Front. Med. 2017, 11(4): 522–527
https://doi.org/10.1007/s11684-017-0526-7

REVIEW

Application of liquid biopsy in precision medicine: opportunities and challenges

Junyun Wang, Shuang Chang, Guochao Li, Yingli Sun (✉)

Key Laboratory of Genomic and Precision Medicine, China Gastrointestinal Cancer Research Center, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing 100101, China; University of Chinese Academy of Sciences, Beijing 100049, China

Abstract **Precision medicine for cancer patients aims to adopt the most suitable treatment options during diagnosis and treatment of individuals. Detecting circulating tumor cell (CTC) or circulating tumor DNA (ctDNA) in plasma or serum could serve as liquid biopsy, which would be useful for numerous diagnostic applications. Liquid biopsies can help clinicians screen and detect cancer early, stratify patients to the most suitable treatment and real-time monitoring of treatment response and resistance mechanisms in the tumor, evaluate the risk for metastatic relapse, and estimate prognosis. We summarized the advantages and disadvantages of tissue and liquid biopsies. We also further compared and analyzed the advantages and limitations of detecting CTCs, ctDNAs, and exosomes. Furthermore, we reviewed the literature related with the application of serum or plasma CTCs, ctDNAs, and exosomes for diagnosis and prognosis of cancer. We also analyzed their opportunities and challenges as future biomarkers. In the future, liquid biopsies could be used to guide cancer treatment. They could also provide the ideal scheme to personalize treatment in precision medicine.**

Keywords liquid biopsy; circulating tumor cells; cell-free ctDNA; exosomes; precision medicine

Introduction

Diagnosing and screening tumors through non-invasive methods represent an important paradigm shift in precision medicine. Tumors are highly heterogeneous, and sampling in its entirety is challenging. Tissue biopsy is invasive and cannot reflect current tumor dynamics or sensitivity to the treatment. With the development of sensitive techniques that can detect rare mutations, the heterogeneous landscape of tumor can be determined using a blood sample [1].

Liquid biopsy refers to the analysis of circulating tumor cells (CTCs) and cell-free circulating nucleic acids (in particular, circulating tumor DNA (ctDNA) and exosomes) released in the peripheral blood from the primary tumor and/or metastatic deposits. A liquid biopsy or blood sample can provide the genetic landscape and epigenetic characteristics of all cancerous lesions and offer the opportunity to track genomic evolution systematically [2]. The response to treatments and the development of acquired resistance should also be predicted. Details of the tumor genetic profile can enable the prediction of disease progression and response to therapies. Liquid biopsy can be used in cancer screening, patient stratification, and monitoring [3]. In addition, short ctDNA fragments harbor the footprints of transcription factors, which could indicate the cell-type origin of pathological states, such as cancer [4].

Table 1 provides a summary of the advantages and disadvantages of tissue and liquid biopsies.

Received November 18, 2016; accepted February 8, 2017
Correspondence: sunyl@big.ac.cn

CTCs

CTCs were first detected in cancer patients in 1869 [7]. CTCs, ctDNAs, and exosomes are released from the primary tumor and/or metastatic sites into the bloodstream, as shown in Fig. 1. Numerous studies in the past decade showed that CTCs may be used as a biomarker to predict cancer progression and survival in metastatic [7–9] and possibly in early-stage cancer patients [5,10].

Detection and clinical application of CTC

An average metastatic carcinoma patient shows 5–50

Table 1 Advantages and disadvantages of tissue and liquid biopsies [2,5,6]

Subject	Advantage	Disadvantage
Tissue biopsy	Standard detection procedure	Suffered and invasive
	Specific mutations that are helpful to target therapies	Not real-time detection
		Some tumors, such as that in lung, are not accessible for biopsy
		Information provided by biopsy is static, and it becomes inaccurate with cancer progression
		Needs multiple biopsies and is expensive
		Unavailable tumor sample
		Intratumor heterogeneity
		Inability to obtain tumor samples at different stages of disease
Liquid biopsy	Non-invasive	Significantly low amounts of circulating tumor DNA (ctDNA) and circulating tumor cell (CTC) and considerable amount of blood sample needed
	Real-time detection	
	Collects samples repeatedly and multiple detection during treatment	
	Provides considerably comprehensive information	ctDNA and CTC levels remarkably vary among individuals, and they are difficult to detect
	Responds to surgery and therapy effects	
	More sensitive as biomarkers than that of tissue biopsy	Needs validation in large studies
		Confined to the clinical application of advanced cancer

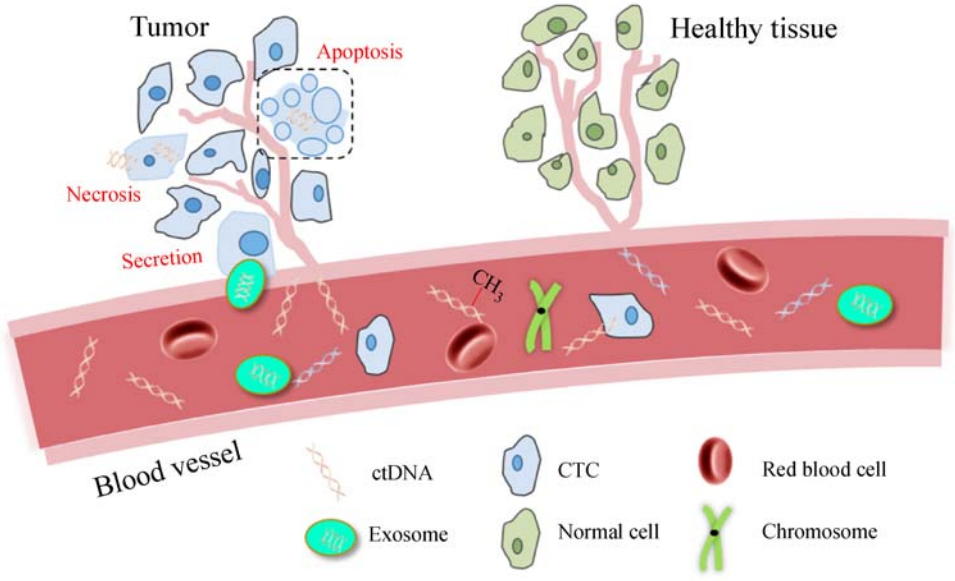

Fig. 1 Origin of CTCs and ctDNAs in blood plasma. CtDNAs and exosomes were secreted from necrotic and apoptotic tumor cells, and CTCs were released from tumor tissue. Gene mutation and epigenetic DNA methylation can be detected as diagnostic and prognostic biomarkers of cancer.

CTCs for approximately 7.5 ml of blood. High numbers of CTC correlate with aggressive disease, increased metastasis, and decreased time to relapse [11]. CTCs potentially carry valuable information about tumor composition, invasiveness, drug susceptibility, and resistance to therapy [12]. Given that high CTC burden can predict poor prognosis in metastatic disease, efforts regarding aggressive chemotherapeutic intervention are also being tested [13].

Therefore, CTCs could serve as a real-time marker for disease progression and survival. CTCs can also screen and detect cancer early, guide therapeutic management, determine therapy effectiveness or necessity even in the absence of detectable metastases, and provide insights into the mechanisms of drug resistance.

Opportunities and challenges of CTCs

The potential clinical value of CTCs is clear: early detection and treatment of metastatic spread are key for disease outcome, and CTCs can target metastasis in real time [14]. CTC enumeration and characterization with

certified systems offer reliable information on prognosis, and liquid biopsy may be considered to identify therapeutic targets or mechanisms of resistance on metastatic cells, such as mutations in *KRAS* [15]. CTC enumeration correlates with poor clinicopathological features and exhibits prognostic and predictive values.

Despite this considerable potential, CTC application is confronted with many challenges [15]. The possibility of using tumor metastasis with CTCs for detection should also be assessed. The few CTCs in serum and current detection technologies limit the further clinical application of CTCs. The genetic and epigenetic variation data collected by analyzing solid tumor biopsies provide valuable insights into a patient's disease and therapy options. In the near future, CTCs may significantly improve our ability to monitor malignancies and indicate whether or not a treatment may work, thereby providing the complementary roles of conventional and liquid biopsies [16].

ctDNA

ctDNA can be released from primary tumors, CTCs, micrometastasis, or overt metastases into the blood of cancer patients. The majority of ctDNA originates from apoptotic and necrotic tumor cells that release their fragmented DNA in the blood. ctDNA concentration in serum or blood is significantly increased compared with that of healthy individuals [17]; this change is correlated with malignancy [18].

Detection and clinical application of ctDNA

Increased concentrations of ctDNA fragments are observed in blood plasma and serum of cancer patients with various tumor types, and they are correlated with unfavorable outcome [19]. Analysis of tumor-linked genetic alterations is increasingly used for diagnostic, prognostic, and treatment purposes. ctDNA can be a promising biomarker in the liquid biopsy of cancer [20]. In addition, ctDNA methylation assay can significantly promote diagnoses of cancer of unknown primary origin and guide remarkably precise therapies associated with improved outcomes [21].

DNA methylation profiling can also be applied in clinic, but confronted with challenges [22]. DNA methylation profiling can be used for disease diagnosis. Non-invasive DNA methylation markers for diagnosis of many cancers, such as *GSTP1* hypermethylation as a biomarker of prostate and colorectal cancers with a sensitivity of 82% and a specificity of 95% [23], frequently show hypermethylation of *APC*, *MGMT, RASSF2*, and *WNT* inhibitory factor 1 [22].

Opportunities and challenges of ctDNA

Analyses of ctDNA may replace CTC detection for monitoring cancer progression in the future [6,24–26]. The major challenge with ctDNA analysis is assay sensitivity and specificity. Multigene panel analysis of ctDNA may increase test sensitivity. Many researchers indicated that ctDNA is more sensitive than CTCs in early detection; ctDNA also presents large dynamic range and considerable relation with changes in tumor burden [24,27].

The challenges of genome-wide DNA methylation analysis are as follows [28]: whether ctDNA reflects a representative characteristic of a cancer, whether metastatic tumors release the same amount of DNA as the primary tumors, whether all the cells in a tumor release the same amount of ctDNA as the other, and whether an accurate profile of tumor burden or a real-time monitoring of emerging or epigenetic mutations saves patients or improves their quality of life [29].

However, results regarding these biomarkers should be validated in independent cohorts of cancer patients. Furthermore, the clinical relevance in daily practice is difficult to determine. The dynamic changes in the ability to classify different subtypes of cancers and measure their evolution functional properties by non-invasive blood monitoring strongly relate cancer therapeutics and diagnostics.

Exosomes

Exosomes are lipid-bilayer-enclosed extracellular vesicles (carrying RNA, DNA, and protein) secreted by cells and circulated in the blood; these vesicles can also function as intercellular messengers, which contribute to their increased recognition in the scientific field [30–35]. Microvesicles, such as exosomes, contain miRNAs, proteins, and DNAs; they have also become promising diagnostic tools of liquid biopsy [36,37]. In addition, exosomes mediate tumor metastasis [38,39].

Detection and clinical application of exosomes

Exosomes present considerable application prospects, such as depiction and capture of tumorigenesis, tumor progression, and predictive markers, as shown in Fig. 2. The isolation of cancer exosomes from cancer patients remains a challenge owing to the lack of specific markers that can differentiate cancer from noncancer exosomes. Melo *et al.* provided evidence that GPC1 may serve as a pan-specific marker of cancer exosomes. Therefore, GPC1 may be an attractive candidate for detection and isolation of exosomes in the blood serum of patients with cancer for genetic analysis of specific alterations [36].

Fig. 2 Clinical applications of liquid biopsy for precision medicine. CTCs, ctDNAs, and exosomes as liquid biopsy for precision medicine, including screening and early detection of cancer, real-time monitoring of therapy, stratification and therapeutic intervention, therapeutic target and resistance mechanism, risk for metastatic relapse (prognosis), and improvement of drug delivery with exosomes.

Opportunities and challenges of exosomes

Detection of exosomes regarding somatic genetic alterations in the blood has been challenging, but new approaches for such analyses have improved the sensitive and specific detection at low levels.

Table 2 presents a summary of the advantages and limitations of detecting CTCs, cell-free ctDNAs, and exosomes [3,40].

Perspectives

CTCs, ctDNAs, and circulating exosomes [36,42] might serve as promising novel candidates as blood-based biomarkers. However, when and how the liquid biopsy will be used as a routine method for cancer patient care should be further determined. Currently, a real gap exists between the gained attraction of liquid biopsy in media and the increased number of publications in this field and its application to the patients. Finally, future blueprint of liquid biopsy in precision medicine should include the affordable cost, reproducibility and reliability of the results, and performance comparison of liquid biopsy and the existing solid biopsy among cancer patients. On the basis of this perspective, we can adopt the optimal strategy or remarkably effective drugs based on patient characteristics. Nevertheless, in the absence of subsequent treatment strategies or drugs, significant benefit from the early detection and precision medication is not obtained, despite that many risk factors related with the cancer or other diseases are screened.

In summary, the molecular and functional analyses of CTCs and circulating nucleic acids could be used as promising diagnostics and prognostics to enhance the stratification of therapies and acquire insights into precision therapy.

Table 2 Advantages and limitations of detecting CTCs, ctDNAs, and exosomes [20,38,41]

Subject	Advantages	Limitations
CTCs	Phenotypic studies of intact cells from tumor, including cell morphology and protein localization	Heterogeneity of the CTC populations (e.g., detection of CTCs with tumor-initiating capacity)
	Relevance for metastatic process and disease progression	Low abundance and fragility
	Allow functional *in vitro*/*in vivo* assays	Require considerably sensitive and specific analytic methods
	Opportunity for molecular characterization at both cellular and subcellular levels	False-negative (due to tumor metastasis) and false-positive results
	Allow immunolabeling-based approaches	Multiplicity of technologies used for CTC isolation
	Complementary with ctDNA: CTCs can escape from current chemotherapy	
	Potentially influence changes in treatment modalities	
ctDNAs	Sensitivity for detection of disease burden	False-negative/false-positive results (e.g., no specific isolation of ctDNA or mutations of tumor-associated genes in frequent benign diseases)
	Complementary with CTCs for detection of minimal residual disease after surgery or therapy with curative intent	No functional assays
	Might predict acquired drug resistance	Lack of standardization of preanalytical conditions
	Potentially influence changes in treatment modalities	
Exosomes	Analyzing RNA, DNA, and protein profiles from tumor cells	Did not analyze the phenotypic studies of cells from tumor
	Analyzing inflammatory response, stromal, and other systemic changes	Difficult extraction
	Used for drug delivery	

Acknowledgements

This work was supported by the Precision Medicine Research Program of the Chinese Academy of Sciences (No. KJZD-EW-L14), the Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDA12020343), the National Basic Research Program (973 Program, Nos. 2013CB911001 and 2012CB518 302), and the National Natural Science Foundation of China (No. 31540033).

Compliance with ethics guidelines

Junyun Wang, Shuang Chang, Guochao Li, and Yingli Sun declare that they have no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

References

1. Chan KCA, Jiang PY, Zheng YWL, Liao GJW, Sun H, Wong J, Siu SSN, Chan WC, Chan SL, Chan ATC, Lai PBS, Chiu RWK, Lo YMD. Cancer genome scanning in plasma: detection of tumor-associated copy number aberrations, single-nucleotide variants, and tumoral heterogeneity by massively parallel sequencing. Clin Chem 2013; 59(1): 211–224
2. Crowley E, Di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. Nat Rev Clin Oncol 2013; 10(8): 472–484
3. Brock G, Castellanos-Rizaldos E, Hu L, Coticchia C, Skog J. Liquid biopsy for cancer screening, patient stratification and monitoring. Transl Cancer Res 2015; 4(3): 280–290
4. Snyder MW, Kircher M, Hill AJ, Daza RM, Shendure J. Cell-free DNA comprises an *in vivo* nucleosome footprint that informs its tissues-of-origin. Cell 2016; 164(1–2): 57–68
5. Alix-Panabieres C, Pantel K. Clinical applications of circulating tumor cells and circulating tumor DNA as liquid biopsy. Cancer Discov 2016; 6(5): 479–491
6. Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol 2014; 32(6): 579–586
7. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, Reuben JM, Doyle GV, Allard WJ, Terstappen LW, Hayes DF. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004; 351(8): 781–791
8. Cohen SJ, Punt CJA, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, Picus J, Morse M, Mitchell E, Miller MC, Doyle GV, Tissing H, Terstappen LWMM, Meropol NJ. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol 2008; 26(19): 3213–3221
9. De Bono JS, Scher HI, Montgomery RB, Parker C, Miller MC, Tissing H, Doyle GV, Terstappen LW, Pienta KJ, Raghavan D. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clinic Cancer Res 2008; 14(19):6302–6309
10. Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, Reichert M, Beatty GL, Rustgi AK, Vonderheide RH, Leach SD, Stanger BZ. EMT and dissemination precede pancreatic tumor formation. Cell 2012; 148(1–2): 349–361
11. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. Science 2011; 331(6024): 1559–1564
12. Gold B, Cankovic M, Furtado LV, Meier F, Gocke CD. Do circulating tumor cells, exosomes, and circulating tumor nucleic acids have clinical utility? A report of the association for molecular pathology. J Mol Diagn 2015; 17(3): 209–224
13. Bidard FC, Fehm T, Ignatiadis M, Smerage JB, Alix-Panabieres C, Janni W, Messina C, Paoletti C, Muller V, Hayes DF, Piccart M, Pierga JY. Clinical application of circulating tumor cells in breast cancer: overview of the current interventional trials. Cancer Metastasis Rev 2013; 32(1–2): 179–188
14. Plaks V, Koopman CD, Werb Z. Cancer. Circulating tumor cells. Science 2013; 341(6151): 1186–1188
15. Alix-Panabieres C, Pantel K. Challenges in circulating tumour cell research. Nat Rev Cancer 2014; 14(9): 623–631
16. Schlange T, Pantel K. Potential of circulating tumor cells as blood-based biomarkers in cancer liquid biopsy. Pharmacogenomics 2016; 17(3): 183–186
17. Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. Free DNA in the serum of cancer patients and the effect of therapy. Cancer Res 1977; 37(3): 646–650
18. Stroun M, Anker P, Lyautey J, Lederrey C, Maurice PA. Isolation and characterization of DNA from the plasma of cancer patients. Eur J Cancer Clin Oncol 1987; 23(6): 707–712
19. Scher HI, Heller G, Molina A, Attard G, Danila DC, Jia XY, Peng WM, Sandhu SK, Olmos D, Riisnaes R, McCormack R, Burzykowski T, Kheoh T, Fleisher M, Buyse M, de Bono JS. Circulating tumor cell biomarker panel as an individual-level surrogate for survival in metastatic castration-resistant prostate cancer. J Clin Oncol 2015; 33(12): 1348–1355
20. Cheng F, Su L, Qian C. Circulating tumor DNA: a promising biomarker in the liquid biopsy of cancer. Oncotarget 2016; 7(30): 48832–48841
21. Moran S, Martínez-Cardús A, Sayols S, Musulén E, Balañá C, Estival-Gonzalez A, Moutinho C, Heyn H, Diaz-Lagares A, de Moura MC, Stella GM, Comoglio PM, Ruiz-Miró M, Matias-Guiu X, Pazo-Cid R, Antón A, Lopez-Lopez R, Soler G, Longo F, Guerra I, Fernandez S, Assenov Y, Plass C, Morales R, Carles J, Bowtell D, Mileshkin L, Sia D, Tothill R, Tabernero J, Llovet JM, Esteller M. Epigenetic profiling to classify cancer of unknown primary: a multicentre, retrospective analysis. Lancet Oncol 2016; 17 (10):1386–1395
22. Heyn H, Esteller M. DNA methylation profiling in the clinic: applications and challenges. Nat Rev Genet 2012; 13(10): 679–692
23. Van Neste L, Herman JG, Otto G, Bigley JW, Epstein JI, Van Criekinge W. The epigenetic promise for prostate cancer diagnosis. Prostate 2012; 72(11): 1248–1261
24. Dawson SJ, Tsui DW, Murtaza M, Biggs H, Rueda OM, Chin SF, Dunning MJ, Gale D, Forshew T, Mahler-Araujo B, Rajan S, Humphray S, Becq J, Halsall D, Wallis M, Bentley D, Caldas C, Rosenfeld N. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 2013; 368(13): 1199–1209
25. Kidess E, Jeffrey SS. Circulating tumor cells versus tumor-derived cell-free DNA: rivals or partners in cancer care in the era of single-

cell analysis? Genome Med 2013; 5(8):70

26. Tan CR, Zhou L, El-Deiry WS. Circulating tumor cells versus circulating tumor DNA in colorectal cancer: pros and cons. Curr Colorectal Cancer Rep 2016; 12(3): 151–161
27. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang YX, Agrawal N, Bartlett BR, Wang H, Luber B, Alani RM, Antonarakis ES, Azad NS, Bardelli A, Brem H, Cameron JL, Lee CC, Fecher LA, Gallia GL, Gibbs P, Le D, Giuntoli RL, Goggins M, Hogarty MD, Holdhoff M, Hong SM, Jiao YC, Juhl HH, Kim JJ, Siravegna G, Laheru DA, Lauricella C, Lim M, Lipson EJ, Marie SKN, Netto GJ, Oliner KS, Olivi A, Olsson L, Riggins GJ, Sartore-Bianchi A, Schmidt K, Shih IM, Oba-Shinjo SM, Siena S, Theodorescu D, Tie JN, Harkins TT, Veronese S, Wang TL, Weingart JD, Wolfgang CL, Wood LD, Xing DM, Hruban RH, Wu J, Allen PJ, Schmidt CM, Choti MA, Velculescu VE, Kinzler KW, Vogelstein B, Papadopoulos N, Luis AJ. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014; 6(224): 224ra24
28. Laird PW. Principles and challenges of genomewide DNA methylation analysis. Nat Rev Genet 2010; 11(3): 191–203
29. Yong E. Cancer biomarkers: written in blood. Nature 2014; 511 (7511): 524–526
30. Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, Guha A, Rak J. Intercellular transfer of the oncogenic receptor EGFrvIII by microvesicles derived from tumour cells. Nat Cell Biol 2008; 10(5): 619–624
31. Baj-Krzyworzeka M, Szatanek R, Weglarczyk K, Baran J, Urbanowicz B, Branski P, Ratajczak MZ, Zembala M. Tumour-derived microvesicles carry several surface determinants and mRNA of tumour cells and transfer some of these determinants to monocytes. Cancer Immunol Immunother 2006; 55(7): 808–818
32. Miranda KC, Bond DT, McKee M, Skog J, Paunescu TG, Da Silva N, Brown D, Russo LM. Nucleic acids within urinary exosomes/microvesicles are potential biomarkers for renal disease. Kidney Int 2010; 78(2): 191–199
33. Noerholm M, Balaj L, Limperg T, Salehi A, Zhu LD, Hochberg FH, Breakefield XO, Carter BS, Skog J. RNA expression patterns in serum microvesicles from patients with glioblastoma multiforme and controls. BMC Cancer 2012; 12(1): 22
34. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9 (6): 654–659
35. van der Vos KE, Balaj L, Skog J, Breakefield XO. Brain tumor microvesicles: insights into intercellular communication in the nervous system. Cell Mol Neurobiol 2011; 31(6): 949–959
36. Melo SA, Luecke LB, Kahlert C, Fernandez AF, Gammon ST, Kaye J, LeBleu VS, Mittendorf EA, Weitz J, Rahbari N, Reissfelder C, Pilarsky C, Fraga MF, Piwnica-Worms D, Kalluri R. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. Nature 2015; 523(7559): 177–182
37. Williams C, Rodriguez-Barrueco R, Silva JM, Zhang WJ, Hearn S, Elemento O, Paknejad N, Manova-Todorova K, Welte K, Bromberg J, Peinado H, Lyden D. Double-stranded DNA in exosomes: a novel biomarker in cancer detection. Cell Res 2014; 24(6): 766–769
38. Syn N, Wang LZ, Sethi G, Thiery JP, Goh BC. Exosome-mediated metastasis: from epithelial-mesenchymal transition to escape from immunosurveillance. Trends Pharmacol Sci 2016; 37(7): 606–617
39. Peinado H, Aleckovic M, Lavotshkin S, Matei I, Costa-Silva B, Moreno-Bueno G, Hergueta-Redondo M, Williams C, Garcia-Santos G, Ghajar CM, Nitadori-Hoshino A, Hoffman C, Badal K, Garcia BA, Callahan MK, Yuan JD, Martins VR, Skog J, Kaplan RN, Brady MS, Wolchok JD, Chapman PB, Kang YB, Bromberg J, Lyden D. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. Nat Med 2012; 18(6): 883–891
40. Ilie M, Hofman V, Long E, Bordone O, Selva E, Washetine K, Marquette CH, Hofman P. 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? Ann Transl Med 2014; 2(11): 107
41. Pantel K, Alix-Panabieres C. Liquid biopsy: potential and challenges. Mol Oncol 2016; 10(3): 371–373
42. Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic Mark M, Molina H, Kohsaka S, Di Giannatale A, Ceder S, Singh S, Williams C, Soplop N, Uryu K, Pharmer L, King T, Bojmar L, Davies AE, Ararso Y, Zhang T, Zhang H, Hernandez J, Weiss JM, Dumont-Cole VD, Kramer K, Wexler LH, Narendran A, Schwartz GK, Healey JH, Sandstrom P, Jørgen Labori K, Kure EH, Grandgenett PM, Hollingsworth MA, de Sousa M, Kaur S, Jain M, Mallya K, Batra SK, Jarnagin WR, Brady MS, Fodstad O, Muller V, Pantel K, Minn AJ, Bissell MJ, Garcia BA, Kang Y, Rajasekhar VK, Ghajar CM, Matei I, Peinado H, Bromberg J, Lyden D. Tumour exosome integrins determine organotropic metastasis. Nature 2015; 527(7578): 329–335