

World Journal of *Clinical Oncology*

World J Clin Oncol 2020 November 24; 11(11): 854-967



FRONTIER

- 854 Tumor-specific lytic path “hyperploid progression mediated death”: Resolving side effects through targeting retinoblastoma or p53 mutant
Hong F, Castro M, Linse K

REVIEW

- 868 Liquid biopsy in ovarian cancer: Catching the silent killer before it strikes
Feeney L, Harley IJ, McCluggage WG, Mullan PB, Beirne JP

MINIREVIEWS

- 890 Updates on “Cancer Genomics and Epigenomics”
Sharma A, Sharma KL, Bansal C, Kumar A
- 898 Deep diving in the PACIFIC: Practical issues in stage III non-small cell lung cancer to avoid shipwreck
Mielgo-Rubio X, Rojo F, Mezquita-Pérez L, Casas F, Wals A, Juan M, Aguado C, Garde-Noguera J, Vicente D, Couñago F

ORIGINAL ARTICLE**Retrospective Study**

- 918 Artificial intelligence in dentistry: Harnessing big data to predict oral cancer survival
Hung M, Park J, Hon ES, Bounsanga J, Moazzami S, Ruiz-Negrón B, Wang D

Prospective Study

- 935 Assessment of breast cancer immunohistochemistry and tumor characteristics in Nigeria
Aliyu UM, Musa AA
- 945 Functional Gait Assessment scale in the rehabilitation of patients after vestibular tumor surgery in an acute hospital
Kos N, Brçar M, Velnar T

CASE REPORT

- 959 Chemotherapy rechallenge in metastatic colon cancer: A case report and literature review
Reddy TP, Khan U, Burns EA, Abdelrahim M

ABOUT COVER

Editorial board member of *World Journal of Clinical Oncology*, Dr. Fabricio Freire de Melo is a Professor at the Multidisciplinary Institute of Health of the Federal University of Bahia, Brazil. He undertook his postgraduate training at the Faculty of Medicine of the Federal University of Minas Gerais, where he received his Master's degree (2007) and PhD (2011), and completed his Postdoctoral Fellowship (2013) in Microbiology. His ongoing research interests involve the host-pathogen interactions in *Helicobacter pylori* gastric infection and the features associated with development of duodenal ulcer and gastric cancer. In addition, he has been investigating diagnostic methods for and the immune response in COVID-19. Currently, he serves as Research and Extension Coordinator of the Multidisciplinary Institute of Health of the Federal University of Bahia. (L-Editor: Filipodia)

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Oncology* (*WJCO*, *World J Clin Oncol*) is to provide scholars and readers from various fields of oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJCO mainly publishes articles reporting research results and findings obtained in the field of oncology and covering a wide range of topics including art of oncology, biology of neoplasia, breast cancer, cancer prevention and control, cancer-related complications, diagnosis in oncology, gastrointestinal cancer, genetic testing for cancer, gynecologic cancer, head and neck cancer, hematologic malignancy, lung cancer, melanoma, molecular oncology, neurooncology, palliative and supportive care, pediatric oncology, surgical oncology, translational oncology, and urologic oncology.

INDEXING/ABSTRACTING

The *WJCO* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), China National Knowledge Infrastructure (CNKI), China Science and Technology Journal Database (CSTJ), and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Li-Li Wang*; Production Department Director: *Yun-Xiaojuan Wu*; Editorial Office Director: *Jia-Ping Yan*.

NAME OF JOURNAL

World Journal of Clinical Oncology

ISSN

ISSN 2218-4333 (online)

LAUNCH DATE

November 10, 2010

FREQUENCY

Monthly

EDITORS-IN-CHIEF

Hiten RH Patel

EDITORIAL BOARD MEMBERS

<https://www.wjgnet.com/2218-4333/editorialboard.htm>

PUBLICATION DATE

November 24, 2020

COPYRIGHT

© 2020 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS

<https://www.wjgnet.com/bpg/gerinfo/204>

GUIDELINES FOR ETHICS DOCUMENTS

<https://www.wjgnet.com/bpg/GerInfo/287>

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH

<https://www.wjgnet.com/bpg/gerinfo/240>

PUBLICATION ETHICS

<https://www.wjgnet.com/bpg/GerInfo/288>

PUBLICATION MISCONDUCT

<https://www.wjgnet.com/bpg/gerinfo/208>

ARTICLE PROCESSING CHARGE

<https://www.wjgnet.com/bpg/gerinfo/242>

STEPS FOR SUBMITTING MANUSCRIPTS

<https://www.wjgnet.com/bpg/GerInfo/239>

ONLINE SUBMISSION

<https://www.f6publishing.com>

Updates on “Cancer Genomics and Epigenomics”

Aarti Sharma, Kiran Lata Sharma, Cherry Bansal, Ashok Kumar

ORCID number: Aarti Sharma 0000-0002-8744-1268; Kiran Lata Sharma 0000-0002-1988-389X; Cherry Bansal 0000-0002-0734-7221; Ashok Kumar 0000-0003-3959-075X.

Author contributions: Aarti S and Kiran LS wrote the manuscript, revised it critically for important intellectual content, and contributed to the conception or design of the work, acquisition, analysis, and interpretation of data; Cherry B and Ashok K drafted the work, revised it critically for important intellectual content, and contributed to the conception or design of the work, acquisition, and analysis of data.

Conflict-of-interest statement: The authors declare no conflicts of interest for this article.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Aarti Sharma, Ashok Kumar, Department of Surgical Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, India

Kiran Lata Sharma, Department of Pathology, Baylor College of Medicine, Houston, TX 77030, United States

Cherry Bansal, Department of Pathology, Era’s Medical College and Hospital, Lucknow 226003, India

Corresponding author: Ashok Kumar, FACS, MD, Professor, Department of Surgical Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Raibareli Rd, Lucknow 226014, India. akgupta@sgpgi.ac.in, doc.ashokgupta@gmail.com

Abstract

The field of “Cancer Genomics and Epigenomes” has been widely investigated for their involvement in cancer to understand the basic processes of different malignancies. The aggregation of genetic and epigenetic alterations also displays a wide range of heterogeneity making it quite necessary to develop personalized treatment strategies. The complex interplay between DNA methylation and chromatin dynamics in malignant cells is one of the major epigenetic mechanisms that lead to gene activation and repression. Hence, each tumor needs to be fully characterized to satisfy the ideas of personalized treatment strategies. The present article addresses various aspects of genome characterization methods and their potential role in the field of cancer genomics and epigenomics.

Key Words: Genetics; Epigenetics; Chromatin; Cancer; Copy number variations; Transcription; Histone-modifications

©The Author(s) 2020. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Various genetic, as well as epigenetic alterations such as mutations, copy number variations, structural variations, and epigenetic dysregulations, are described as hallmarks of cancer. Understanding the complex interplay of genetic and epigenetic changes is exceedingly important for improving cancer care and the development of personalized medicine. Advanced genome analysis tools have enabled researchers to obtain a comprehensive picture of the cancer genome. However, the understanding of tumor genomics/epigenomics and the correct use of technology are tightly coupled processes. The present article summarizes various genome analytic methods utilized in

Manuscript source: Invited manuscript

Specialty type: Oncology

Country/Territory of origin: United States

Peer-review report's scientific quality classification

Grade A (Excellent): 0

Grade B (Very good): 0

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

Received: February 25, 2020

Peer-review started: February 25, 2020

First decision: June 13, 2020

Revised: July 3, 2020

Accepted: August 1, 2020

Article in press: August 1, 2020

Published online: November 24, 2020

P-Reviewer: de Talamoni NGT

S-Editor: Zhang L

L-Editor: Filipodia

P-Editor: Wang LL



cancer biology.

Citation: Sharma A, Sharma KL, Bansal C, Kumar A. Updates on "Cancer Genomics and Epigenomics". *World J Clin Oncol* 2020; 11(11): 890-897

URL: <https://www.wjnet.com/2218-4333/full/v11/i11/890.htm>

DOI: <https://dx.doi.org/10.5306/wjco.v11.i11.890>

INTRODUCTION

"Cancer Genomics and Epigenomes" has played a critical role in our understanding of the basic processes of different malignancies. Multistep tumorigenesis is a progression of events, which result from the dysregulation of signaling mechanisms and alterations in the processing of genetic information. Cancer is a heterogeneous genetic disease caused by alterations in the genes that control cell growth and division. The major changes involve mutations in our genetic material *i.e.* deoxyribonucleic acid (DNA) which makes our genes. The risk of developing cancer increases if genetic changes are inherited as germline changes and present in the germ cells. These changes are present in the entire cells of the progeny. Cancer-causing genetic changes can also be acquired during a person's life because of the errors in DNA repair mechanisms, which are caused by chemicals in tobacco, smoke, and radiation like ultraviolet rays. The genetic changes, which happen after conception at any time during the life of a person, are called somatic or acquired changes. The tumor cells can undergo various genetic changes like chromosomal rearrangements or genetic mutations.

Epigenetics is defined as heritable changes in gene activity that occur without alterations in the DNA sequence. These changes in gene expression are stable between cell divisions^[1]. Some of these non-genetic variations are strongly controlled by two main epigenetic mechanisms, *i.e.* chemical modifications to the cytosine residues of DNA called DNA methylation and histone post-translational modifications associated with DNA. The complex interplay between DNA methylation and chromatin dynamics in malignant cells is one of the major epigenetic mechanisms that lead to gene activation and repression. These modifications play critical roles in the epigenetic inheritance of transcriptional memory. Complex patterns of epigenetic modifications can act as epigenetic markers to characterize gene expression, gene activity, and chromatin state^[2,3]. Most epigenetic alterations are the global regulator of gene expression, affect the properties and behavior of the cells, and severely affect cancer progression.

The Cancer Genome Atlas, a database of comprehensive, multi-dimensional maps of the crucial genomic changes in 33 different cancers, that has been developed by a collaboration between the National Cancer Institute and National Human Genome Research Institute, comprises 2.5 petabytes data describing paired tumors and normal tissues of more than 11,000 patients. This database is freely available and is widely used by researchers (<https://cancergenome.nih.gov/>). The cBio Cancer Genomics Portal is a web-based (<http://www.cbioportal.org/>) open-access interactive platform of cancer genomics multidimensional datasets, which offer access to 5000 tumor samples from 20 various cancer studies. These efforts have lowered the walls between big genomic multifarious data and cancer researchers to translate the important information into biologic visions with applications to the clinic^[4,5]. Nevertheless, the online cancer databases have recently evolved as a portal for integrative oncogenomics that stores data regarding gene information, microRNA (miRNA), protein expression profiling, copy number variations for several cancer forms, as well as protein-protein interaction information (<http://www.canevolve.org/>). It allows interrogating the consequences of the primary analysis, integrative analysis as well as network analysis of oncogenomics data^[6]. A recent study published by Agarwal *et al*^[7] created a Colorectal cancer Database, which contains the information from 2056 colorectal cancer (CRC)-related genes associated with different CRC stages, obtained from available literature with current information. This database is helping the clinicians obtain a better understanding of diagnosis as well as treatment and classification of CRCs <http://lms.snu.edu.in/corecg/>^[7].

Next-generation sequencing (NGS)-based platforms such as the Ion Torrent PGM

sequencer, MiSeq, HiSeq, reduced representation bisulfite sequencing, chromatin immunoprecipitation sequencing, methylation sequencing, methylated DNA immunoprecipitation, RNASeq, and array-based techniques are recently in use and have greatly assisted the discovery of biomarkers for the early diagnosis, prognosis, responses to chemotherapy, and treatment strategies in various cancers. In addition, the establishment of advanced NGS methodologies for less explored domains of cancer genomics such as non-coding RNA (ncRNA), miRNA, long ncRNA, small nucleolar RNA, and circular RNA (circRNA) have also revolutionized cancer research.

The present article addresses various aspects of genome characterization methods and their potentials role in the field of cancer genomics and epigenomics.

SINGLE-CELL SEQUENCING IN CANCER

The biological functions of cells are attained from transcription and translation. However, errors in their process can give rise to many genetic issues. Differences in gene regulation, stochastic variation, or environmental perturbations, are reflected at the genomic, transcriptomic, and proteomic levels. Cellular heterogeneity among cells within the same tumor is a result of genetic changes influenced by the environment. The heterogeneous nature of tumors is the primary reason for complicated treatment outcomes since a treatment that targets one tumor cell population may not be effective against another. To understand the role of rare cells in tumor progression single-cell sequencing is an emerging technology. Single-cell sequencing is a strong tool for investigating clonal evolution and heterogeneousness in cancer. With the help of currently available technologies for single-cell sequencing, it has become quite possible to characterize the intra-tumor cellular heterogeneity, measure mutation rates, and identify rare cell types that give rise to cellular heterogeneity. The hypothesis behind the development of carcinoma is the process of clonal evolution from mutated cells. Currently available technologies to identify rare cell types *e.g.*, microfluidic-based single-cell sorting methods for stem cells, mass cytometry-based proteomic strategies, and high-throughput multiplexed quantitative polymerase chain reaction or sequencing methodologies. These methods provided new possibilities to look into the dynamical processes of cell-fate transitions^[6].

Single-cell analysis in cancer genomics is highly important and new research orientation these days. Literature shows a good number of studies that have tried to explore the single-cell analysis approach to characterize cellular heterogeneity especially in the field of cancer genetics^[9,10]. Bartoschek *et al*^[10] studied the spatially and functionally distinct subclasses of breast cancer-associated fibroblasts by a single-cell RNA sequencing approach and found that there was an improved resolution of the widely defined cancer-associated fibroblast (CAF) population. The authors concluded that the single-cell RNA sequencing approach paves the way for the development of biomarker-related drugs directly for precision targeting of CAFs. Moreover, a recent review article by Macaulay *et al*^[11] concluded that both genomic and transcriptomic heterogeneity within an organism has been considerably underestimated, and single-cell approaches now stand poised to illuminate this new layer of biological complexity during normal development and disease^[11].

Exploring the field of cellular non-uniformity by single-cell sequencing approach will be very useful to impart fundamental insights into biological processes. Besides, it will also have important applications in the development of cancer-related therapies which is till now challenged by inter/intra heterogeneity.

COPY NUMBER VARIATIONS IN CANCER

Human genomes were once thought to be stable but current literature is full of publications with opposite views. An unexpectedly frequent, dynamic, and complex form of genetic diversity called copy number variations (CNVs) is an example of the dynamic nature of the genome. CNVs are a class of structurally variant regions within two human genomes. These discoveries have suddenly reversed the idea of a single diploid "reference genome." The DNA CNVs are an important constituent of genetic variation, affecting a larger fraction of the genome as compared to single nucleotide variation. CNVs are greater than 1 kb base in size and defined as gains/losses of genomic DNA, which are of microscopic or submicroscopic level, therefore, not necessarily visible by standard G-banding karyotyping. The figures from March 2009 until the date of the Database of Genomic Variants have ascertained approximately

21000 CNVs or around 6500 unique CNV loci. The CNVs are believed to cover around 10% of the human genome. The cutting-edge technologies (NGS and microarray) have greatly facilitated the study of various aspects of human genomes. The recent advancements in medical research have made it possible to characterize the widespread constitutional CNVs, which have highlighted their role in susceptibility to a wide spectrum of diseases. The role of CNVs in cancer is currently underestimated and less understood. The CNV data can be used to identify regions of the genome involved in disease phenotypes because it affects the larger fraction of the genome. The advent of high-resolution technologies has made it possible to identify genome-wide CNVs in comparatively less amount of time and are actively trying to determine the clinical impact of CNVs in patient populations. In particular, presence of CNVs leads to gene dosage variations and large-scale genome-wide investigations have demonstrated the large impact of CNVs on severe developmental disorders such as Down, Prader Willi, Angelman, and Cri du Chat syndromes, which result from gain or loss of one copy of a chromosome or chromosomal region. In cancer, copy number changes have also been involved in altered expression of tumor-suppressor as well as oncogenes. Thus, detection and mapping of copy number abnormalities provide significant information about the link of copy number aberrations with the disease phenotype, which ultimately helps the researchers in localizing critical genes.

TUMOR GENOTYPING IN CANCER

The development of targeted therapies for cancer patients greatly depends on tumor genotyping. A collaboration of pathologists and molecular biologists with clinical practitioners has shed a light on better, cost-effective, and comparatively faster methods to "genotype" malignant tumors to understand the genetic architecture of many cancers. Identifying the genetic abnormalities that drive particular tumors is leading to significant improvement in treatment-related modalities. It is evident from current data that genetic mutation based on targeted therapies work much better than conventional treatment methods. Moreover, personalized therapies can be common for two or more different cancers, irrespective of their tissue of origin.

MICROSATELLITE INSTABILITY AND CANCER

Genetic instability is a characteristic of most known malignancies, which involves a higher rate of mutations in the genome of cellular lineage. Genetic instability pathways are known to underlie mechanisms behind the development pattern of CRC. However, existing data concerning microsatellite instability (MSI) in hereditary colorectal cancer (85%) shows a well-marked distinction compared to sporadic CRC (up to 20%). It may be of particular interest that inactivated DNA mismatch repair (MMR) apparatus plays a crucial role in MSI occurrence, as loss or altered function of MMR proteins causes more error-prone DNA replication leading to the sequential accumulation of mutations.

MiRNA AND CANCER

miRNA or small ncRNA regularizes various target genes and hence engages in a variety of biological and pathological processes, like cancer. The role of miRNA in cancer has been discovered in several studies. Recent studies have identified mir-139-5p as a novel serum biomarker for CRC progression recurrence and metastasis^[12]. CircRNA "ciRS-7-A" is a potential therapeutic prognostic biomarker for CRC^[13]. A study from the Yang group identified some novel approaches for studying circRNA functions. The authors found that circ-Foxo3 regulates the cellular functions of tissue cells and suppresses cancer cell progression in breast cancer^[14]. The long ncRNA, H19, is significantly associated with CRC patient survival^[15]. H19 silencing blocks the G1-S transition, inhibits cell migration, and reduces cell proliferation^[15]. Regulation of Let-7^[16], RB1-E2F1, and activity of β -catenin are also crucial upstream regulators mediating H19 function. Moreover, these ultra-conserved elements (*i.e.* miRNA) signify a newly recognized class of ncRNA, which assist in regulating miRNAs by direct interaction. These ultra-conserved genes expression signatures can help in better classification of tumors, including CRCs^[17].

Cancer pharmacogenomics targets the understanding of how genetic variants influence drug efficacy and toxicity in cancer patient treatments. The inter-individual genetic variation affects a drug's pharmacokinetics, pharmacodynamics, and response to treatment^[18]. As every patient responds to drug treatment differently, the genotype-phenotype association can decide the drug dose. Pharmacogenomics-based databases have been developed in recent years for identifying patient target genes and small molecule candidates for cancer therapeutics^[17]. A review article published on "cancer pharmacogenomics" in *Nature genetics* 2013 explains about various strategies and challenges including recent developments in sequencing techniques, clinical trial designs, and application of germline genetics analysis with bio-statistical genetics analysis methods that have potential for the detection of genetic variants associated with inter-individual response to the drug^[19].

The list of genome/epigenome characterization techniques with their respective advantages and disadvantages is available in [Table 1](#)^[20-30].

LIQUID BIOPSIES

Cells undergoing apoptosis or necrosis shed cell-free fragments of DNA into the bloodstream, which gives rise to circulating cell-free DNA. Recently, the technology has enabled to detect low levels of such a minor amount of cell-free DNA. In cancer, the load of circulating free DNA increases as the cells are increasingly undergoing cell division. The DNA shed by cancerous cells is known as circulating tumor DNA (ctDNA). ct-DNA contains the genetic information about the tumor and it has been evident from previous studies that ctDNA can also depict a wide range of clinical information like tumor genotyping, tumor staging, tumor grading, and prognosis, *etc*^[31-34]. There are two major methods to study ct-DNA: One is by targeting ctDNA to find particular gene mutations/structural rearrangements in specific genome regions and the other one is the detection of *de novo* ctDNA mutations and somatic CNVs. These approaches are known as targeted and non-targeted approaches, respectively. The untargeted approach does not require any prior knowledge of molecular alteration, for instance, whole-genome sequencing. Both of these approaches have advantages and limitations. Genotyping ctDNA can prove a useful tool to detect any malignancy at the initial stage as well as the effect of ongoing treatment on patients without the involvement of invasive techniques. [Table 2](#) lists the available CTC isolation methods^[35-39].

CONCLUSION

In a nutshell, mutations, genome imbalances, and disruption of epigenetic machinery cumulatively characterize cancer. This evolutionary procedure for cancer progression requires interplay between the Cancer Genome and Epigenome. The newly formed malignant traits stably accumulate in the clonal lineage of cancer cells. Oncogenic events like mutation, copy number alteration, deletion, and insertion are particularly well recognized in the field of cancer biology. Cancer has been primarily studied based on genetics only. However, epigenetics turned out to be an alternative way of cancer that plays a significant role in acquiring stable oncogenic traits. Genetic and epigenetic mechanisms have a combined role in the formation of oncogenic traits by working in a multitude of ways as a team. Therefore, tumor characterization must include epigenomics along with genomics to get more accurate insights about the molecular pathology of the disease.

Table 1 Genome characterization technologies

Technique	Application	Advantages/remarks	Disadvantages	Ref.
GTG banding	Chromosomal abnormalities, such as translocations, genomic, autosomal trisomies, polyploidy, sex chromosome monosomy and double trisomies	Can only detect rearrangements that involve > 3 Mb of DNA; -low Sensitivity	-Limited to mitotically active cells; -Difficulties in decoding highly rearranged chromosomes	[20,21]
FISH	Gene fusions, aneuploidy, loss of a chromosomal region or a whole chromosome	Can identify genetic changes that are too small. High sensitivity and specificity	Heavily influenced by cellular phenomena and hybridization artifacts	[22,23]
aCGH	Deletions, amplifications, breakpoints, and ploidy abnormalities	Detect DNA copy changes simultaneously at multiple loci in a genome- sensitivity was estimated to be 96.7% - specificity was 99.1%	Inappropriate for the detection of mosaicism, balanced chromosomal translocations, and inversions	[24]
Sanger seq	Single nucleotide change, small indels (insertions or deletions)	Gold standard platform widely used for SNV detection; --High sensitivity and Specificity	Unable to detect mosaic alleles below a threshold of 15%–20% (Limit of detection 15%–20%); Low discovery power	[25]
NGS	Whole genome sequencing, de novo assembly sequencing, resequencing, and transcriptome sequencing at the DNA or RNA level	Higher sequencing depth enables higher sensitivity (down to 1%); More data produced with the same amount of input DNA; -High sensitivity and Specificity	Expensive method; High rate of sequencing errors	[26,27]
Bisulfite conversion	DNA methylation	Resolution at DNA level. Effective method providing information about cytosine methylation	Beginning with high quality DNA is critical, Impossible to distinguish methylated and hemimethylated cytosine	[28]
MDRE	Restrict CpG methylation analysis only to methylated regions of genome	Easy to use availability and assortment of endonucleases	DNA methylation assay is circumscribed by the use of a particular enzyme	[29]
ChIP-seq	-Analyze protein interactions with DNA; - ChIP-seq combines chromatin immunoprecipitation (ChIP) with NGS for identification of binding sites of DNA-binding proteins	Fast well studied. Compatible with array-or sequencing-based analysis, <i>i.e.</i> it is possible to perform genome-wide analysis	Relies on antibody specificity Microarray assay relies on particular probes	[30]

FISH: Fluorescence in situ hybridization; aCGH: Array comparative genomic hybridization; NGS: Next generation sequencing; MDRE: Methylation-dependent restriction enzyme; ChIP-seq: Chromatin immunoprecipitation sequencing; Sanger seq: Sanger sequencing; SNV: Single nucleotide variation.

Table 2 Circulating tumor cell isolation techniques

Technology	Platform	Methods	Ref.
Physical properties	Density gradient, physical filter, dielectric, photoacoustic microfluidic	Size, density	[35,36]
High-throughput imaging	Imaging cytometry	Scanning of cells on the slide	[36]
Leukocyte depletion	Batch cell lysis; microfluidic CTC-iChip; immunomagnetic separation	Negative depletion of leukocytes	[37]
Antibody capture	Cell search; magsweeper; microfluidic CTC-chip	Selection for tumor-specific markers	[37]
Functional characteristics	Epispot assay, invasion assay	Protein secretion, cell migration	[38]
Nanotechnology	Immunomagnetic nanobeads, nano-structures substrates in microchip	Nanomaterials able to increase interactions with CTCs and specific antibodies, and to enable their electrical conductivity	[39]

CTC: Circulating tumor cell.

REFERENCES

- 1 **Goldberg AD**, Allis CD, Bernstein E. Epigenetics: a landscape takes shape. *Cell* 2007; **128**: 635-638 [PMID: 17320500 DOI: 10.1016/j.cell.2007.02.006]
- 2 **Bernstein BE**, Meissner A, Lander ES. The mammalian epigenome. *Cell* 2007; **128**: 669-681 [PMID: 17320505 DOI: 10.1016/j.cell.2007.01.033]
- 3 **Kouzarides T**. Chromatin modifications and their function. *Cell* 2007; **128**: 693-705 [PMID: 17320507 DOI: 10.1016/j.cell.2007.02.005]
- 4 **Gao J**, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C, Schultz N. Integrative analysis of complex cancer genomics and clinical profiles using

- the cBioPortal. *Sci Signal* 2013; **6**: p11 [PMID: 23550210 DOI: 10.1126/scisignal.2004088]
- 5 **Cerami E**, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C, Schultz N. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2012; **2**: 401-404 [PMID: 22588877 DOI: 10.1158/2159-8290.CD-12-0095]
 - 6 **Samur MK**, Yan Z, Wang X, Cao Q, Munshi NC, Li C, Shah PK. canEvolve: a web portal for integrative oncogenomics. *PLoS One* 2013; **8**: e56228 [PMID: 23418540 DOI: 10.1371/journal.pone.0056228]
 - 7 **Agarwal R**, Kumar B, Jayadev M, Raghav D, Singh A. CoReCG: a comprehensive database of genes associated with colon-rectal cancer. *Database (Oxford)* 2016; 2016 [PMID: 27114494 DOI: 10.1093/database/baw059]
 - 8 **Wang Y**, Navin NE. Advances and applications of single-cell sequencing technologies. *Mol Cell* 2015; **58**: 598-609 [PMID: 26000845 DOI: 10.1016/j.molcel.2015.05.005]
 - 9 **Singer J**, Kuipers J, Jahn K, Beerenwinkel N. Single-cell mutation identification via phylogenetic inference. *Nat Commun* 2018; **9**: 5144 [PMID: 30514897 DOI: 10.1038/s41467-018-07627-7]
 - 10 **Bartoschek M**, Oskolkov N, Bocci M, Lötvot J, Larsson C, Sommarin M, Madsen CD, Lindgren D, Pekar G, Karlsson G, Ringnér M, Bergh J, Björklund Å, Pietras K. Spatially and functionally distinct subclasses of breast cancer-associated fibroblasts revealed by single cell RNA sequencing. *Nat Commun* 2018; **9**: 5150 [PMID: 30514914 DOI: 10.1038/s41467-018-07582-3]
 - 11 **Macaulay IC**, Voet T. Single cell genomics: advances and future perspectives. *PLoS Genet* 2014; **10**: e1004126 [PMID: 24497842 DOI: 10.1371/journal.pgen.1004126]
 - 12 **Miyoshi J**, Toden S, Yoshida K, Toiyama Y, Alberts SR, Kusunoki M, Sinicrope FA, Goel A. MiR-139-5p as a novel serum biomarker for recurrence and metastasis in colorectal cancer. *Sci Rep* 2017; **7**: 43393 [PMID: 28262692 DOI: 10.1038/srep43393]
 - 13 **Weng W**, Wei Q, Toden S, Yoshida K, Nagasaka T, Fujiwara T, Cai S, Qin H, Ma Y, Goel A. Circular RNA ciRS-7-A Promising Prognostic Biomarker and a Potential Therapeutic Target in Colorectal Cancer. *Clin Cancer Res* 2017; **23**: 3918-3928 [PMID: 28174233 DOI: 10.1158/1078-0432.CCR-16-2541]
 - 14 **Lu WY**. Roles of the circular RNA circ-Foxo3 in breast cancer progression. *Cell Cycle* 2017; **16**: 589-590 [PMID: 28278047 DOI: 10.1080/15384101.2017.1278935]
 - 15 **Kallen AN**, Zhou XB, Xu J, Qiao C, Ma J, Yan L, Lu L, Liu C, Yi JS, Zhang H, Min W, Bennett AM, Gregory RI, Ding Y, Huang Y. The imprinted H19 lncRNA antagonizes let-7 microRNAs. *Mol Cell* 2013; **52**: 101-112 [PMID: 24055342 DOI: 10.1016/j.molcel.2013.08.027]
 - 16 **Ohtsuka M**, Ling H, Ivan C, Pichler M, Matsushita D, Goblirsch M, Stiegelbauer V, Shigeyasu K, Zhang X, Chen M, Vidhu F, Bartholomeusz GA, Toiyama Y, Kusunoki M, Doki Y, Mori M, Song S, Gunther JR, Krishnan S, Slaby O, Goel A, Ajani JA, Radovich M, Calin GA. H19 Noncoding RNA, an Independent Prognostic Factor, Regulates Essential Rb-E2F and CDK8-β-Catenin Signaling in Colorectal Cancer. *EBioMedicine* 2016; **13**: 113-124 [PMID: 27789274 DOI: 10.1016/j.ebiom.2016.10.026]
 - 17 **Rossi S**, Kopetz S, Davuluri R, Hamilton SR, Calin GA. MicroRNAs, ultraconserved genes and colorectal cancers. *Int J Biochem Cell Biol* 2010; **42**: 1291-1297 [PMID: 19497386 DOI: 10.1016/j.biocel.2009.05.018]
 - 18 **Dickmann LJ**, Ware JA. Pharmacogenomics in the age of personalized medicine. *Drug Discov Today Technol* 2016; **21-22**: 11-16 [PMID: 27978982 DOI: 10.1016/j.ddtec.2016.11.003]
 - 19 **Wheeler HE**, Maitland ML, Dolan ME, Cox NJ, Ratain MJ. Cancer pharmacogenomics: strategies and challenges. *Nat Rev Genet* 2013; **14**: 23-34 [PMID: 23183705 DOI: 10.1038/nrg3352]
 - 20 **Barkholt L**, Flory E, Jekerle V, Lucas-Samuel S, Ahnert P, Bisset L, Büscher D, Fibbe W, Foussat A, Kwa M, Lantz O, Mačiulaitis R, Palomäki T, Schneider CK, Sensebé L, Tachdjian G, Tarte K, Tosca L, Salmikangas P. Risk of tumorigenicity in mesenchymal stromal cell-based therapies—bridging scientific observations and regulatory viewpoints. *Cytotherapy* 2013; **15**: 753-759 [PMID: 23602595 DOI: 10.1016/j.jcyt.2013.03.005]
 - 21 **Bejjani BA**, Shaffer LG. Application of array-based comparative genomic hybridization to clinical diagnostics. *J Mol Diagn* 2006; **8**: 528-533 [PMID: 17065418 DOI: 10.2353/jmoldx.2006.060029]
 - 22 **Yan J**, Guilbault E, Massé J, Bronsard M, DeGrandpré P, Forest JC, Drouin R. Optimization of the fluorescence in situ hybridization (FISH) technique for high detection efficiency of very small proportions of target interphase nuclei. *Clin Genet* 2000; **58**: 309-318 [PMID: 11076056 DOI: 10.1034/j.1399-0004.2000.580409.x]
 - 23 **Eastmond DA**, Schuler M, Rupa DS. Advantages and limitations of using fluorescence in situ hybridization for the detection of aneuploidy in interphase human cells. *Mutat Res* 1995; **348**: 153-162 [PMID: 8544867 DOI: 10.1016/0165-7992(95)90003-9]
 - 24 **Möhlendick B**, Stoecklein NH. Analysis of Copy-Number Alterations in Single Cells Using Microarray-Based Comparative Genomic Hybridization (aCGH). *Curr Protoc Cell Biol* 2014; **65**: 22.19.1-22.19.23 [PMID: 25447076 DOI: 10.1002/0471143030.cb2219s65]
 - 25 **Sanger F**, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A* 1977; **74**: 5463-5467 [PMID: 271968 DOI: 10.1073/pnas.74.12.5463]
 - 26 **Kircher M**, Kelso J. High-throughput DNA sequencing—concepts and limitations. *Bioessays* 2010; **32**: 524-536 [PMID: 20486139 DOI: 10.1002/bies.200900181]
 - 27 **Shendure J**, Ji H. Next-generation DNA sequencing. *Nat Biotechnol* 2008; **26**: 1135-1145 [PMID: 18846087 DOI: 10.1038/nbt1486]
 - 28 **Leontiou CA**, Hadjidaniel MD, Mina P, Antoniou P, Ioannides M, Patsalis PC. Bisulfite Conversion of DNA: Performance Comparison of Different Kits and Methylation Quantitation of Epigenetic Biomarkers that Have the Potential to Be Used in Non-Invasive Prenatal Testing. *PLoS One* 2015; **10**: e0135058 [PMID: 26247357 DOI: 10.1371/journal.pone.0135058]
 - 29 **Laird PW**. Principles and challenges of genomewide DNA methylation analysis. *Nat Rev Genet* 2010; **11**: 191-203 [PMID: 20125086 DOI: 10.1038/nrg2732]
 - 30 **Furey TS**. ChIP-seq and beyond: new and improved methodologies to detect and characterize protein-DNA interactions. *Nat Rev Genet* 2012; **13**: 840-852 [PMID: 23090257 DOI: 10.1038/nrg3306]

- 31 **Gabriel E**, Bagaria SP. Assessing the Impact of Circulating Tumor DNA (ctDNA) in Patients With Colorectal Cancer: Separating Fact From Fiction. *Front Oncol* 2018; **8**: 297 [PMID: 30128304 DOI: 10.3389/fonc.2018.00297]
- 32 **Saluja H**, Karapetis CS, Pedersen SK, Young GP, Symonds EL. The Use of Circulating Tumor DNA for Prognosis of Gastrointestinal Cancers. *Front Oncol* 2018; **8**: 275 [PMID: 30087854 DOI: 10.3389/fonc.2018.00275]
- 33 **Vafaei S**, Fattahi F, Ebrahimi M, Janani L, Shariftabrzi A, Madjd Z. Common molecular markers between circulating tumor cells and blood exosomes in colorectal cancer: a systematic and analytical review. *Cancer Manag Res* 2019; **11**: 8669-8698 [PMID: 31576171 DOI: 10.2147/CMAR.S219699]
- 34 **Rapado-González Ó**, Álvarez-Castro A, López-López R, Iglesias-Canle J, Suárez-Cunqueiro MM, Muñelo-Romay L. Circulating microRNAs as Promising Biomarkers in Colorectal Cancer. *Cancers (Basel)* 2019; **11** [PMID: 31252648 DOI: 10.3390/cancers11070898]
- 35 **Jacob K**, Sollier C, Jabado N. Circulating tumor cells: detection, molecular profiling and future prospects. *Expert Rev Proteomics* 2007; **4**: 741-756 [PMID: 18067413 DOI: 10.1586/14789450.4.6.741]
- 36 **Aguiar-Avelar C**, Soto-García B, Aráiz-Hernández D, Yee-de León JF, Esparza M, Chacón F, Delgado-Balderas JR, Alvarez MM, Trujillo-de Santiago G, Gómez-Guerra LS, Velarde-Calvillo LP, Abarca-Blanco A, Wong-Campos JD. High-Throughput Automated Microscopy of Circulating Tumor Cells. *Sci Rep* 2019; **9**: 13766 [PMID: 31551445 DOI: 10.1038/s41598-019-50241-w]
- 37 **Bankó P**, Lee SY, Nagygyörgy V, Zrínyi M, Chae CH, Cho DH, Telekes A. Technologies for circulating tumor cell separation from whole blood. *J Hematol Oncol* 2019; **12**: 48 [PMID: 31088479 DOI: 10.1186/s13045-019-0735-4]
- 38 **Millner LM**, Linder MW, Valdes R Jr. Circulating tumor cells: a review of present methods and the need to identify heterogeneous phenotypes. *Ann Clin Lab Sci* 2013; **43**: 295-304 [PMID: 23884225]
- 39 **Lou XL**, Sun J, Gong SQ, Yu XF, Gong R, Deng H. Interaction between circulating cancer cells and platelets: clinical implication. *Chin J Cancer Res* 2015; **27**: 450-460 [PMID: 26543331 DOI: 10.3978/j.issn.1000-9604.2015.04.10]



Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: <https://www.f6publishing.com/helpdesk>
<https://www.wjgnet.com>

