stages. Such observations imply that mutation acquisition may follow a regulated sequence rather than occurring in a completely stochastic manner. However, in breast cancer, mutations in key driver genes such as PIK3 CA, TP53, PTEN, BRCA2, and MYC have been observed at both early and late stages of tumor development. This suggests that subclonal evolution in breast cancer does

not adhere to a strict temporal hierarchy but instead follows a nonlinear evolutionary pattern influenced by stochastic events, selective pressures from the tumor microenvironment, and interclonal interactions [13]. C>T transitions at CpG sites represent one of the most common mutational signatures in cancer. Studies indicate that these mutations frequently arise during the early phases of tumor development, contributing to the initial formation of ITH. As the tumor progresses, the proportion of C>T transitions continues to increase, ultimately becoming a predominant source of mutational burden in later stages [26]. This dynamic mutational landscape is likely shaped by progressive genomic instability and selective pressures, further illustrating the complexity of tumor evolution over time.

Distinct subclones within a tumor often exhibit considerable functional diversity, with variations in proliferative capacity, invasiveness, metastatic potential, and therapeutic resistance. This functional heterogeneity plays a crucial role in determining tumor aggressiveness and response to treatment, highlighting the necessity for personalized therapeutic approaches that account for the diverse biological behaviors of different subclonal populations.

Primary methods and technical models for subclone research

Bulk sequencing

Bulk sequencing remains one of the most widely used approaches, leveraging whole-genome sequencing of population-level or mixed-cell samples to characterize the average genetic features of a sample [3]. However, bulk DNA sequencing cannot resolve the mutational combinations present in individual cells, making it challenging to dissect the heterogeneity inherent in tumors (Table 1). In a study on relapsed/refractory multiple myeloma, WGS identified only four subclones, whereas integrating single-cell RNA sequencing and single-cell ATAC sequencing revealed eleven, highlighting the limitations of WGS in accurately resolving intratumor heterogeneity (ITH) [29].

To improve spatial and temporal resolution, multiscale bulk sequencing approaches, including targeted sequencing, multiregion sequencing, and longitudinal sequencing, extend conventional bulk sequencing by refining sampling strategies. Multiregion sequencing of treatment-naïve small cell lung cancer (SCLC) showed high clonal homogeneity, suggesting early clonal selection that establishes a dominant, chemotherapy-sensitive clone. After relapse, clonal diversity increased, indicating that chemotherapy eliminated the dominant clone while promoting the expansion of subclones from the common ancestor clone, explaining the high initial response and poor second-line efficacy [30]. Similarly, longitudinal sequencing of gliomas revealed that CDKN2 A deletion and MYC amplification drive tumor progression and recurrence as early events rather than therapy-induced mutations [31].

Targeted sequencing enhances the detection of low-frequency or rare mutations, which may be missed by conventional bulk sequencing. A study using targeted sequencing first identified a novel frameshift insertion in ZNF384 in high-grade gliomas (HGGs), potentially associated with tumor recurrence or therapy resistance, highlighting the need for high-depth sequencing to detect rare oncogenic drivers [32].

Despite these advantages, multiscale bulk sequencing still has inherent limitations, including spatial and

Table 1 Comparison of primary experimental approaches in subclone research

Method	Advantages	Limitations	Main applications
Bulk sequencing	Provides a comprehensive genomic landscape; detects large-scale genomic alterations	Lacks single-cell resolution; may miss low-frequency mutations	Whole-genome/exome profiling; CNV analysis
Single-cell sequencing	Resolves gene expression or mutation patterns at single-cell level	Costly; complex data; sensitive to technical noise	Rare clone detection; lineage tracing
Spatial transcriptomics	Preserves spatial info of gene expression	Limited resolution; cannot detect genomic alterations directly	Tumor microenvironment and subclone mapping
Histological techniques (mIHC, mIF, MSI)	Detect multiple proteins/metabolites in tissue context	Confined to protein/metabolite level; limited targets	Subclone validation; immune profiling
Liquid biopsy	Non-invasive; dynamic monitoring of evolution	Sensitivity limited by ctDNA level; not all clones shed ctDNA	MRD tracking; resistance mutation detection
Functional genomic approaches	Directly tests effects of gene alterations	May not replicate in vivo environment; off-target effects	Driver gene screening; resistance mechanism study
PDOs and PDXs	Preserve tumor heterogeneity; personalized testing	Time-consuming; culture may alter subclone state	Drug screening; subclone functional validation

temporal sampling biases that may hinder a complete reconstruction of tumor subclonal architecture. Multiregion sequencing may still miss low-abundance subclones due to uneven distribution, while longitudinal sequencing, despite offering insights into tumor evolution, relies on discrete time-point sampling, making continuous tracking of clonal dynamics unfeasible. This limitation may result in undetectable relapse-driving clones at diagnosis, particularly in branching evolution [29].

Single-cell sequencing technologies

Single-cell sequencing technologies, such as single-cell DNA sequencing, provide mutation profiles at the single-cell level, enabling the direct inference of phylogenetic trees without requiring deconvolution [33]. In phylogenetic trees constructed using variant allele frequency (VAF) and cancer cell fraction (CCF), specific mutations are often inferred to belong to the same clone. Single-cell sequencing data can validate whether these mutations indeed coexist within the same cell, thereby confirming or refuting the proposed phylogenetic relationships among tumor subclones [34]. Furthermore, advancements in single-cell whole-genome sequencing have significantly improved the accuracy of detecting copy number alterations (CNAs) and single nucleotide variants (SNVs), leading to more reliable phylogenetic inferences [35]. Unlike bulk sequencing, which averages transcriptomic signals, scRNA-seq identifies a continuum of tumor cell states. In hepatoblastoma, it delineates a differentiation axis from hepatocytic to progenitor to mesenchymal states, with an intermediate transitional population. This refines tumor classification and quantifies cell states that drive progression [36]. Integrating single-cell DNA and RNA sequencing captures both genetic and phenotypic heterogeneity, providing a comprehensive view of tumor evolution.

Spatial transcriptomic sequencing

Spatially resolved transcriptomics (SRT) and in situ sequencing map gene expression across tumor regions, enabling subclonal spatial analysis. While SRT cannot directly detect genomic alterations, arge-scale copy number alterations (CNAs) often leave expression signatures, allowing indirect inference of genomic changes. In high-grade meningiomas, SRT has revealed regional ITH, which leads to discrepancies between molecular and histological classifications. Current classification systems may overlook these molecular differences, suggesting that tumor subtyping should incorporate spatial evolutionary characteristics [37]. To refine tumor spatial evolution analysis, Benjamin J. et al. developed CalicoST, an algorithm that integrates SRT data to infer allele-specific CNAs and reconstruct tumor subclone phylogeography. By analyzing loss of heterozygosity (LOH) events, CalicoST constructs phylogenetic trees and models subclone evolution across temporal and spatial dimensions, enabling three-dimensional spatial mapping [38]. While SRT provides valuable transcriptomic insights, it remains limited in detecting genomic alterations. Spatial DNA sequencing offers a complementary approach by directly capturing genomic changes, enhancing the resolution of tumor evolution studies, though this technology is still in development [39].

Histological techniques

Multiplexed immunohistochemistry and multiplexed immunofluorescence enable the simultaneous detection of multiple protein markers within a single tissue section, facilitating the identification of tumor subclones or validating the accuracy of clonality prediction models [40]. Mass spectrometry imaging (MSI) allows for spatially resolved quantitative analysis of proteins, lipids, and metabolites. For example, MSI analysis of primary tumors and lymph node metastases in papillary thyroid carcinoma revealed that intrapatient heterogeneity between primary tumors and metastases exceeds interpatient heterogeneity among primary tumors [41]. This suggests that tumor cells may undergo clonal selection or phenotypic plasticity during metastasis to adapt to new microenvironments.

Liquid biopsy

Circulating tumor DNA (ctDNA), fragmented DNA released into the bloodstream by tumor cells, reflects the mutational landscape and clonal composition of tumors. Liquid biopsy is a non-invasive approach that enables the analysis of cell-free DNA (cfDNA) and circulating tumor cells (CTCs) to track tumor subclone dynamics. For example, non-small-cell lung cancer is prone to postoperative recurrence, necessitating more sensitive biomarkers for relapse prediction. To address this, researchers developed ECLIPSE, a computational method designed to infer tumor clonal evolution from low-ctDNA samples. ECLIPSE achieves a detection sensitivity of 94% at a 0.1% clonal ctDNA level, enabling precise tracking of tumor subclone dynamics and improving relapse risk assessment [42]. In a study of 42 gastrointestinal cancer patients with acquired resistance to targeted therapy, cfDNA liquid biopsy demonstrated superior sensitivity in detecting tumor heterogeneity compared to conventional tissue biopsies. Resistance mutations undetected in matched tumor biopsies were identified in 78% of cases. Additionally, cfDNA analysis revealed geographic and evolutionary differences among

subclones, underscoring the limitations of single-lesion biopsies in capturing tumor heterogeneity [43]. Advancements in liquid biopsy technologies have further enhanced its utility in cancer research. Breakpoint-specific PCR has enabled the detection of mutant DNA at levels as low as 0.001%, facilitating the successful identification of mutated ctDNA in patient plasma samples [44]. These innovations allow liquid biopsy to detect low-frequency subclones, providing a more comprehensive understanding of tumor evolution and resistance mechanisms.

Functional genomic approaches

Functional genomic approaches, such as CRISPR–Cas9 and shRNA screening, enable direct manipulation of gene expression to systematically investigate key genes involved in tumor progression. A recent study combined in vivo single-cell CRISPR screening with scRNA-seq to analyze the clonal expansion dynamics of 150 frequently mutated genes in squamous cell carcinoma. This study identified a transition in the TNF signaling module from extrinsic stimulation to autocrine activation, suggesting that this shift represents a critical step in the progression from clonal expansion to invasive cancer cells [45].

Establishment of in vitro and in vivo models

Establishing patient-derived organoids (PDOs) and patient-derived xenograft models (PDXs) enables the investigation of tumor subclones while maintaining tumor heterogeneity. In the development of PDO models for castrate-resistant prostate cancer (CRPC), researchers applied lineage tracing to analyze the clonal evolution of CRPC cells. Their findings revealed that distinct CRPC subtypes are driven by specific stem/progenitor cell populations. Furthermore, PDO models identified a subclone-specific transition from luminal adenocarcinoma to neuroendocrine and amphicrine subclones [46]. In pancreatic ductal adenocarcinoma and ampullary cancer, PDOs have been utilized to examine the dynamic evolution of chemotherapy-resistant subclones. Using low-volume screening assays combined with automated spatial alignment algorithms, researchers tracked individual subclonal responses to chemotherapy. The study revealed that resistant subclones not only exhibited enhanced survival but also evaded apoptotic and necrotic signaling, highlighting novel resistance mechanisms [47]. Additionally, integrating PDOs with single-cell technologies allows for a deeper analysis of subclone-specific drug responses and their interactions with the tumor microenvironment. For instance, in colorectal cancer PDOs, cancer-associated fibroblasts were found to promote chemoresistance by altering stem cell states, further emphasizing the crucial role of the microenvironment in subclonal plasticity and therapeutic responses [48].

Bioinformatics analysis

Andor N et al. developed a computational tool, EXPANDS, designed to estimate the proportion of tumor cells carrying specific mutations. By using probabilistic models to analyze cellular frequencies, it identifies mutations that occur before clonal expansion and predicts tumor purity. This method has been successfully applied to various cancers, including breast cancer and glioblastoma, providing valuable insights into tumor heterogeneity and clonal dynamics [49]. PyClone is a Bayesian statistical tool commonly used for subclonal analysis, designed to group deeply sequenced somatic mutations into putative clonal clusters and calculate the cellular prevalence (or Variant Allele Frequency) of each cluster. This enables the assessment of the abundance of different clones within a tumor and the inference of cancer's clonal population structure. Its accuracy has been validated through single-cell sequencing, making it a reliable method for exploring subclonal dynamics [50].

Phylogenetic trees are graphical representations of evolutionary relationships among organisms or cellular populations. In the context of tumors, phylogenetic trees illustrate the evolutionary trajectories of subclones, mapping how they diverged from a parental clone over time. By analyzing the distribution of genetic variations across different tumor regions or cell populations, researchers can infer the evolutionary relationships among subclones and construct a phylogenetic tree. This can be achieved using a variety of data types, including CNVs, SNVs, epigenetic modifications, scRNA-seq data, mitochondrial mutations, and MSI (microsatellite instability). Researchers constructed phylogenetic trees using CNVs and SNVs. The Bayesian Dirichlet Process was adapted to cluster substitution mutations, facilitating the identification of shared and unique subclones. Shared CNVs and substitution mutations between parental and subclones revealed their common ancestry, while subclone-specific CNVs or substitution mutations marked independent evolutionary branches. This approach provided a detailed view of tumor subclonal dynamics and their evolutionary history [51].

Subclones and the formation of tumor heterogeneity

Subclones play a crucial role in the formation of tumor heterogeneity

Tumor heterogeneity refers to the genetic, epigenetic, phenotypic, and functional diversity observed either within a single tumor or between tumors of the same histological type. It is a hallmark of most cancers,

reflecting the complex and dynamic nature of tumor evolution. Tumor heterogeneity can be broadly classified into intertumoral heterogeneity and intratumoral heterogeneity (ITH). Intertumoral heterogeneity describes differences between tumors of the same histological type across different patients, typically driven by patient-specific factors such as germline genetic variations, somatic mutation profiles, and environmental influences. The primary focus here, however, is ITH, a critical indicator of tumor evolution.

The formation of ITH is not random but is profoundly influenced by subclonal evolution. Specifically, branched evolution and independent clonal expansions collectively drive the development of ITH, further increasing the complexity of tumors. Even in multifocal diseases such as prostate cancer, the origins of the disease may trace back to a single parental clone [52]. The coexistence of multiple cancer lineages within the same tumor strongly supports the concept of branched tumor evolution. Each lineage is marked by distinct ERG fusion genes, indicating that the tumor undergoes separate clonal cell expansions along divergent evolutionary trajectories. This combination of branched evolution and separate clonal cell expansions contributes to cancer clone mixing, further amplifying intratumor heterogeneity (ITH) [51]. These findings highlight that tumor heterogeneity is more likely driven by subclonal dynamics rather than by polyclonal origins.

Subclonal evolution as a dynamic process in the formation of tumor heterogeneity

Clonal evolution is a fundamental process driving tumor progression. Different subclones harboring distinct genetic alterations continuously compete, expand, or disappear under selective pressures. This process not only results from tumor heterogeneity but also reinforces and sustains it over time.

During this evolutionary course, certain subclones gain dominance through proliferative advantages, while others with lower adaptive fitness are gradually eliminated. For instance, early-stage colorectal neoplasia often contains multiple coexisting subclones. However, as the disease progresses, interclonal cooperation diminishes and competitive dynamics lead to the emergence of a dominant, more fit lineage [53].

Simultaneously, the acquisition of new mutations can give rise to novel subclonal lineages, redirecting the tumor's evolutionary trajectory. This is exemplified in ALK-rearranged lung cancer. Following initial treatment with crizotinib, the emergence of the C1156Y mutation alters the ALK kinase domain, leading to resistance. Although subsequent treatment with the third-generation inhibitor lorlatinib initially controls the disease, the appearance of the L1198 F mutation confers renewed resistance [54, 55]. Interestingly, this mutation also restores sensitivity to crizotinib by enhancing drug binding, thereby resensitizing the tumor to the original therapy [56].

These findings underscore the importance of tracking subclonal dynamics and resistance mutations, offering rationale for reintroducing previously ineffective treatments. Such insights highlight the clinical potential of personalized strategies in managing therapy-resistant cancers.

The role of subclone formation in tumor growth, invasion, and immune evasion

Subclonal evolution drives tumor growth, invasion, and metastasis

The parental, non-metastatic founder cell, which harbors founder mutations, represents the earliest and most prevalent category of mutations within a tumor. However, a single founder mutation alone is insufficient to fully transform this cell into a malignant tumor. In the early stages of tumorigenesis, these cells exhibit a relatively low proliferation rate. Over time, as additional mutations accumulate, the cell gains the ability to proliferate and form a primary tumor. At this stage, however, the tumor has not yet acquired the genetic alterations required for invasion or metastasis [10].

As tumor evolution progresses, subclones arise from the parental clone, driven by progressor mutations that introduce additional genetic changes. These mutations enhance tumor growth, invasion, and metastatic potential. Compared to the parental population, subclones often exhibit greater adaptability, allowing them to survive under selective pressures, such as hypoxia, immune attacks, and therapeutic interventions. This adaptability not only fuels tumor progression but also plays a crucial role in immune evasion, enabling cancer cells to escape immune surveillance and resist treatment.

Studies utilizing CNA and single nucleotide variant (SNV) data have reconstructed evolutionary trees to model prostate cancer progression. Unsupervised clustering analysis of CNA data has further identified distinct tumor subtypes, highlighting the heterogeneous nature of tumor evolution.

Analysis revealed that mutational subtypes in the trunk and branches of the phylogenetic tree were independent, reflecting the differentiation and adaptation of tumors at different evolutionary stages. Branch-specific mutations were predominantly subclonal, with CNA alterations enriched in genes regulating cellular signaling and response, such as MTOR and BAD. Mutations or amplifications in these genes likely drive excessive

cellular proliferation and inhibit apoptosis, contributing to aggressive tumor growth.

Furthermore, VEGFB amplification in specific subclones has been identified as a key driver of angiogenesis, a process that enhances the supply of nutrients and oxygen within the tumor microenvironment. This facilitates tumor invasion and progression, reinforcing the role of subclonal evolution in driving malignancy [57].

Metastasis is responsible for the majority of cancer-related deaths [58]. The traditional hypothesis suggests that metastatic lesions arise from the dissemination and expansion of a single tumor cell (monoclonal origin) [59]. However, recent evidence indicates that multiple subclones can cooperate to establish metastatic sites, challenging the monoclonal origin theory [60].

Through whole-genome sequencing of both primary and metastatic prostate cancer lesions, researchers applied an n-dimensional Bayesian Dirichlet process to classify clonal and subclonal mutations. The findings revealed that certain mutations exist at varying proportions across different metastatic sites, suggesting that metastases are not derived from a single tumor cell but rather arise from the dissemination of genetically distinct subclones.

Additionally, phylogenetic analyses demonstrated that metastatic lesions frequently originate from minor subclones, with certain genetic alterations conferring selective advantages. Key drivers identified include PPP2R5 A deletion and AR duplication, which play a crucial role in enhancing metastatic potential [61]. These findings align with the "seed and soil" hypothesis, which suggests that metastasis depends on both tumor cell properties (seed) and the host microenvironment (soil) [62].

A study on pancreatic cancer further revealed that certain gene mutations associated with invasion and metastasis, including CNTN5, DOCK2, MEP1 A, and LMTK2, were detected not only in metastatic lesions but also in the primary tumor [10]. This suggests that these mutations may not act as direct drivers of metastasis but rather represent the genetic characteristics of specific subclones within the primary tumor.

These subclones may subsequently undergo independent expansion, ultimately giving rise to metastatic lesions. This observation underscores the complexity of tumor evolution, where metastatic potential may be pre-established in primary tumor subclones, even before overt dissemination occurs.

Subclonal promotion of tumor immune evasion

Dunn et al. proposed the concept of cancer immunoediting, which posits that the immune system not only eliminates tumor cells but also shapes their immunogenic phenotypes through selective pressure. This process comprises three phases: elimination, equilibrium, and escape. During the elimination phase, most tumor cells are recognized and destroyed by the immune system. However, some cells carrying adaptive mutations may evade immune clearance and enter the equilibrium phase, where they coexist with the immune system in a dynamic balance. Eventually, clones with immune-evasive capabilities expand during the escape phase, leading to sustained tumor growth under diminished immune surveillance [63].

Immunoediting plays a critical role in the generation and selection of neoantigens. As mutations accumulate, tumor cells can produce two broad categories of neoantigens: clonal neoantigens, which are present across all tumor cells, and subclonal neoantigens, restricted to specific subpopulations. A high burden of clonal neoantigens enhances immune recognition and is associated with better responses to immunotherapy. In contrast, subclonal neoantigens are more heterogeneously distributed and often expressed in only a fraction of tumor cells, making them less effective in eliciting robust immune responses. In the later stages of immunoediting, selective pressure from the immune system may drive the expansion of subclones with enhanced immune-evasive properties, further impairing immune surveillance and promoting tumor progression [64].

This escape phase is orchestrated through a convergence of diverse immune regulatory mechanisms. Tumor subclones may evade immune elimination by modulating antigen expression, activating immune checkpoints, remodeling the tumor microenvironment, and altering their metabolic programs.

Subclones may escape recognition by downregulating or losing tumor antigens, or by reducing the expression of HLA class I molecules, thereby impairing antigen presentation. For instance, in melanoma patients receiving NY-ESO-1 vaccination, recurrent tumors often exhibit loss of NY-ESO-1 expression [65, 66]. Moreover, differences in the expression of immune checkpoint molecules such as CTLA-4 and PD-L1 across subclones contribute to clonal-level immune tolerance. Yu et al. identified CTLA-4 expression in the cytoplasm and membrane of stromal lymphocytes, as well as in the cytoplasm of breast cancer cells, where its high expression correlated with poor prognosis [67]. PD-L1 is widely expressed across various solid tumors, including lung, melanoma, breast, colorectal, gastric, hepatocellular, and bladder cancers, and is closely associated with immune evasion and therapeutic resistance [68].

In parallel, certain subclones can reshape the tumor microenvironment to promote immune suppression. For example, tumor-derived chemokines such as CCL22 can recruit regulatory T cells (Tregs), while myeloid-derived suppressor cells (MDSCs) in colorectal and prostate cancers generate reactive nitrogen species that nitrosylate CCL2, trapping T cells in the peritumoral stroma and preventing effective infiltration into the tumor core [69, 70]. Additionally, metabolic alterations such as lactate accumulation and dysregulated tryptophan metabolism can suppress T cell proliferation and cytotoxicity, fostering an immunosuppressive environment conducive to tumor progression [71–73].

Clinical significance of tumor subclone research

Tumor subclone research has profound clinical implications, improving diagnostic accuracy, guiding personalized treatment, predicting disease progression, and refining therapeutic strategies. By understanding the genetic diversity and evolutionary dynamics of tumor subclones, clinicians can optimize cancer management and develop more effective interventions.

Advancing early detection

Studying subclonal characteristics in early-stage tumors may facilitate the development of more sensitive and specific early detection methods. For example, chromosome 3p loss is a hallmark of clear cell renal cell carcinoma and often arises due to chromothripsis early in life. However, this alteration alone does not immediately result in cancer-additional mutations accumulate over decades before tumor formation [74]. This prolonged latency period presents an opportunity for early detection and preventive intervention.

Enhancing diagnostic accuracy

Investigating tumor subclones provides a deeper understanding of genetic heterogeneity within tumors, improving diagnostic precision. Identifying different subclones enables more accurate classification of tumor types and subtypes, forming the basis for personalized treatment strategies. For instance, low-grade gliomas (LGGs) are typically slow-growing with favorable prognoses, but some cases progress to aggressive glioblastomas (GBMs). Certain GBMs have been misclassified as LGGs due to atypical pathological features [75]. Studies suggest that tumors with a higher number of clones correlate with poorer survival, indicating that assessing intratumor heterogeneity (ITH) may aid in distinguishing glioblastomas from LGGs, thus improving diagnostic accuracy [76, 77].

Guiding personalized and combination therapy

Understanding the subclonal composition of tumors provides a foundation for selecting the most effective therapeutic strategies. Given the distinct treatment sensitivities of different subclones, targeting resistant subpopulations may enhance efficacy while minimizing unnecessary toxicity.

In diffuse large B-cell lymphoma, molecular profiling has identified distinct genetic subtypes defined by specific mutation combinations, such as the MCD subtype (characterized by MYD88 and CD79B mutations) and the EZB subtype (with EZH2 mutations and BCL2 translocations). These subtypes rely on divergent oncogenic pathways and may respond preferentially to BTK inhibitors or EZH2/BCL2-targeted therapies, highlighting the clinical potential of subclonal-level molecular stratification [78].

In metastatic prostate cancer, androgen deprivation therapy remains a standard approach; however, resistance frequently develops. Subclonal mutations have been recognized as key drivers of castration resistance, involving alterations in AR and related genes (such as FOXA1), MYC amplification, and CTNNB1 mutations that influence the Wnt signaling pathway [61, 79–81].To address the therapeutic challenges posed by subclonal heterogeneity, multiple strategies have been proposed. Combination therapy aims to simultaneously target multiple pathways to prevent clonal escape, while adaptive therapy adjusts treatment over time based on tumor evolution, potentially delaying or overcoming resistance.

Predicting disease progression and recurrence

The evolution and composition of tumor subclones provide crucial insights into disease progression and recurrence risk. Monitoring subclonal dynamics helps predict patient outcomes and enables timely therapeutic interventions. In prostate cancer, wide genetic divergence among subclones is often observed at diagnosis, and patients with poor prognoses frequently exhibit unique genomic architectures [82, 83]. Notably, monoclonal prostate tumors have a significantly lower recurrence risk than polyclonal tumors, indicating that multiple subclones contribute to recurrence, though their presence alone is not always sufficient to predict it [57]. Tracking specific subclones also allows for early detection of minimal residual disease, which is critical for evaluating treatment efficacy and predicting recurrence. As residual resistant subclones persist post-treatment, they can drive relapse, often leading to more aggressive and therapy-resistant tumors.

Optimizing drug development and clinical trials

Subclonal analysis can refine clinical trial design by improving the resolution of drug response assessment and identifying patient subgroups with distinct therapeutic sensitivities. While most trials continue to treat triple-negative breast cancer (TNBC) as a single category, the TBCRC032 study incorporated molecular subtypes such as LAR into its framework [84]. Post hoc analyses further showed that the BLIA RNA subtype was more predictive of response to immune checkpoint inhibitors than conventional PD-L1 immunohistochemistry [85, 86]. Subclonal profiling also informs early-stage drug development by revealing recurrent driver alterations and pathway dependencies, offering a basis for rational target selection.

Conclusions

Tumor subclones play a critical role in cancer progression, therapeutic resistance, and immune evasion. Their emergence is driven by genomic instability, epigenetic alterations, and selective pressures from the tumor microenvironment. Advances in single-cell sequencing, spatial transcriptomics, and computational modeling have substantially improved our understanding of subclonal dynamics and their clinical relevance.

However, translating subclonal analysis into clinical standards requires addressing several key practical challenges. Standardized protocols for liquid biopsy-including sample handling, timing, and data analysis-are essential to ensure reproducibility and reliability in subclonal monitoring. The clinical validation of bioinformatic tools depends on large-scale, multidisciplinary collaboration to establish algorithmic robustness, interpretability, and cross-platform consistency. Clear guidelines for multi-region and longitudinal sampling remain lacking, limiting efforts to reconstruct subclonal evolution across spatial and temporal dimensions. The development of scalable, cost-effective multi-omics platforms compatible with clinical workflows is also needed to facilitate broad implementation. Ultimately, the integration of subclonal data into routine clinical decision-making will depend on the establishment of clinically actionable reporting frameworks and risk stratification models. Addressing these challenges will be essential for embedding subclonal profiling into standard precision oncology practice.

Abbreviations

ITH	Intratumoral heterogeneity
CNV	Copy number variation
EMT	Epithelial-to-mesenchymal transition
TME	Tumor microenvironment
scDNA-seq	Single-cell DNA sequencing
SNVs	Single nucleotide variants
scRNA-seq	Single-cell RNA sequencing
SRT	Spatially resolved transcriptomics
cfDNA	Cell-free DNA
ctDNA	Circulating tumor DNA
CNAs	Copy number alterations
MDSCs	Myeloid-derived suppressor cells
LGGs	Low-grade gliomas
GBMs	Glioblastomas
Tregs	Regulatory T cells
CTLA-4	Cytotoxic T-lymphocyte antigen-4
CRPC	Castrate-resistant prostate cancer
MSI	Mass spectrometry imaging

Author contributions

ZYH conceived and designed the review. ZYH conducted the literature search, data interpretation, and manuscript drafting. WWD provided critical revisions and supervised the study. All authors read and approved the final manuscript. **Yuhong Zhang** and **Weidong Wang** are affiliated with the **Clinical Medical College, Southwest Medical University, Luzhou, China**, and the **Department of Radiation Oncology, Sichuan Clinical Research Center for Cancer, Sichuan Cancer Hospital & Institute, Sichuan Cancer Center, Affiliated Cancer Hospital of University of Electronic Science and Technology of China, Chengdu, China**.

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Competing interests

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