Tumor heterogeneity and evolution

Tumors are marked by high levels of tumor heterogeneity, out-of-control cell growth, and metastasis. They encompass and competing ecosystem, including tumor-cell-related epithelial cells, fibroblasts, infiltrated immune cells, mesenchymal stem cells (MSCs), and surrounding endothelium of blood vessels. Tumor heterogeneity arises from the step-wise accumulation of genomic and epigenetic alterations by genomic instability, which in turn lead to different patterns of clonal evolution, i.e., expansion of certain cell lineages and depletion of other cell populations. Clonal evolution of tumors is a continuous dynamic remodeling process with distinct dimensions of heterogeneity. Additionally, artificial intervention by chemotherapy or radiotherapy and cancer cell interaction with the surrounding microenvironment can reshape tumor cell populations at genomic, transcriptomic, epigenomic, proteomic levels, ultimately leading to tumor heterogeneity. Tumor heterogeneity can be divided into inter-tumor (between different patients) and intra-tumor (within a single tumor) heterogeneity. Both types of tumor heterogeneity mark a key challenge in the selection of specific biomarkers, treatment decision, and monitoring for oncology.

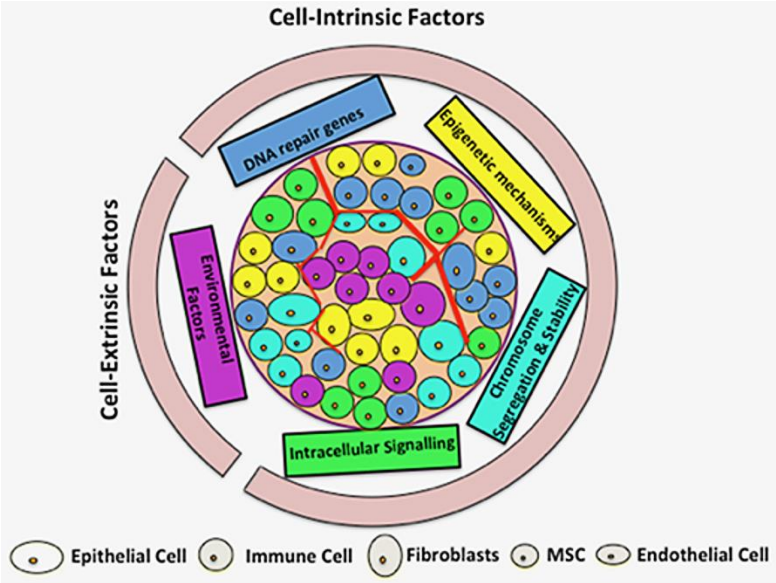

Figure 1. Structure of tumors and factors contributing to tumor heterogeneity.

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Detection of tumor heterogeneity

Heterogeneity can be detected at three levels: genes, cells or tissues, and clinical features. From clinical observation to testing for molecular mutations, a lengthy learning curve has been experienced in exploring the heterogeneity. For example, the first observation of the tumor heterogeneity of breast cancer happened before 3,500 years ago. But until the 21st century did the genetic analysis first confirm the heterogeneity of breast cancer at the genetic level. Genetic heterogeneity can be detected utilizing sequencing, microarrays, and *in situ* hybridization (ISH) / fluorescence *in situ* hybridization (FISH). Genomic profiling technology based on next-generation sequencing (NGS) has been widely used to uncover genomic heterogeneity and provided substantial insights into tumor heterogeneity and evolution at the molecular level. As a huge number of somatic mutations detected by NGS, it is becoming clear that individual tumor is unique, each containing distinct mutation patterns.

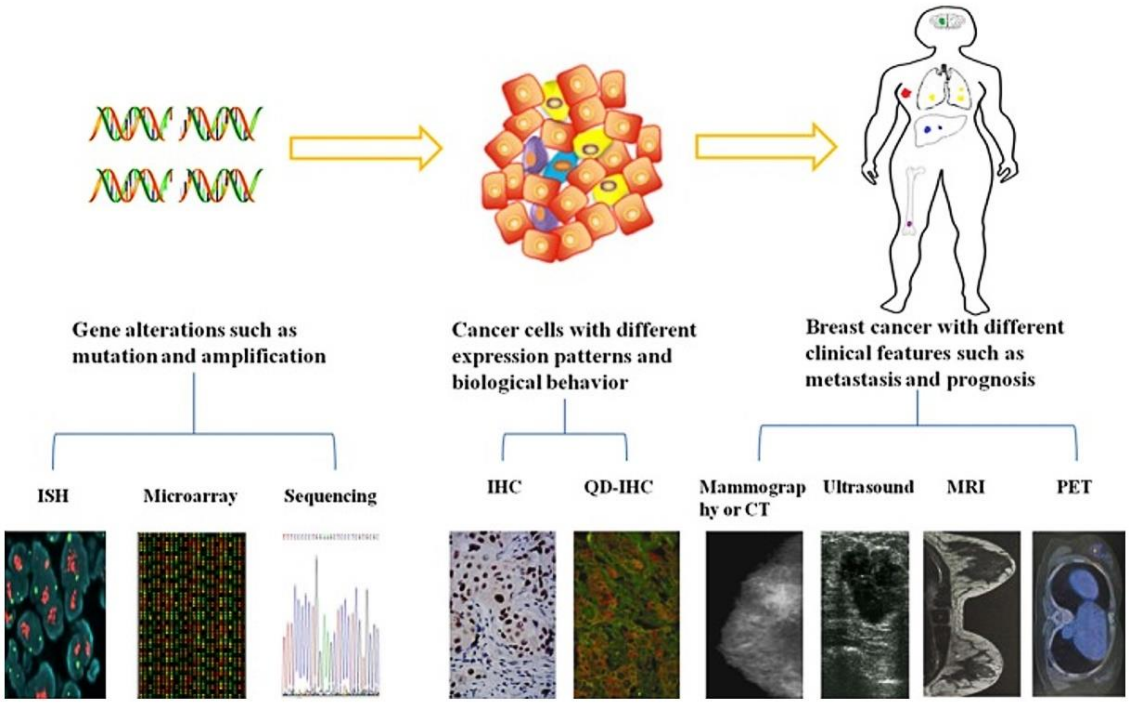

Figure 2. Heterogeneity can be detected t three levels: genes, cells and tissues, and clinical features (Song *et al*. 2016).

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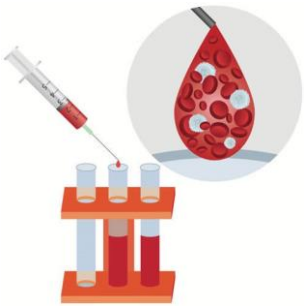
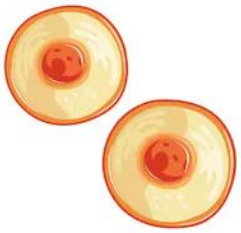
Emerging genomics approaches for interrogating tumor heterogeneity

Liquid biopsies & CTC/ctDNA analysis

The blood-based analysis using intact circulating tumor cells (CTCs) and cell-free ctDNA is a noninvasive approach for tumor genotyping and sequencing. Serial sampling enables to interrogate temporal patterns of tumor heterogeneity. Cell-free ctDNA analysis is the enrichment and sequencing of rare and fragile CTCs without the need for costly tools, allowing to discover clinical biomarkers for oncology. Genetic analysis of CTCs using high-throughput sequencing is the preferred clinical biomarker for interrogating genomic architecture, and can be used for functional models to assess their roles in tumor metastasis. And several studies have demonstrated that gene expression profiling of CTCs can throw light in intratumor heterogeneity.



Single-cell analysis



Single-cell analysis offers the most definitive tool for revealing tumor heterogeneity. The key advantage of single-cell analysis over bulk tumor analysis is the preservation of gene expression information lost in bulk tumor analysis. Single-cell sequencing consists of four major steps: single-cell isolation, DNA/RNA isolation, amplification of DNA/RNA, high-throughput sequencing, and bioinformatics analysis. MALBAC and MDA can be used for single-cell whole-genome amplification. MALBAC offers the lower false-negative rate for detection of single nucleotide variations (SNVs) but results in higher false positives owing to a lower fidelity polymerase. Zahn *et al*. (2017) introduced a novel approach called direct library preparation (DLP), which does not require amplification and prepares libraries directly from single-cell genome.

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Tumor heterogeneity to translational research

The Cancer Genome Atlas (TCGA) project was a comprehensive effort to promote our understanding of the genetic changes specific for each cancer through the application of genome-wide analysis technologies. The resulting data provided valuable insights into tumor genetic heterogeneity and the cellular processes contributing to tumor heterogeneity. Tumor heterogeneity and its related pathogenesis present a major area for novel therapeutic approaches such as targeting P13K, mTOR, ERK/MAPK pathways, and checkpoint immunotherapy. There have been more than 40 drugs approved by FDA or under clinical trials, targeting individual or combined P13K, Akt, mTOR, ERK1/2 and MAPK, and NFκB for treating human cancers. And there are more than 16 checkpoint inhibitors approved by FDA or under clinical trials, targeting PD-1, PD-L1, CTLA-4, CDK4/6 to treat solid tumor and hematologic malignancies.

Our solutions

NGS has now be extensively applied to molecular mapping of tumor heterogeneity, tumor diagnosis and prognosis prediction in clinical settings, as well as new drug discoveries. Furthermore, there has been a remarkable acceleration in the use of NGS to develop individualized treatment. To support medical research and translation, we provide a full range of sequencing/microarray-based approaches including gene expression profiling, RNA-seq, whole-genome sequencing (WGS), whole exome sequencing (WES), epigenomics, and single-cell sequencing for detecting tumor heterogeneity.

◆ Gene-expression profiling

We provide high-resolution array scanning (Affymetrix arrays) and automation to detect differentially expressed genes in tumor samples, allowing to elucidate tumor pathogenesis, discover biomarkers, and design drugs.

◆ RNA-seq

RNA-seq is able to provide insights into genetic and transcriptional heterogeneity, reveal genetic alterations that drive disease progression and drug resistance, improve prognostic stratification, and help design novel and rational treatments.

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◆ WGS / WES

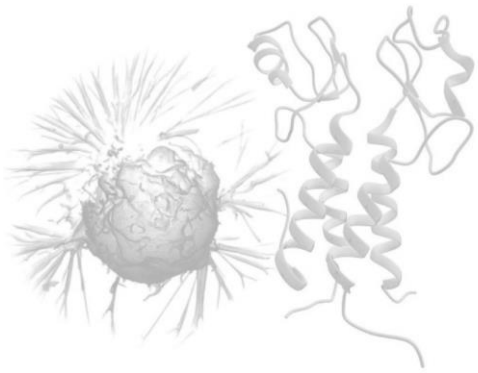
We provide both WGS and WES to identify genomic or exomic heterogeneity at the nucleotide and chromosomal level in tumor samples, including copy number variations (CNVs), SNPs, insertions and deletions (InDels), and structural variations (SVs).

◆ Epigenomics

Epigenetic alterations, especially DNA methylation, and directly influence gene function and may contribute to tumor cell heterogeneity. We provide a range of epigenomics services to help you identify key epigenomic modifications in promoting tumor heterogeneity.

◆ Single-cell sequencing

We utilize single-cell technology to provide a range of NGS-based services including single-cell DNA sequencing, RNA sequencing, and DNA methylation sequencing, for quantitative characterization of genetic alterations in individual tumor cells.



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