We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Circulating Cell-Free DNA

Yingli Sun, Ke An and Caiyun Yang

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.80730>

Abstract

Circulating cell-free DNA (cfDNA) refers to extracellular DNA present in body fluid that may be derived from both normal and diseased cells. The concentration, integrity, genetic, and epigenetic alternations in the cfDNA may suggest pathological conditions of the body, such as inflammation, autoimmune diseases, stress, or even malignancies. cfDNA from patients with malignancies contains variants as those in the tumor tissue cells, thus allowing noninvasive assessment of tumor in real time. The clinical detection of cfDNA is one major application of liquid biopsy and has great application value in the early diagnosis of clinical tumors, real-time progression monitoring, curative effect observation and evaluation, prognosis assessment, and metastasis risk analysis. This chapter summarizes the origin of cell-free DNA and its important clinical applications as a noninvasive biomarker.

Keywords: liquid biopsy, circulating cell-free DNA, cancer, biomarker

1. Introduction

Liquid biopsy, a term relative to tissue biopsy, is a technical way to analyze the nonsolid biological tissue by detection of cells and free DNA that enter body fluids. Liquid biopsy refers to the real-time monitoring of the dynamic alterations of disease by detecting circulating tumor cells (CTCs), circulating cell-free DNA (cfDNA), exosomes and so on. This technique has great application value as a tool for disease early diagnosis, real-time progression monitoring, curative effect observation and evaluation, prognosis assessment, and metastasis risk analysis, with the added benefit of being noninvasive and flexible for repeat tumor sampling [1–3].

Circulating cell-free DNA (cfDNA) is released as single-stranded DNA and double-stranded DNA into body fluids, including the blood [4], sputum [5], urine [6], cerebrospinal fluid [7], or ascites [8] from apoptotic and necrotic cells [9]. cfDNA was first identified by Mandel and Metais

in the human blood in 1948 [10]. In 1977, Leon discovered that circulating cell-free DNA was also existed in cancer patients [11]. In 1997, Lo et al. found the presence of a small percentage of cfDNA originating from the fetus in the maternal plasma and serum. Then cfDNA was first used for noninvasive prenatal testing, including fetal sex assessment which can identify sex for fetus [12], RhD blood group genotyping, detection of chromosomal aneuploidy, and fetal-related diseases. These diseases include systemic lupus erythematosus (SLE), an autoimmune disease involving multiple systems, multiple organs, and multiple autoantibodies [13], and monogenic diseases, such as β -globin gene and *HBB* gene [14]. At present, as an important aspect of liquid biopsy, the detection of circulating cell-free DNA displays its irreplaceable advantages in clinic, including simpleness and accessibility. Compared with the solid biopsy, the liquid biopsy by detection of cfDNA is noninvasive and easily repeated. The detection of cfDNA as a clinic marker has amounts of advantages. The intra-abnormalities can be detected in cfDNA at an earlier time, thus enabling early diagnosis of disease. And the detection of cfDNA makes repeated sampling possible for the monitoring of disease progression, drug response, and prognostic tracking.

2. Clinical applications of circulating cell-free DNA

The concentration, integrity, genetic, and epigenetic alternations in the cfDNA may suggest pathological conditions of the body, such as inflammation, autoimmune diseases, stress, or even malignancies. Different disease-associated molecular characteristics can be detected as the indicators of pathological conditions in the plasma of patients, including the total level and fragment, copy-number aberrations [15–18], methylation changes [19–21], single-nucleotide mutations [16, 22–25], cancer-derived viral sequences [26, 27], and chromosomal rearrangements [28, 29]. cfDNA from patients with malignancies (cell-free tumor DNA, ctDNA) contains variants as those in the tumor tissue cells, thus allowing noninvasive assessment of tumor in real time. ctDNA is a very promising tumor biomarker for cancer diagnosis and monitoring, prognosis assessment, and personalized medication guidance compared with conventional serum markers.

2.1. The size of cfDNA

The length of cfDNA from patients differs from that of healthy groups, which may suggest some kinds of physiological or pathological conditions, including pregnancy, cancer, liver/bone marrow transplantation, SLE, and many other clinical scenarios such as stroke, autoimmune disorders, and myocardial infarction [30–37]. The length of cfDNA was previously identified by gel electrophoresis and electron microscopy (EM) in 1998. Giacona et al. found that most abundant cfDNA fragments from pancreatic cancer patients displayed stronger ladder patterns compared with that from healthy controls, which was equivalent to whole-number multiples (1–5×) of nucleosomal DNA (185–200 bp). The average strand length distributions of DNA (DNA-SL) in pancreatic cancer patients were also obviously shorter (231 nm; median, 185 nm) than average plasma DNA-SL in controls (311 nm; median, 273 nm). There were more excess of short DNA at approximately 63, approximately 126, approximately 189, approximately 252, and approximately 315 nm, corresponding to small multiples of lengths

associated with nucleosomes, in the pancreatic cancer patient plasma than in the plasma of healthy control [38]. The molecular size-distribution profiles of plasma DNA in systemic lupus erythematosus (SLE) patients exhibited a significantly increased proportion of short DNA fragments [22]. Jiang et al. found that the plasma DNA molecules from hepatocellular carcinoma patients were aberrantly short or long through massively parallel sequencing and the aberrantly short ones preferentially carried tumor-associated copy-number aberrations [23]. The study now confirms that the overall size of cfDNA was approximately 166 or 143 bp or even shorter with a periodicity of 10 bp [37]. The size distributions of cfDNA prominent peak were focused in 166 bp for hepatocellular carcinoma (HCC) patients and hepatitis B virus (HBV) carriers [39]. The size of cfDNA fragment was different from the systemic lupus erythematosus patients that the height of the 166 bp peak was reduced and has smaller peaks and healthy individuals [40]. These abundant cfDNA molecules were most likely generated from apoptosis cells accompanied with certain enzymatic cleavage processes shaped by nucleosome-associated DNA packing [34, 40–43]. With the technology development and refinement for the determination of cfDNA fragment size, cfDNA fragment size and its distribution provide important information associated with pathological conditions and display to be a promising indicator for clinical diagnosis.

2.2. cfDNA concentration

The concentration or level of cfDNA could change with different physiological conditions. The study described the concentration of cfDNA in patients with non-small cell lung cancer (NSCLC) was higher than healthy controls, and the average level was 95.67 and 59.60 ng/ μ l, respectively [44]. The concentrations of overall cfDNA in cancer patients have a significant increase with a wide range (hundreds to thousands ng/ml in the blood) compared with in the healthy controls (a relative level of 30 ng/ml) [45–48]. The level of cfDNA in cancer patients, such as in ovarian cancer, colorectal cancer, and pancreatic cancer, is significantly associated with the cancer-specific survival and can be used as an independent predictor for death [22, 46, 47]. The study found preoperative cell-free DNA levels are significantly elevated in patients with epithelial ovarian carcinoma (EOC), and the cell-free DNA level is a potential predictor for clinical outcome in patients with ovarian cancer. For colorectal cancer patients, the cfDNA level is correlated with a shorter survival and may be a biomarker for survival when it is above 1000 ng/ml [47]. The level of cfDNA is the highest in pancreatic ductal adenocarcinoma compared with pancreatic neuroendocrine tumor and chronic pancreatitis using Alu repeat amplicon [1]. The cfDNA level also can be used as a marker of cellular trauma and inflammation from anesthesia and surgery in clinic. The concentration of cfDNA displayed significant differences and fluctuation pattern during serial perioperative process in donors and recipients undergoing living donor liver transplantation (LDLT). The cfDNA concentration is higher in recipients than in donor undergoing living donor liver transplantation and is an indicative marker for liver injury. The cfDNA level fluctuated from a baseline 37.62 ng/ml to a relative high level of 94.72 ng/ml in recipient who developed postoperative sepsis [49]. In the study of lung cancer, the patients with high baseline cfDNA concentration had a significantly worse disease-free and overall survival than those with lower concentrations [50].

2.3. cfDNA genetic variations

Cell-free DNA generates from apoptosis or necrosis cells and contains the same genetic variations with intra-tissues. cfDNA is widely used as a genetic biomarker for disease diagnosis and monitoring by detection of the copy-number variations, SNPs, and mutation occurred in cfDNA.

2.3.1. Copy-number variations of cfDNA

Copy-number variations (CNV) are always associated with the occurrence of complex disorders. The CNV of urine cfDNA in advanced prostate cancer patients is significantly associated with tumor burden, and the CNV change after stage-specific therapies reflected disease progression status and overall survival [51]. Copy-number variations of HLA-DRB5 in 135 systemic lupus erythematosus (SLE) patients were higher than that in 219 healthy controls and were associated with the risk of SLE. The copy-number at 6p21.32 is aberrant in the majority of SLE patients [52]. In the plasma of neuroblastoma patients, the copy-number alterations of cfDNA displayed concordant high patterns and can be used as a cost-effective, noninvasive, rapid, robust, and sensitive biomarker for neuroblastoma prognosis [53].

2.3.2. Mutation of cfDNA

Mutation is a widespread phenomenon in biology, the effect of which is permanent alteration of nucleotide sequence. Mutations play a vital role in both normal and abnormal biological processes, such as evolution and cancer. Many studies display that the mutation detection in cfDNA will enable noninvasive tumor diagnosis and monitoring with higher sensitivity and specificity in advance [54, 55]. Many patients with advanced lung cancers that are resistant to AZD9291 therapy carried *EGFR* C797S mutation in cfDNA [56]. *EGFR* mutations in cfDNA were significantly associated with overall survival (OS), progression-free survival (PFS), and response to therapy in the EURTAC trial. The *EGFR* L858R mutations in cfDNA proved to be a novel prognostic marker [57]. In melanoma, *BRAF* mutation in cfDNA can be detected earlier than primary lesion [58]. *KRAS* mutation in cfDNA for pancreatic ductal adenocarcinoma (PDAC) provided a new diagnostic marker and could optimize therapeutic strategies for patients [59].

2.3.3. SNP of cfDNA

Single-nucleotide polymorphism is a variation in a single nucleotide occurring in the genome at a specific position. Detection of SNP in circulating cell-free DNA has been widely used in prenatal screening. The detection of SNP located in *SRY* gene or *TSPY* gene in Y chromosome proved to be a highly accurate and clinically applicable noninvasive prenatal diagnosis (NIPD) marker for fetal gender determination [60–62]. The study reported first trimester contingent screening used nuchal translucency and cell-free DNA, the latter has higher detection rate that is up to 98% for trisomy 21, but noninvasive prenatal testing will not be cost-effective associated with traditional [63, 64].

2.4. cfDNA methylation as epigenetic biomarker

Epigenetic modifications are heritable molecular events that affect gene expression without changing DNA sequences, including DNA methylation, histone modification, and so on.

They are stable through cell division. DNA methylation refers to the addition of methyl group to cytosine residues in DNA sequence, and it is the best-studied epigenetic event [65, 66]. The quantitative DNA methylation analysis of tumor-derived cell lines was conducted in 1999 for the first time, and the possibility of using them as noninvasive biomarkers for cancer was examined [21, 67, 68]. Various studies were performed to detect cfDNA to assess the performance of cfDNA methylation as a biomarker [64].

The level of cfDNA methylation for GSTP1 and APC in the castration-resistant prostate cancer patients could be used as a marker reflecting treatment response and prognosis [69]. The BRMS1 promotor in cfDNA could provide prognostic information from the plasma of NSCLC and highly methylated from advanced NSCLC patients [70]. cfDNA epigenetic pattern can be used as an early diagnostic marker for breast cancer [71].

3. Conclusion

At present, cfDNA has been used as an independent marker for prenatal screening and also has great applicable value in the disease prognosis and monitoring, particularly in cancer. When the concentration of cfDNA is above a baseline 30 ng/ml and is closed to hundreds or even thousands ng/ml, or/and when the size of cfDNA is obviously short and displays ladder pattern, or/and when vital genetic and epigenetic mutations are reported in cfDNA, patients should be recommended for further examination. The appearance of cfDNA conforms to the current trend of precision medicine in the disease and achieves accurate diagnosis and precise treatment. However, there are many challenges in the real clinic applications. Firstly, the detected method is not uniform and the standardization process is lacking [72]. Secondly, the level of cfDNA can be too low, so the detection technology needs to be improved to increase the sensitivity and specificity [73, 74].

The study of cfDNA is still in its infancy, and a lot of in-depth research is needed to further confirm its clinical application value.

Conflict of interest

The authors declare that they have no conflict of interest.

Author details

Yingli Sun^{1,2*}, Ke An^{1,2} and Caiyun Yang^{1,2}

*Address all correspondence to: sunyl@big.ac.cn

1 Key Laboratory of Genomic and Precision Medicine, China Gastrointestinal Cancer Research Center, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, China

2 University of Chinese Academy of Sciences, Beijing, China

References

- [1] Sikora K, Bedin C, Vicentini C, et al. Evaluation of cell-free DNA as a biomarker for pancreatic malignancies. *The International Journal of Biological Markers*. 2015;**30**(1):E136-E141
- [2] Zhang X, Shi S, Zhang B, et al. Circulating biomarkers for early diagnosis of pancreatic cancer: Facts and hopes. *American Journal of Cancer Research*. 2018;**8**(3):332-353
- [3] Li BT, Drilon A, Johnson ML, et al. A prospective study of total plasma cell-free DNA as a predictive biomarker for response to systemic therapy in patients with advanced non-small-cell lung cancers. *Annals of Oncology*. 2016;**27**(1):154-159
- [4] Cree IA, Uttley L, Buckley Woods H, et al. The evidence base for circulating tumour DNA blood-based biomarkers for the early detection of cancer: A systematic mapping review. *BMC Cancer*. 2017;**17**(1):697
- [5] Su Y, Fang H, Jiang F. Integrating DNA methylation and microRNA biomarkers in sputum for lung cancer detection. *Clinical Epigenetics*. 2016;**8**:109
- [6] Foj L, Ferrer F, Serra M, et al. Exosomal and non-exosomal urinary miRNAs in prostate cancer detection and prognosis. *Prostate*. 2017;**77**(6):573-583
- [7] Pentsova EI, Shah RH, Tang J, et al. Evaluating cancer of the central nervous system through next-generation sequencing of cerebrospinal fluid. *Journal of Clinical Oncology*. 2016;**34**(20):2404-2415
- [8] Husain H, Nykin D, Bui N, et al. Cell-free DNA from ascites and pleural effusions: Molecular insights into genomic aberrations and disease biology. *Molecular Cancer Therapeutics*. 2017;**16**(5):948-955
- [9] Jahr S, Hentze H, Englisch S, et al. DNA fragments in the blood plasma of cancer patients: Quantitations and evidence for their origin from apoptotic and necrotic cells. *Cancer Research*. 2001;**61**(4):1659-1665
- [10] Mandel PMP. Les acides nucléiques du plasma sanguin chez l'homme. *Comptes Rendus. Académie des Sciences*. 1948;**142**:241-243
- [11] Leon SA, Shapiro B, Sklaroff DM, et al. Free DNA in the serum of cancer patients and the effect of therapy. *Cancer Research*. 1977;**37**(3):646-650
- [12] Costa JM, Benachi A, Gautier E. New strategy for prenatal diagnosis of X-linked disorders. *The New England Journal of Medicine*. 2002;**346**(19):1502
- [13] Atamaniuk J, Hsiao YY, Mustak M, et al. Analysing cell-free plasma DNA and SLE disease activity. *European Journal of Clinical Investigation*. 2011;**41**(6):579-583
- [14] Lam KWG, Jiang P, Liao GJW, et al. Noninvasive prenatal diagnosis of monogenic diseases by targeted massively parallel sequencing of maternal plasma: Application to beta-thalassemia. *Clinical Chemistry*. 2012;**58**(10):1467-1475
- [15] Heitzer E, Auer M, Hoffmann EM, et al. Establishment of tumor-specific copy number alterations from plasma DNA of patients with cancer. *International Journal of Cancer*. 2013;**133**(2):346-356

- [16] Chan KCA, Jiang PY, Zheng YWL, et al. Cancer genome scanning in plasma: Detection of tumor-associated copy number aberrations, single-nucleotide variants, and tumoral heterogeneity by massively parallel sequencing. *Clinical Chemistry*. 2013;**59**(1):211-224
- [17] Heitzer E, Ulz P, Belic J, et al. Tumor-associated copy number changes in the circulation of patients with prostate cancer identified through whole-genome sequencing. *Genome Medicine*. 2013;**5**(4):30
- [18] Leary RJ, Sausen M, Kinde I, et al. Detection of chromosomal alterations in the circulation of cancer patients with whole-genome sequencing. *Science Translational Medicine*. 2012;**4**(162):162ra154
- [19] Chan KCA, Jiang PY, Chan CWM, et al. Noninvasive detection of cancer-associated genome-wide hypomethylation and copy number aberrations by plasma DNA bisulfite sequencing. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;**110**(47):18761-18768
- [20] Chan KCA, Lai PBS, Mok TSK, et al. Quantitative analysis of circulating methylated DNA as a biomarker for hepatocellular carcinoma. *Clinical Chemistry*. 2008;**54**(9):1528-1536
- [21] Wong IHN, Lo YMD, Zhang J, et al. Detection of aberrant p16 methylation in the plasma and serum of liver cancer patients. *Cancer Research*. 1999;**59**(1):71-73
- [22] Diehl F, Li M, Dressman D, et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;**102**(45):16368-16373
- [23] Yung TKF, Chan KCA, Mok TSK, et al. Single-molecule detection of epidermal growth factor receptor mutations in plasma by microfluidics digital pcr in non-small cell lung cancer patients. *Clinical Cancer Research*. 2009;**15**(6):2076-2084
- [24] Murtaza M, Dawson SJ, Tsui DWY, et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature*. 2013;**497**(7447):108-112
- [25] Forsheo T, Murtaza M, Parkinson C, et al. Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. *Science Translational Medicine*. 2012;**4**(136):136ra68
- [26] Lo YMD, Chan LYS, Lo KW, et al. Quantitative analysis of cell-free Epstein-Barr virus DNA in plasma of patients with nasopharyngeal carcinoma. *Cancer Research*. 1999;**59**(6):1188-1191
- [27] Chan KCA, Hung ECW, Woo JKS, et al. Early detection of nasopharyngeal carcinoma by plasma Epstein-Barr virus DNA analysis in a surveillance program. *Cancer*. 2013;**119**(10):1838-1844
- [28] McBride DJ, Orpana AK, Sotiriou C, et al. Use of cancer-specific genomic rearrangements to quantify disease burden in plasma from patients with solid tumors. *Genes, Chromosomes and Cancer*. 2010;**49**(11):1062-1069
- [29] Leary RJ, Kinde I, Diehl F, et al. Development of personalized tumor biomarkers using massively parallel sequencing. *Science Translational Medicine*. 2010;**2**(20):20ra14

- [30] Rainer TH, Wong LKS, Lam W, et al. Prognostic use of circulating plasma nucleic acid concentrations in patients with acute stroke. *Clinical Chemistry*. 2003;**49**(4):562-569
- [31] Zhong XY, von Muhlenen I, Li Y, et al. Increased concentrations of antibody-bound circulatory cell-free DNA in rheumatoid arthritis. *Clinical Chemistry*. 2007;**53**(9):1609-1614
- [32] Bartoloni E, Ludovini V, Alunno A, et al. Increased levels of circulating DNA in patients with systemic autoimmune diseases: A possible marker of disease activity in Sjogren's syndrome. *Lupus*. 2011;**20**(9):928-935
- [33] Antonatos D, Patsilnakos S, Spanodimos S, et al. Cell-free DNA levels as a prognostic marker in acute myocardial infarction. *Annals of the New York Academy of Sciences*. 2006;**1075**:278-281
- [34] Fan HC, Blumenfeld YJ, Chitkara U, et al. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. *Proceedings of the National Academy of Sciences of the United States of America*. 2008;**105**(42):16266-16271
- [35] Mouliere F, Robert B, Peyrotte EA, et al. High fragmentation characterizes tumour-derived circulating DNA. *PLoS One*. 2011;**6**(9):e23418
- [36] Chan KCA, Zhang J, Hui ABY, et al. Size distributions of maternal and fetal DNA in maternal plasma. *Clinical Chemistry*. 2004;**50**(1):88-92
- [37] Suzuki N, Kamataki A, Yamaki J, et al. Characterization of circulating DNA in healthy human plasma. *Clinica Chimica Acta*. 2008;**387**(1-2):55-58
- [38] Giacona MB, Ruben GC, Iczkowski KA, et al. Cell-free DNA in human blood plasma: Length measurements in patients with pancreatic cancer and healthy controls. *Pancreas*. 1998;**17**(1):89-97
- [39] Jiang P, Chan CW, Chan KC, et al. Lengthening and shortening of plasma DNA in hepatocellular carcinoma patients. *Proceedings of the National Academy of Sciences of the United States of America*. 2015;**112**(11):E1317-E1325
- [40] Chan RWY, Jiang P, Peng X, et al. Plasma DNA aberrations in systemic lupus erythematosus revealed by genomic and methylomic sequencing. *Proceedings of the National Academy of Sciences of the United States of America*. 2014;**111**(49):E5302-E5311
- [41] Lo YMD, Chan KCA, Sun H, et al. Maternal plasma DNA sequencing reveals the genome-wide genetic and mutational profile of the fetus. *Science Translational Medicine*. 2010;**2**(61):61ra91
- [42] Zheng YWL, Chan KCA, Sun H, et al. Nonhematopoietically derived DNA is shorter than hematopoietically derived DNA in plasma: A transplantation model. *Clinical Chemistry*. 2012;**58**(3):549-558
- [43] Yu SCY, Lee SWY, Jiang PY, et al. High-resolution profiling of fetal DNA clearance from maternal plasma by massively parallel sequencing. *Clinical Chemistry*. 2013;**59**(8):1228-1237
- [44] Leng SY, Zheng JJ, Jin YH, et al. Plasma cell-free DNA level and its integrity as biomarkers to distinguish non-small cell lung cancer from tuberculosis. *Clinica Chimica Acta*. 2018;**477**:160-165

- [45] Schwarzenbach H, Hoon DSB, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. *Nature Reviews Cancer*. 2011;**11**(6):426-437
- [46] Kamat AA, Baldwin M, Urbauer D, et al. Plasma cell-free DNA in ovarian cancer an independent prognostic biomarker. *Cancer*. 2010;**116**(8):1918-1925
- [47] Schwarzenbach H, Stoecklacher J, Pantel K, et al. Detection and monitoring of cell-free DNA in blood of patients with colorectal cancer. *Annals of the New York Academy of Sciences*. 2008;**1137**:190-196
- [48] Fournie GJ, Courtin JP, Laval F, et al. Plasma DNA as a marker of cancerous cell-death—Investigations in patients suffering from lung-cancer and in nude-mice bearing human tumors. *Cancer Letters*. 1995;**91**(2):221-227
- [49] Prakash K, Aggarwal S, Bhardwaj S, et al. Serial perioperative cell-free DNA levels in donors and recipients undergoing living donor liver transplantation. *Acta Anaesthesiologica Scandinavica*. 2017;**61**(9):1084-1094
- [50] Tissot C, Toffart AC, Villar S, et al. Circulating free DNA concentration is an independent prognostic biomarker in lung cancer. *The European Respiratory Journal*. 2015;**46**(6):1773-1780
- [51] Xia Y, Huang CC, Dittmar R, et al. Copy number variations in urine cell free DNA as biomarkers in advanced prostate cancer. *Oncotarget*. 2016;**7**(24):35818-35831
- [52] Wu LJ, Guo SC, Yang DQ, et al. Copy number variations of HLA-DRB5 is associated with systemic lupus erythematosus risk in Chinese Han population. *Acta Biochimica et Biophysica Sinica*. 2014;**46**(2):155-160
- [53] Van Roy N, Van der Linden M, Menten B, et al. Shallow whole genome sequencing on circulating cell-free DNA allows reliable noninvasive copy-number profiling in neuroblastoma patients. *Clinical Cancer Research*. 2017;**23**(20):6305-6314
- [54] De Mattos-Arruda L, Weigelt B, Cortes J, et al. Capturing intra-tumor genetic heterogeneity by de novo mutation profiling of circulating cell-free tumor DNA: A proof-of-principle. *Annals of Oncology*. 2014;**25**(9):1729-1735
- [55] Hamakawa T, Kukita Y, Kurokawa Y, et al. Monitoring gastric cancer progression with circulating tumour DNA. *British Journal of Cancer*. 2015;**112**(2):352-356
- [56] Thress KS, Paweletz CP, Felip E, et al. Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. *Nature Medicine*. 2015;**21**(6):560-562
- [57] Karachaliou N, Mayo-de las Casas C, Queralt C, et al. Association of EGFR L858R mutation in circulating free DNA with survival in the EURTAC trial. *JAMA Oncology*. 2015;**1**(2):149-157
- [58] Ashida A, Uhara H, Mikoshiba A, et al. Melanoma with braf mutation in circulating cell-free DNA despite no mutation in the primary lesion: A case report. *Acta Dermato-Venereologica*. 2016;**96**(1):128-129

- [59] Takai E, Totoki Y, Nakamura H, et al. Clinical utility of circulating tumor DNA for molecular assessment and precision medicine in pancreatic cancer. *Circulating Nucleic Acids in Serum and Plasma—Cnaps Ix*. 2016;**924**:13-17
- [60] Fernandez-Martinez FJ, Galindo A, Garcia-Burguillo A, et al. Noninvasive fetal sex determination in maternal plasma: A prospective feasibility study. *Genetics in Medicine*. 2012;**14**(1):101-106
- [61] Perlado-Marina S, Bustamante-Aragones A, Horcajada L, et al. Overview of five-years of experience performing non-invasive fetal sex assessment in maternal blood. *Diagnostics (Basel)*. 2013;**3**(2):283-290
- [62] Ramezanzadeh M, Khosravi S, Salehi R. Cell-free fetal nucleic acid identifier markers in maternal circulation. *Advanced Biomedical Research*. 2017;**6**:89
- [63] Conner P, Gustafsson S, Kublickas M. First trimester contingent testing with either nuchal translucency or cell-free DNA. Cost efficiency and the role of ultrasound dating. *Acta Obstetrica et Gynecologica Scandinavica*. 2015;**94**(4):368-375
- [64] Miltoft CB, Rode L, Tabor A. Positive view and increased likely uptake of follow-up testing with analysis of cell-free fetal DNA as alternative to invasive testing among danish pregnant women. *Acta Obstetrica et Gynecologica Scandinavica*. 2018;**97**(5):577-586
- [65] Callinan PA, Feinberg AP. The emerging science of epigenomics. *Human Molecular Genetics*. 2006;**15**:R95-R101
- [66] Jones PA, Laird PW. Cancer epigenetics comes of age. *Nature Genetics*. 1999;**21**(2):163-167
- [67] Lo YMD, Wong IHN, Zhang J, et al. Quantitative analysis of aberrant p16 methylation using real-time quantitative methylation-specific polymerase chain reaction. *Cancer Research*. 1999;**59**(16):3899-3903
- [68] Esteller M, Sanchez-Cespedes M, Rosell R, et al. Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. *Cancer Research*. 1999;**59**(1):67-70
- [69] Hendriks R, Dijkstra S, Smit F, et al. Cell free DNA methylation markers as predictors of treatment response and prognosis for castration-resistant prostate cancer. *European Urology Supplements*. 2017;**16**(3):e847
- [70] Balkouranidou I, Chimonidou M, Milaki G, et al. Breast cancer metastasis suppressor-1 promoter methylation in cell free DNA provides prognostic information in non-small cell lung cancer. *Cancer Research*. 2014;**110**(8):2054-2062
- [71] Uehiro N, Sato F, Pu F, et al. Circulating cell-free DNA-based epigenetic assay can detect early breast cancer. *Breast Cancer Research*. 2016;**18**(1):129
- [72] Ilie M, Hofman V, Long E, et al. Current challenges for detection of circulating tumor cells and cell-free circulating nucleic acids, and their characterization in non-small cell lung carcinoma patients. What is the best blood substrate for personalized medicine? *Annals of Translational Medicine*. 2014;**2**(11):107

- [73] Casoni GL, Ulivi P, Mercatali L, et al. Increased levels of free circulating DNA in patients with idiopathic pulmonary fibrosis. *The International Journal of Biological Markers*. 2010;**25**(4):229-235
- [74] Chang CPY, Chia RH, Wu TL, et al. Elevated cell-free serum DNA detected in patients with myocardial infarction. *Clinica Chimica Acta*. 2003;**327**(1-2):95-101

IntechOpen

IntechOpen