REVIEW

Circulating Exosomal miRNAs as a Promising Diagnostic Biomarker in Cancer

Regína LOHAJOVÁ BEHULOVÁ[1,2,3], Aneta BUGALOVÁ[3,5], Juraj BUGALA[3,5], Eva STRUHÁRŇANSKÁ[3,6], Martin ŠAFRANEK[3,7], Ivan JURÁŠ[3,4]

[1]Department of Clinical Genetics, St Elizabeth's Cancer Institute, Bratislava, Slovakia, [2]Institute of Genetics and Molecular Medicine, Faculty of Medicine, Slovak Medical University, Bratislava, Slovakia, [3]Lambda Life a. s., Bratislava, Slovakia, [4]Centre of personalized medicine, Lambda Life a. s., Bratislava, Slovakia, [5]Comenius University Science Park, Bratislava, Slovakia, [6]Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia, [7]Department of Experimental Plant Biology, Institute of Botany, Plant Science and Biodiversity Center, Slovak Academy of Sciences, Bratislava, Slovakia

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Summary
Cancer belongs to multifactorial diseases characterized by uncontrolled growth and proliferation of abnormal cells. Breast cancer, non-small cell lung cancer, and colorectal cancer are the most frequently diagnosed malignancies with a high mortality rate. These carcinomas typically contain multiple genetically distinct subpopulations of tumor cells leading to tumor heterogeneity, which promotes the aggressiveness of the disease. Early diagnosis is necessary to increase patient progression-free survival. Particularly, miRNAs present in exosomes derived from tumors represent potential biomarkers suitable for early cancer diagnosis. Identification of miRNAs by liquid biopsy enables a personalized approach with the subsequent better clinical management of patients. This review article highlights the potential of circulating exosomal miRNAs in early breast, non-small cell lung, and colorectal cancer diagnosis.

Keywords
Exosome • miRNA • Breast cancer • Non-small cell lung cancer • Colorectal cancer

Corresponding author
J. Bugala, Lambda Life a.s., Levočská 3, 851 01 Bratislava, Slovakia. E-mail: bugala.juraj@persmed.online

Introduction

Cancer is the second most common cause of death worldwide. In 2020, 2.7 million people were diagnosed with cancer and another 1.3 million people died in the European Union. Lung, prostate, colorectal, stomach, and liver cancers are the most common cancers in men, while breast, colorectal, lung, cervical, and thyroid cancer are the most prevalent among women [1].

Cancer is a multifactorial, systemic, genetic disease - that means it is caused by changes in genes involved in cell growth, cell division, proliferation, migration, differentiation, etc. Each cancer cell contains a unique combination of genetic changes. As a tumor grows, additional genetic changes are formed, which are responsible for the tumor multiclonality with a diverse spectrum of unique gene or genomic alterations. Epigenetic mechanisms are essential for appropriate and functional regulation of gene expression. Dysregulation of epigenetic processes can lead to altered gene function and subsequent malignant transformation of cells. Several mechanisms are involved in epigenetic regulation such as DNA methylation, histone modifications, nucleosome positioning, non-coding RNA molecules, and expression of specific microRNAs [2].

In addition to numerous genetic alterations, human cancer cells also acquire epigenetic abnormalities. These genetic and epigenetic changes interact at all stages of cancer development. Moreover, recent studies suggest that epigenetic regulation may be essential for the origin

and development of some tumors. Epigenetic alterations, unlike genetic mutations, are potentially reversible and can be restored to a normal state by several mechanisms [3]. Epigenetic regulation becomes a promising diagnostic and therapeutic biomarker for emerging epigenetic therapies.

Personalized medicine, through a broad and early approach to biomarker testing, has the potential to improve cancer patient healthcare.

Personalized medicine

The term personalized medicine first appeared in 1999 in The Wall Street Journal in an article titled: '*New Era of Personalized Medicine: Targeting Drugs for Each Unique Genetic Profile.*' For more than 20 years, the opportunity of personalized medicine has arisen from technical innovations in genetics, proteomics, and other "omics" fields challenging the paradigm of evidence-based medicine and its concept of gold standards in diagnosis and treatment. In 2015 conclusions of personalized medicine, the Council of the European Union stated that although there is no broad consensus on the definition of the term 'personalized medicine', it is widely understood as a medical model using the characterization of phenotypes and genotypes to tailor the right diagnostic and therapeutic strategy for the right person at the right time [4].

Personalized medicine in clinical oncology refers to the application of the molecular characteristics of the tumor and its microenvironment together with other information about the patient's medical condition to design and provide customized medical therapy that is more effective and less toxic than conventional treatments [5].

Biomarker identification is considered as the most important aspect of personalized medicine in clinical oncology. The standard diagnosis of most types of solid tumors is by a combination of imaging methods followed by a biopsy to confirm the diagnosis. Different types of biomarkers are distinguished in clinical practice according to their predictive value. While prognostic markers indicate the possible development of the disease, pharmacological markers indicate the treatment efficacy. Predictive markers are used to estimate the patient's prognosis for the chosen treatment. Each of these biomarkers is relevant at different stages of the disease [6]. The ideal biomarker must meet certain criteria. First, the biomarker must be easily and repeatedly available in biological material and measurable by a non-invasive method. Another important requirement is its specificity and sensitivity to the pathology. Ideal biomarker should be also detectable before the first onset of clinical symptoms and its levels in biological material should display dynamics depending on the progression of the disease and/or the patient's response to treatment [7].

Extracellular vesicles

Extracellular vesicles (EVs) represent a highly heterogeneous group of phospholipid membrane-bound structures produced by body cells that are released into the extracellular space. They are important mediators in both local and systemic intercellular communication. Based on their biogenesis, extracellular vesicles are generally divided into apoptotic bodies (50-5000 nm), microvesicles (50-1000 nm) and exosomes (30-200 nm). Unlike microvesicles, which are generated by direct budding of the plasma membrane, exosomes are formed by invagination of the endosome membrane and are released by fusion of the multivesicular bodies and the plasma membrane. Compared to microvesicles, exosome biosynthesis is a highly specific and regulated process [8]. However, it is important to emphasize that particular subpopulations of EVs may be formed by different biogenesis pathways. The EVs size within the primary classification also varies widely, making their standardized classification very challenging. Establishing a comprehensive characterization of EVs is highly demanded. The International Society for Extracellular Vesicles (ISEV), which comprises nearly 2,000 researchers and scientists involved in the study of extracellularly secreted vesicles, make an effort in a field of EVs more accurate and specific nomenclature [9].

Exosomes are actively secreted by many cell types such as erythrocytes, dendritic cells, epithelial and endothelial cells, nerve cells, mesenchymal stem cells as well as tumor cells [10]. Exosomes are present in most biological fluids including blood, synovial fluid, pleural fluid, saliva, urine, bronchoalveolar lavage fluid, amniotic fluid, and breast milk [11].

Enriched with biologically active molecules such as cytoplasmic proteins, lipids, cellular metabolites as well as nucleic acids, including dsDNA and small non-coding RNAs (miRNAs, circRNAs, lncRNAs), exosomes deliver biological information in intra- and intercellular communication [12]. Exosomes released by cells inherit the content and function of parental cells. Transfer of

biomolecules from parental to recipient cells may contribute to many pathophysiological processes. Exosomes produced by cancer cells play a fundamental role in oncogenesis and cancer progression [13]. Exosome-mediated exchange of biological material and signals affects diverse tumor cell functions, promotes angiogenesis and tumor invasion, inhibits host anti-tumor responses, and mediates immune evasion and treatment resistance [14,15]. In tumor microenvironment, tumor as well as normal stromal cells may generate exosomes that modulate malignant behavior and tumor cell responses to stress conditions [16]. Moreover, tumor cells can promote an immunosuppressive environment *in situ* or create a pre-metastatic environment in the metastatic niches of distal organs through exosome release [17].

Tumor-derived exosomes contain cargo related to cell pathophysiological state and actual changes in signaling pathways. These properties make them potential biomarkers suitable for early cancer diagnosis, disease progression, detection of highly metastatic tumor cell activity, and monitoring patient treatment response contributing to the personalized medical approaches. Secretion of exosomes into the bloodstream reflects cellular activity, which is increased in cancer patients. Higher exosome amount corresponds to the activity of oncological process, which makes exosome more accessible and informative biomarker in diagnosis [18,19]. Not only the quantity of exosomes, but also their cargo content, especially proteins, miRNAs and lncRNAs, are important in cancer diagnosis.

Liquid biopsy is a minimally invasive approach to collect biological material, usually body fluids, to detect molecular changes of tumor cells and their metabolites. Liquid biopsy is currently gaining significant attention in clinical management, including diagnosis and disease monitoring, since it is much more accessible than tumor tissue samples and allows collecting of biological material repeatedly. This may contribute to earlier detection of disease and relapses, systemic dissemination and resistance to treatment. Therefore, liquid biopsy followed by molecular-biology analysis of the exosomal cargos reflecting tumor heterogeneity seems to be a promising approach in cancer medicine [20].

Recently, especially exosomal miRNAs are gaining attention in oncology since they influence tumor growth and are involved in various processes of tumorigenesis [21]. MiRNAs are small, 20-22 nucleotides long, highly conserved, non-coding RNA molecules that regulate gene expression at the transcriptional, post-transcriptional and post-translational levels. MiRNAs also work as secreted molecules that trigger a receptor-mediated response in a different cell or tissue through autocrine and paracrine signaling mechanisms. They can be released into the extracellular environment within exosomes that are present in many body fluids. In this way, their mode of action can be compared to hormones [22]. It is well-known that miRNAs regulate malignant cell transformation at all levels, and their aberrant expression in different tumor types can have oncogenic (oncomiRNAs) or suppressor (tumor-suppressor miRNAs) effects (Figure 1) [23]. The same miRNAs may have oncogenic potential in one cell type while being a tumor-suppressor in another [24]. In human cells, about 60 % of interactions between miRNAs and target mRNA sequences are non-canonical, meaning that their sequences are not always fully complementary. Therefore, a particular miRNA can regulate numerous mRNAs, and at the same time, a specific mRNA can contain multiple binding sites for different miRNAs [25]. Since dysregulated miRNA levels have been described in many malignancies and tumor-derived exosomes reflect the miRNA expression in original tumor cells, tumor-

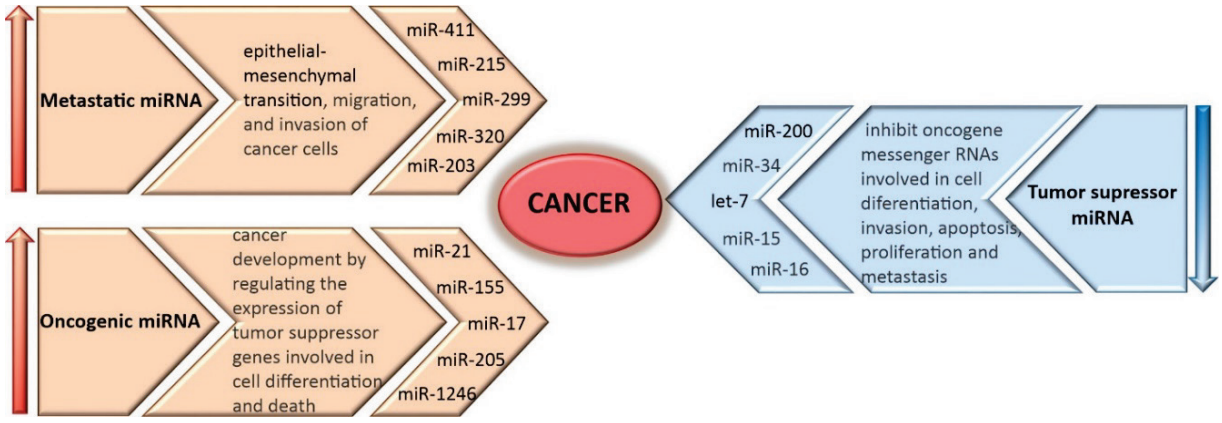

Fig. 1. Different functions of miRNAs in oncogenesis

associated exosomal miRNAs can be used as blood biomarkers suitable for personalized cancer diagnosis and treatment [26]. As a biomarker, exosomal miRNAs have other advantages, including relative stability and inaccessibility to RNases due to the protection of extracellular vesicles. Their analysis by qPCR methods is relatively inexpensive and rapid. For example, highly oncogenic miR-21 appears to be one of the potential biomarkers, as several studies have observed increased levels of exosomal miR-21 in various malignancies, including breast cancer (BC), colorectal cancer (CRC), and non-small cell lung cancer (NSCLC) [27]. These three malignancies accounted for up to 33 % of all newly diagnosed cancers worldwide according to the 2020 GLOBOCAN study. Slightly higher prevalence was observed in Slovakia (37 %) and Czech Republic (34 %) [28].

Breast cancer

Breast cancer (BC) is the most commonly occurring cancer in women and the most common cancer overall. There were more than 2.26 million new cases of breast cancer in women in 2020 [28]. Most breast cancers represent sporadic cases, only 10-15 % are caused by germline mutations in genes associated with hereditary forms of BC such as *BRCA1/2, CHEK2, PALB2, PTEN, STK11, TP53* etc. [29].

Breast cancer is a highly heterogeneous disease with specific clinical, histological and molecular features. According to activation of hormone estrogen (ER) and progesterone (PgR) receptors and human epidermal growth factor receptor 2 (HER2), breast cancer can be divided into subtypes: luminal type A-like (ER and PgR positive, HER2 negative tumors), luminal type B-like (ER and PgR positive, HER2 positive or negative tumors), HER2-positive (ER and PgR negative, HER2 positive tumors) and basal-like (triple-negative tumors) [30]. This classification is useful for selection of targeted therapies, as each BC subtype is characterized by a different ability to proliferate and form metastases [31]. Despite new insights into molecular subtypes and improvements in therapy of BC patients, metastatic breast cancer is one of the most common causes of cancer-related death [32]. Currently, miRNA analysis represents a potential biomarker in the diagnosis and clinical management of breast cancer patients.

A key role of miRNAs in the regulation and/or dysregulation of gene expression leading to the development of breast cancer have been demonstrated. The expression of specific genes can be dysregulated in both primary tumor and metastatic cells. The first studies describing potential involvement of miRNAs in the pathogenesis of BC were published in 2005 [33,34]. A study done by Cookson *et al.* [35] was among first investigating the association of specific miRNAs with BC development and progression, suggesting that dysregulated plasma levels of specific miRNAs may reflect the presence of a solid tumor. Therefore, the first efforts to use miRNAs were focused on early detection of the disease.

Three plasma exosomal miRNAs (miR-16, miR-30b and miR-93) are dysregulated in BC patients, especially miR-16 represented a reliable biomarker candidate for BC diagnosis [36]. In a comprehensive study, Li *et al.* identified two exosomal miRNA diagnostic panels to distinguish between BC patients and healthy controls. The first one comprises four plasma miRNAs (miR-106a-3p, miR-106a-5p, miR-20b-5p, and miR-92a-2-5p) and the second included four serum miRNAs (miR-106a-5p, miR-19b-3p, miR-20b-5p, and miR-92a-3p). The Receiver Operating Characteristic (ROC) correlation result, which is powerful statistical tool to evaluate the biomarker diagnostic accuracy to distinguish patients from healthy individuals, was 0.858 for the plasma panel and 0.949 for the serum panel. These results supports the use of both miRNA panels as a promising biomarker in BC early detection [37].

The clinical potential of miRNAs is not only in the early identification of breast cancer, but also in determination of different stages of the disease and their expression pattern can correlate with staging. In BC early stages patients, miR-425 [38], miR-182 [39], miR-223 [40], miR-155 [41], miR-1246 and miR-21 [42] were significantly dysregulated. In particular, oncogenic miR-21 and miR-155 have recently been of high interest in various applied studies targeting cancer patients. Among miRNAs with dysregulated expression in breast cancer, miR-155 and miR-21 showed significantly elevated levels in the plasma of BC patients, and meta-analyses confirmed a correlation between increased expression of these biomolecules and detection of early stages of disease [43-45].

MiR-21 is a highly oncogenic miRNA that functions as an anti-apoptotic factor promoting survival of multiple tumor cell types [46]. Consistent overexpression of miR-21 in tumor tissue is associated with metastatic progression in BC [47,48]. In a study by Yuan *et al.* the role of exosomal miR-21 in bone metastasis was confirmed. Significantly higher levels of

mir-21 were detected in the serum exosomes of BC patients with bone metastases compared to those with localized disease or with other sites of relapse [49]. MiR-155 is evolutionary conserved and closely associated with the development and progression of various solid tumors, such as carcinomas of breast and ovarian, colon or lung cancer [50–52]. Elevated levels of exosomal miR-155 are associated with metastasis and invasive features of BC. Since elevated levels of exosomal miR-21 and 155 have also been confirmed in various types of cancer, diagnostic potential to detect BC can be improved by their addition to the diagnostic panel. Serum exosomal miR-21, miR-155 and miR-222 were used to differentiate BC patients from healthy controls, and according to miR-21 level localized BC tumors from cases of distant metastasis could be distinguished. This study also found a positive correlation between miR-21, BC tumor size and disease stage [53]. Early detection of metastatic BC can lead to suitable treatment therapy and eventually to increased progression free survival of patients. Several more exosomal miRNAs have been identified that act as activators of metastasis in BC and show diagnostic value, including miR-148a [54] miR-411, miR-215, and miR-299–5p [55].

Criteria for clinical management of BC include tumor size, presence of distant metastases, as well as hormone ER, PgR and HER2 expression levels. Hormone receptor positivity and the estrogen-signalling pathway play important roles in BC development, progression, and therapeutic response [56]. The luminal subtypes, mainly dominated by hormone receptor positive tumors, are one of the most common and accounts for approximately 60 % of all BC tumors [57]. Rodríguez-Martínez *et al.* demonstrated higher miR-222 expression levels in luminal B-like compared to luminal A-like tumors. Moreover, PgR-negative patients showed a positive association with miR-222 expression [53]. Triple-negative tumors accounts for about 10-15 % of all breast cancers. However, this subtype tends to grow and spread faster, has fewer treatment options leading to a worse prognosis and patient survival rate [58]. The results of study by Eichelser *et al.* demonstrated higher expression levels of circulating exosomal miR-373 in Triple-negative subtype compared to Luminal-like cases. Higher expression of miR-373 was also observed in ER-/PgR-negative BC compared to hormonal positive tumors. In addition, overexpression of miR-373 inhibited apoptosis in cell models *in vitro*, highlighting the association of miR-373 with more aggressive BC phenotype [59]. The significantly higher levels of plasma exosomal miR-376c and miR-382 were shown in Triple-negative patients comparing to HER2-positive BC patients. In HER2-positive patients higher exosomal occurrence of miR-27b was observed [60].

MiRNAs have been identified as key regulators involved in the pathogenesis and progression of breast cancer. Their dysregulated plasma or serum levels are gaining increasing attention as biomarkers suitable for early detection of BC, also with regard to specific BC subtypes.

Non-small cell lung cancer

Lung cancer is the second most common cancer worldwide and the leading cause of cancer mortality, with 2.2 million new cases and 1.8 million deaths in 2020 [28]. Smoking is a major risk factor for lung carcinoma and is associated with approximately 80 % of all cases [61]. The development of lung cancer is a complex process influenced by environmental as well as genetic risk factors [62].

There are different types of primary lung cancer. Approximately 15 % of cases are classified as small cell lung cancer (SCLC) and 85 % are non-small cell lung cancer (NSCLC) [63]. NSCLC is further categorized into adenocarcinoma (ADC), squamous cell carcinoma (SCC) and large cell carcinoma (LCC), based on o the tumor origin and the type of cellular pathology observed [64]. NSCLC is typically diagnosed at advanced stages and the 5-year survival rate for patients is only 23.6 %. For patients who undergo radical resection at an early stage, the 5-year survival rate increases to 40-70 % [65]. Early detection of NSCLC significantly reduces the mortality of patients. However, the diversity and complexity of this disease requires patient-specific diagnostic and therapeutic methods. In this regard, particularly miRNAs have attracted increasing attention as its dysregulation and aberrant expression play a key role in NSCLC proliferation, invasion and metastasis [66,67].

Many studies have confirmed that exosomal miRNAs could be a useful diagnostic marker for early detection of NSCLC. Let-7 miRNA is one of the most well-known examples. The let-7 miRNA family is evolutionary conserved and often present in multiple copies in genomes. In humans, the let-7 family consists of ten members (let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-98, and miR-202) that are involved in the regulation of gene expression of many proto-oncogenes or oncogenes [68]. Let-7 target genes include c-Myc proto-oncogene [69], Signal Transducer and

Activator of Transcription 3 (STAT3) [70], Janus kinase 2 (JAK2) [71], or genes involved in the cell cycle [72]. By targeting RAS family, let-7 is involved in control of cell proliferation and immune response regulation through interleukin-6 and interleukin-10 expression[73]. Low level of the let-7 miRNA family is associated with metastasis, advanced stages of disease, and poor survival of NSCLC patients [74]. MiRNAs of this family were used as a part of a diagnostic panel consisting of miR-let-7b-5p, miR-let-7e-5p, miR-23a-3p and miR-486-5p suitable to differentiate stage I NSCLC patients from healthy individuals with 92.3 % specificity and 80.5 % sensitivity [75]. High diagnostic value was also observed with exosomal miR-5684 and miR-125b-5p, which showed significantly reduced levels in NSCLC patients compared to healthy controls. Moreover, miR-125b-5p was capable to distinguish between early and late stage disease, as well as lymph node metastasis and distant metastasis [76].

An example of upregulated miRNA in NSCLC is miR-17-5p with significantly increased levels in patients compared to healthy individuals [77]. MiR-17-5p is part of a polycistronic cluster miR-17-92 consisting of six miRNAs (miR-17-5p, miR-18a-5p, miR-19a-3p, miR-19b-1-5p, miR-20a-5p, and miR-92a-1-5p). This oncogenic cluster located within the third intron of C13orf25 gene on chromosome 13q31.3. is frequently overexpressed in NSCLC [78]. The upregulated miR-17-92 cluster negatively regulates E2F and Myc family of transcription factors [78], Hypoxia-inducible factor 1-alpha (HIF-1α) [79] and Phosphatase and Tensin Homolog (PTEN) [80], which increase malignant potential of the disease. MiR-17-5p is the most important member of the miR-17-92 cluster as a key regulator of cellular processes such as proliferation, cell cycle, apoptosis and autophagy. Elevated levels of oncogenic miR-17-5p are associated with worse survival of lung cancer patients [81]. This knowledge led to the development of an diagnostic panel monitoring the expression level of miR-17-5p and three other protein biomarkers, carcinoembryonic antigen (CEA), water-soluble cytokeratin 19 fragment (CYFRA 21-1), and Squamous Cell Cancer Antigen (SCCA). According to gene expression panel profiling, NSCLC patients could be distinguished from healthy controls with both specificity and sensitivity of 75 %. These results suggest that the combination of exosomal miRNA and conventional tumor markers have clinical potential in NSCLC diagnosis [77]. Lai and Friedman [82] developed a mathematical model that identified the three most highly expressed exosomal miRNAs (miR-21, miR-205, and miR-155) suitable for early NSCLC detection. Oncogenic function of miR-21 and miR-155 has also been proved in lung cancer. High levels of miR-21 and miR-155 are associated with advanced clinical stages and metastasis as they promote tumor cell growth and invasion by inhibition of PTEN tumor suppressor [50].

Studies focusing on differential diagnostics of lung cancer confirmed different miRNA expression profile between SCLC and NSCLC. MiR-203 was used to differentiate between SCLC and NSCLC patients with 100 % specificity and 80 % sensitivity [83].

Poroyko *et al.* identified a panel of thirteen exosomal miRNAs that could correctly distinguish between patients with SCLC and NSCLC. Of these, three miRNAs (miR-331-5p, miR-451a, miR-363-3p) were able to discriminate SCLC and NSCLC cases with 100 % specificity and 100 % sensitivity, highlighting the potential of miRNAs as a valuable biomarkers suitable for differential diagnosis of lung cancer [84].

In addition, miRNAs have also been applied as biomarkers to distinguish the NSCLC subtypes. Four miRNAs (miR-181-5p, miR-30a-3p, miR-30e-3p, and miR-361-5p) specific for ADC patients and three miRNAs (miR-10b-5p, miR-15b-5p, and miR-320b) associated with SCC was identified from plasma exosomes using miRNA-sequencing [75]. Zhang *et al.* [85] identified elevated levels of miR-205, miR-93, miR-221 and miR-30e in SCC cases, while ADC showed high expression of miR-29b, miR-29c, let-7, miR-100 and miR-125a-5p. Moreover, a panel of exosomal miRNAs (miR-19b-3p, miR-21-5p, miR-221-3p, miR-409-3p, miR-425-5p, and miR-584-5p) was used to discriminate ADC patients and healthy controls [86]. Exosomal miR-4448 showed diagnostic potential for metastatic ADC identification, since its lower levels was observed in this group of patients [87].

Many studies published in recent years has clarified the key role of miRNAs in process of tumorigenesis and highlight their clinical potential as biomarkers useful in the early NSCLC detection NSCLC.

Colorectal cancer

Colorectal cancer (CRC) is the third most common cancer worldwide, with 1.1 million new cases per year, and is the second leading cause of cancer death [28]. CRC occurs more frequently in middle- to high-income countries with an eight-fold variation in incidence across the world. This rise may be associated with known

risk factors, including alcohol intake, tobacco use, obesity, sedentariness and dietary patterns (diets low in fruits, vegetables and unrefined plant food, and high in red meat, processed foods and fat) [88]. The absence of early CRC screening results in localized or distant metastases, which is the main cause of death. Approximately 15-30 % of patients present with metastases, and 20-50 % of patients with initially localized disease will develop metastases. The most common location of metastases is liver, then lung, peritoneum and distant lymph nodes. The 5-year survival rate of patients diagnosed at disease stage I and II without metastases is approximately 90 % [89].

Early diagnosis of colorectal cancer is necessary to reduce patient mortality. Therefore, the identification of new non-invasive, specific and sensitive biomarkers suitable for detection of CRC at early stages is required. Exosomal miRNAs obtained by liquid biopsy seem to be potentially valuable clinical biomarkers.

MiRNAs have important functional roles in various biological processes associated with CRC carcinogenesis. For example, miR-494 [90], miR-598 [91], and miR-17-3p [92] are involved in CRC tumorigenesis. Overexpression of miR-1246 contributes to tumor cells differentiation and invasion by suppressing the production of cyclin G2, which is involved in cell cycle control [93]. Matsumura *et al.* [94] observed 2.23-fold increased levels of serum exosomal miR-1246 in 209 CRC patients, of whom 107 were at disease stages I and II, compared to healthy controls. Overexpression of miR-1246 was also observed in stage II CRC patients compared to healthy individuals [95], supporting the diagnostic application of this miRNA.

MiR-150-5p, miR-195-5p and miR-203 are involved in gene dysregulation of nuclear factor-kappa B (NF-κB) signaling pathway, a regulator of immune response and inflammation that plays an important role in CRC carcinogenesis [96]. Exosomal oncomiR-21-5p (miR-21) also contributes to promotion of pro-inflammatory environment by regulating Toll-Like Receptor Signaling Pathway 7 (TLR7), which polarizes macrophages leading to the synthesis and release of pro-inflammatory cytokines such as interleukin-6. This process creates a pre-metastatic niche in the liver where circulating CRC cells can survive and subsequently develop metastases [97]. Increased exosomal miR-21 expression was also reported in the serum of patients suffering from colonic adenoma. Differentiation of patients from healthy controls reached 73.1 % sensitivity and 68.1 % specificity. These data indicates that miR-21 could be elevated in serum exosomes from early development of adenoma and remains elevated in late CRC stages, suggesting the diagnostic potential of miR-21 [98]. Mir-21 overexpression have been demonstrated in various cancer types, which may be a barrier to its application as a population-screening tool due to lack of specificity for CRC. However, such markers can still be used for CRC screening in combination with already established methods. An example is oncogenic miR-150-5p with lower serum level observed in CRC patients. Diagnostic accuracy of miR-150-5p was improved by combined detection with protein marker CEA [99].

Another possibility to enhance diagnostic accuracy is detection of several CRC related miRNA that are part of a diagnostic panel. Combining already mentioned oncogenic miR-1246 and miR-21 with five other miRNAs (let-7a, miR-1229, miR-150, miR-223 and miR-23a), Ogata-Kawata *et al.* created a panel that was effective in discriminating patients at different CRC stages from healthy controls. High sensitivity and specificity of this panel was confirmed by ROC analysis supporting clinical diagnostic potential. In addition, serum exosomal levels of these miRNAs were significantly reduced after surgical resection of tumor [100]. MiRNA diagnostic panel consisting of Let7, miR-16 and miR-23 also significantly differed between CRC and healthy controls [101]. Min *et al.* identified miRNA-139-3p, let-7b-3p, and miRNA-145-3p in plasma exosomes with increased expression in early-stage CRC patients compared to healthy controls. High reliability based on score 0.927 was shown by ROC test [102]. Yan *et al.* [103] observed upregulation of miR-486 and downregulation of miR-548c when comparing exosomal serum miRNA levels in 77 CRC patients, of whom 26 were at CRC stage I and II, with healthy controls. Increased miR-486 levels in CRC patients were also confirmed in a study by Liu *et al.* [104]. In concordance with data by Yan *et al.* [103] decreased levels of miR-548c were reported by Peng *et al.* [105] in 108 CRC patients. These independent studies confirm the diagnostic potential of miR-486 and miR-548c.

Metastasis formation represents a key process in CRC tumorigenesis. In colorectal cancer, miRNAs are involved in multiple cellular processes related to metastasis, including epithelial-mesenchymal transition [106], angiogenesis [107], and interactions with the tumor microenvironment [108]. The spread of CRC tumor cells with subsequent metastasis formation in liver is a major cause of disease progression and death in patients [109]. In particular, miR-320b was significantly upregulated in

CRC patients with liver metastasis leading to increased expression of metastasis-promoting genes. Serum miR-320b levels in stage IV CRC patients were elevated compared to stage I and II patients [110]. In a study focused on different expression pattern of exosomal miRNAs in CRC patients with liver metastases, Tang *et al.* observed elevated levels of miR-320d. Discriminatory power of miR-320d to differentiate patients with metastases from those at stage I and II, reached 62 % sensitivity and nearly 65 % specificity. Combination of miR-320d with CEA led to an increase in diagnostic efficiency with a sensitivity of 63 % and specificity of 91 %, suggesting that miR-320d could be a suitable biomarker for metastatic CRC in the future [111]. Takano *et al.* [112] analyzed by qRT-PCR expression of exosomal miR-203 in serum of 240 CRC patients. They observed that increased expression of miR-203 correlated with disease stage. Its elevated levels were associated with pathological tumor progression, including lymph node metastasis, venous invasion, and distant metastasis. Serum levels of exosomal miR-6803-5p were also significantly elevated in stage II and III patients with liver and lymph node metastases. As in previous studies, high miR-6803-5p expression levels were associated with disease progression and worsen patients overall survival rate [113].

Based on increasing knowledge, exosomal miRNAs are considered as novel biomarkers with clinical potential for accurate identification of early-stage colorectal cancer as well as possibility of disease staging, which may lead to better management of oncological treatment.

Conclusion

Exosomes with abundant miRNAs have become the topic of tumor research in recent years. Exosomal miRNAs produced by tumor cells play a key role in tumorigenesis and cancer progression and their clinical potential lies particularly in early cancer diagnosis. Non-invasive liquid biopsy together with stable and real-time biological properties of circulating miRNAs are the key factors enabling not only diagnosis but also cancer staging and patient prognosis prediction. However, extensive miRNA research led to identification of almost 40000 records in miRBASE (v22.1) database, so for clinical usage is necessary its further characterization and quantification. As seen in this review, exosomal miRNAs have great potential in the field of personalized cancer medicine including diagnostic and prognostic clinical evaluation.

Conflict of Interest

There is no conflict of interest.

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References

1. Cancer Burden Statistics and Trends Across Europe. 2020. p. ECIS. https://ecis.jrc.ec.europa.eu/
2. Cao J, Yan Q. Cancer epigenetics, tumor immunity, and immunotherapy. Trends in Cancer 2020;6:580-592. https://doi.org/10.1016/j.trecan.2020.02.003
3. Takeshima H, Ushijima T. Accumulation of genetic and epigenetic alterations in normal cells and cancer risk. Precis Oncol 2019;3:1-8. https://doi.org/10.1038/s41698-019-0079-0
4. Eu Commision. Council conclusions on personalised medicine for patients. Off J Eur Union 2015;58:1-32.
5. Gambardella V, Tarazona N, Cejalvo JM, Lombardi P, Huerta M, Roselló S, Fleitas T, Roda D, Cervantes A. Personalized medicine: recent progress in cancer therapy. cancers (Basel) 2020;12:1009. https://doi.org/10.3390/cancers12041009
6. Awad K, Dalby M, Cree I, Challoner B, Ghosh S, Thurston D. The precision medicine approach to cancer therapy: part 1-solid tumours. Pharm J 2019;303. https://doi.org/10.1211/PJ.2019.20207119
7. Goossens N, Nakagawa S, Sun X, Hoshida Y. Cancer biomarker discovery and validation. Transl Cancer Res 2015;4:256. https://doi.org/10.3978/j.issn.2218-676X.2015.06.04
8. Jadli AS, Ballasy N, Edalat P, Patel VB. Inside(sight) of tiny communicator: exosome biogenesis, secretion, and uptake. Mol Cell Biochem 2020;467:77-94. https://doi.org/10.1007/s11010-020-03703-z

9. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, Antoniou A, Arab T, Archer F, Atkin-Smith GK, Ayre DC, Bach JM, Bachurski D, Baharvand H, Balaj L, Baldacchino S, Bauer NN, Baxter AA, Bebawy M, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles 2018;7:1535750. https://doi.org/10.1080/20013078.2018.1535750
10. Farooqi AA, Desai NN, Qureshi MZ, Librelotto DRN, Gasparri ML, Bishayee A, Nabavi SM, Curti V, Daglia M. Exosome biogenesis, bioactivities and functions as new delivery systems of natural compounds. Biotechnol Adv 2018;36:328-34. https://doi.org/10.1016/j.biotechadv.2017.12.010
11. Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, Galas DJ, Wang K. The MicroRNA Spectrum in 12 Body Fluids. Clin Chem 2010;56:1733-1741. https://doi.org/10.1373/clinchem.2010.147405
12. Mathieu M, Martin-Jaular L, Lavieu G, Théry C. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. Nat Cell Biol 2019;21:9-17. https://doi.org/10.1038/s41556-018-0250-9
13. Stefanius K, Servage K, Orth K. Exosomes in cancer development. Curr Opin Genet Dev 2021;66:83-92. https://doi.org/10.1016/j.gde.2020.12.018
14. Zhang L, Yu D. Exosomes in cancer development, metastasis, and immunity. Biochim Biophys Acta - Rev Cancer 2019;1871:455-68. https://doi.org/10.1016/j.bbcan.2019.04.004
15. Paskeh MDA, Entezari M, Mirzaei S, Zabolian A, Saleki H, Naghdi MJ, Sabet S, Khoshbakht MA, Hashemi M, Hushmandi K, Sethi G, Zarrabi A, Kumar AP, Tan SC, Papadakis M, Alexiou A, Islam MA, Mostafavi E, Ashrafizadeh M. Emerging role of exosomes in cancer progression and tumor microenvironment remodeling. J Hematol Oncol 2022;15:1-39. https://doi.org/10.1186/s13045-022-01305-4
16. Vallabhaneni KC, Hassler MY, Abraham A, Whitt J, Mo YY, Atfi A, Pochampally R. Mesenchymal stem/stromal cells under stress increase osteosarcoma migration and apoptosis resistance via extracellular vesicle mediated communication. PLoS One 2016;11:e0166027. https://doi.org/10.1371/journal.pone.0166027
17. Guo J, Duan Z, Zhang C, Wang W, He H, Liu Y, Wu P, Wang S, Song M, Chen H, Chen C, Si Q, Xiang R, Luo Y. Mouse 4T1 breast cancer cell-derived exosomes induce proinflammatory cytokine production in macrophages via miR-183. J Immunol 2020;205:2916-2925. https://doi.org/10.4049/jimmunol.1901104
18. Caivano A, Laurenzana I, De Luca L, La Rocca F, Simeon V, Trino S, D'Auria F, Traficante A, Maietti M, Izzo T, D'Arena G, Mansueto G, Pietrantuono G, Laurenti L, Musto P, Del Vecchio L. High serum levels of extracellular vesicles expressing malignancy-related markers are released in patients with various types of hematological neoplastic disorders. Tumor Biol 2015;36:9739-9752. https://doi.org/10.1007/s13277-015-3741-3
19. Yamamoto CM, Oakes ML, Murakami T, Muto MG, Berkowitz RS, Ng SW. Comparison of benign peritoneal fluid- and ovarian cancer ascites-derived extracellular vesicle RNA biomarkers. J Ovarian Res 2018;11:1-9. https://doi.org/10.1186/s13048-018-0391-2
20. Preethi KA, Selvakumar SC, Ross K, Jayaraman S, Tusubira D, Sekar D. Liquid biopsy: Exosomal microRNAs as novel diagnostic and prognostic biomarkers in cancer. Mol Cancer 2022;21:1-15. https://doi.org/10.1186/s12943-022-01525-9
21. Sun Z, Shi K, Yang S, Liu J, Zhou Q, Wang G, Song J, Li Z, Zhang Z, Yuan W. Effect of exosomal miRNA on cancer biology and clinical applications. Mol Cancer 2018;17:1-19. https://doi.org/10.1186/s12943-018-0897-7
22. Bayraktar R, Roosbroeck K Van, Calin GA. Cell-to-cell communication : microRNAs as hormones. Mol Oncol 2017;11:1673-1686. https://doi.org/10.1002/1878-0261.12144
23. Iorio MV, Croce CM. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. EMBO Mol Med 2012;4:143-59. https://doi.org/10.1002/emmm.201100209
24. Giza DE, Vasilescu C, Calin GA. Key principles of miRNA involvement in human diseases. Discoveries 2014;2:e34. https://doi.org/10.15190/d.2014.26
25. Cortez MA, Bueso-ramos C, Ferdin J, Lopez-berestein G, Anil K, Calin GA. MicroRNAs in body fluids-the mix of hormones and biomarkers. Nat Rev Clin Oncol 2011;8:467-477. https://doi.org/10.1038/nrclinonc.2011.76
26. Wang X, Tian L, Lu J, Ng IOL. Exosomes and cancer - Diagnostic and prognostic biomarkers and therapeutic vehicle. Oncogenesis 2022;11:1-12. https://doi.org/10.1038/s41389-022-00431-5

27. Bautista-Sánchez D, Arriaga-Canon C, Pedroza-Torres A, De La Rosa-Velázquez IA, González-Barrios R, Contreras-Espinosa L, Montiel-Manríquez R, Castro-Hernández C, Fragoso-Ontiveros V, Álvarez-Gómez RM, Herrera LA. The promising role of miR-21 as a cancer biomarker and its importance in RNA-based therapeutics. Mol Ther - Nucleic Acids 2020;20:409-420. https://doi.org/10.1016/j.omtn.2020.03.003
28. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-749. https://doi.org/10.3322/caac.21660
29. De Silva S, Tennekoon KH, Karunanayake EH. Overview of the genetic basis toward early detection of breast cancer. Breast Cancer Targets Ther 2019;11:71-80. https://doi.org/10.2147/BCTT.S185870
30. Dai X, Li T, Bai Z, Yang Y, Liu X, Zhan J, Shi B. Breast cancer intrinsic subtype classification, clinical use and future trends. Am J Cancer Res 2015;5:2929.
31. Wu Q, Li J, Zhu S, Wu J, Chen C, Liu Q, Wei W, Zhang Y, Sun S. Breast cancer subtypes predict the preferential site of distant metastases: a SEER based study. Oncotarget 2017;8:27990. https://doi.org/10.18632/oncotarget.15856
32. Gennari A, André F, Barrios CH, Cortés J, de Azambuja E, DeMichele A, Dent R, Fenlon D, Gligorov J, Hurvitz SA, Im SA, Krug D, Kunz WG, Loi S, Penault-Llorca F, Ricke J, Robson M, Rugo HS, Saura C, et al. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer ☆. Ann Oncol 2021;32:1475-95. https://doi.org/10.1016/j.annonc.2021.09.019
33. Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Ménard S, Palazzo JP, Rosenberg A, Musiani P, Volinia S, Nenci I, Calin GA, Querzoli P, Negrini M, et al. MicroRNA gene expression deregulation in human breast cancer. Cancer Res 2005;65:7065-7070. https://doi.org/10.1158/0008-5472.CAN-05-1783
34. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. Nature 2005;435:834-838. https://doi.org/10.1038/nature03702
35. Cookson VJ, Bentley MA, Hogan B V., Horgan K, Hayward BE, Hazelwood LD, Hughes TA. Circulating microRNA profiles reflect the presence of breast tumours but not the profiles of microRNAs within the tumours. Cell Oncol 2012;35:301-308. https://doi.org/10.1007/s13402-012-0089-1
36. Ni Q, Stevic I, Pan C, Müller V, Oliviera-Ferrer L, Pantel K, Schwarzenbach H. Different signatures of miR-16, miR-30b and miR-93 in exosomes from breast cancer and DCIS patients. Sci Rep 2018;8:1-10. https://doi.org/10.1038/s41598-018-31108-y
37. Li M, Zhou Y, Xia T, Zhou X, Huang Z, Zhang H, Zhu W, Ding Q, Wang S. Circulating microRNAs from the miR-106a-363 cluster on chromosome X as novel diagnostic biomarkers for breast cancer. Breast Cancer Res Treat 2018;170:257-270. https://doi.org/10.1007/s10549-018-4757-3
38. Zhu Y, Dou H, Liu Y, Yu P, Li F, Wang Y, Xiao M. Breast Cancer Exosome-Derived miR-425-5p Induces cancer-associated fibroblast-like properties in human mammary fibroblasts by TGF β 1/ROS signaling pathway. Oxid Med Cell Longev 2022;2022. https://doi.org/10.1155/2022/5266627
39. Mihelich BL, Dambal S, Lin S, Nonn L. miR-182, of the miR-183 cluster family, is packaged in exosomes and is detected in human exosomes from serum, breast cells and prostate cells. Oncol Lett 2016;12:1197-203. https://doi.org/10.3892/ol.2016.4710
40. Yoshikawa M, Iinuma H, Umemoto Y, Yanagisawa T, Matsumoto A, Jinno H. Exosome-encapsulated microRNA-223-3p as a minimally invasive biomarker for the early detection of invasive breast cancer. Oncol Lett 2018;15:9584-9592. https://doi.org/10.3892/ol.2018.8457
41. Sun Y, Wang M, Lin G, Sun S, Li X, Qi J, Li J. Serum MicroRNA-155 as a potential biomarker to track disease in breast cancer. PLoS One 2012;7:e47003. https://doi.org/10.1371/journal.pone.0047003
42. Hannafon BN, Trigoso YD, Calloway CL, Zhao YD, Lum DH, Welm AL, Zhao ZJ, Blick KE, Dooley WC, Ding WQ. Plasma exosome microRNAs are indicative of breast cancer. Breast Cancer Res 2016;18:1-14. https://doi.org/10.1186/s13058-016-0753-x

43. Li S, Yang X, Yang J, Zhen J, Zhang D. Serum microRNA-21 as a potential diagnostic biomarker for breast cancer: a systematic review and meta-analysis. Clin Exp Med 2016;16:29-35. https://doi.org/10.1007/s10238-014-0332-3
44. Markou A, Zavridou M, Sourvinou I, Yousef G, Kounelis S, Malamos N, Georgoulias V, Lianidou E. Direct Comparison of metastasis-related miRNAs expression levels in circulating tumor cells, corresponding plasma, and primary tumors of breast cancer patients. Clin Chem 2016;62:1002-1011. https://doi.org/10.1373/clinchem.2015.253716
45. Nguyen THN, Nguyen TTN, Nguyen TTM, Nguyen LHM, Huynh LH, Phan HN, Nguyen HT. Panels of circulating microRNAs as potential diagnostic biomarkers for breast cancer: a systematic review and meta-analysis. Breast Cancer Res Treat 2022;196:1-15. https://doi.org/10.1007/s10549-022-06728-8
46. Feng YH, Tsao CJ. Emerging role of microRNA-21 in cancer (Review). Biomed Reports 2016;5:395-402. https://doi.org/10.3892/br.2016.747
47. Wang H, Tan Z, Hu H, Liu H, Wu T, Zheng C, Wang X, Luo Z, Wang J, Liu S, Lu Z, Tu J. MicroRNA-21 promotes breast cancer proliferation and metastasis by targeting LZTFL1. BMC Cancer 2019;19:1-13. https://doi.org/10.1186/s12885-019-5951-3
48. Yan LX, Huang XF, Shao Q, Huang MY, Deng L, Wu QL, Zeng YX, Shao JY. MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. RNA 2008;14:2348-2360. https://doi.org/10.1261/rna.1034808
49. Yuan X, Qian N, Ling S, Li Y, Sun W, Li J, Du R, Zhong G, Liu C, Yu G, Cao D, Liu Z, Wang Y, Qi Z, Yao Y, Wang F, Liu J, Hao S, Jin X, et al. Breast cancer exosomes contribute to pre-metastatic niche formation and promote bone metastasis of tumor cells. Theranostics 2021;11:1429. https://doi.org/10.7150/thno.45351
50. Xue X, Liu Y, Wang Y, Meng M, Wang K, Zang X, Zhao S, Sun X, Cui L, Pan L, Liu S. MiR-21 and MiR-155 promote non-small cell lung cancer progression by downregulating SOCS1, SOCS6, and PTEN. Oncotarget 2016;7:84508. https://doi.org/10.18632/oncotarget.13022
51. Pfeffer SR, Yang CH, Pfeffer LM. The Role of miR-21 in Cancer. Drug Dev Res 2015;76:270-277. https://doi.org/10.1002/ddr.21257
52. Kong W, He L, Coppola M, Guo J, Esposito NN, Coppola D, Cheng JQ. MicroRNA-155 regulates cell survival, growth, and chemosensitivity by targeting FOXO3a in breast cancer. J Biol Chem 2010;285:17869-17879. https://doi.org/10.1074/jbc.M110.101055
53. Rodríguez-Martínez A, De Miguel-Pérez D, Ortega FG, García-Puche JL, Robles-Fernández I, Exposito J, Martorell-Marugan J, Carmona-Sáez P, Garrido-Navas MDC, Rolfo C, Ilyine H, Lorente JA, Legueren M, Serrano MJ. Exosomal miRNA profile as complementary tool in the diagnostic and prediction of treatment response in localized breast cancer under neoadjuvant chemotherapy. Breast Cancer Res 2019;21:1-9. https://doi.org/10.1186/s13058-019-1109-0
54. Zhu Y, Dou H, Liu Y, Yu P, Li F, Wang Y, Xiao M. Identification of serum exosomal miR-148a as a novel prognostic biomarker for breast cancer. Eur Rev Med Pharmacol Sci 2020;24:7303-7309.
55. van Schooneveld E, Wouters MCA, Van der Auwera I, Peeters DJ, Wildiers H, Van Dam PA, Vergote I, Vermeulen PB, Dirix LY, Van Laere SJ. Expression profiling of cancerous and normal breast tissues identifies microRNAs that are differentially expressed in serum from patients with (metastatic) breast cancer and healthy volunteers. Breast Cancer Res 2012;14:1-16. https://doi.org/10.1186/bcr3127
56. Zelli V, Compagnoni C, Capelli R, Cannita K, Sidoni T, Ficorella C, Capalbo C, Zazzeroni F, Tessitore A, Alesse E. Circulating micrornas as prognostic and therapeutic biomarkers in breast cancer molecular subtypes. J Pers Med 2020;10:1-18. https://doi.org/10.3390/jpm10030098
57. Sisti JS, Collins LC, Beck AH, Tamimi RM, Rosner BA, Eliassen AH. Reproductive risk factors in relation to molecular subtypes of breast cancer: Results from the nurses' health studies. Int J Cancer 2016;138:2346-2356. https://doi.org/10.1002/ijc.29968
58. Howard FM, Olopade OI. Epidemiology of triple-negative breast cancer: a review. Cancer J (United States) 2021;27:8-16. https://doi.org/10.1097/PPO.0000000000000500
59. Eichelser C, Stückrath I, Müller V, Milde-Langosch K, Wikman H, Pantel K, Schwarzenbach H, Eichelser C, Stückrath I, Müller V, Milde-Langosch K, Wikman H, Pantel K, Schwarzenbach H. Increased serum levels of

circulating exosomal microRNA-373 in receptor-negative breast cancer patients. Oncotarget 2014;5:9650-9663. https://doi.org/10.18632/oncotarget.2520

60. Stevic I, Müller V, Weber K, Fasching PA, Karn T, Marmé F, Schem C, Stickeler E, Denkert C, Van Mackelenbergh M, Salat C, Schneeweiss A, Pantel K, Loibl S, Untch M, Schwarzenbach H. Specific microRNA signatures in exosomes of triple-negative and HER2-positive breast cancer patients undergoing neoadjuvant therapy within the GeparSixto trial. BMC Med 2018;16:1-16. https://doi.org/10.1186/s12916-018-1163-y
61. Shankar A, Dubey A, Saini D, Singh M, Prasad CP, Roy S, Bharati SJ, Rinki M, Singh N, Seth T, Khanna M, Sethi N, Kumar S, Sirohi B, Mohan A, Guleria R, Rath GK. Environmental and occupational determinants of lung cancer. Transl Lung Cancer Res 2019;8:S31. https://doi.org/10.21037/tlcr.2019.03.05
62. Corrales L, Rosell R, Cardona AF, Martín C, Zatarain-Barrón ZL, Arrieta O. Lung cancer in never smokers: The role of different risk factors other than tobacco smoking. Crit Rev Oncol Hematol 2020;148:102895. https://doi.org/10.1016/j.critrevonc.2020.102895
63. Osmani L, Askin F, Gabrielson E, Li QK. Current WHO guidelines and the critical role of immunohistochemical markers in the subclassification of non-small cell lung carcinoma (NSCLC): Moving from targeted therapy to immunotherapy. Semin Cancer Biol 2018;52:103-109. https://doi.org/10.1016/j.semcancer.2017.11.019
64. Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. Nature 2018;553:446-454. https://doi.org/10.1038/nature25183
65. Naylor EC. Adjuvant Therapy for Stage I and II Non-Small Cell Lung Cancer. Surg Oncol Clin N Am 2016;25:585-99. https://doi.org/10.1016/j.soc.2016.03.003
66. Chen P, Li Y, Liu R, Xie Y, Jin Y, Wang M, Yu Z, Wang W, Luo X. Non-small cell lung cancer-derived exosomes promote proliferation, phagocytosis, and secretion of microglia via exosomal microRNA in the metastatic microenvironment. Transl Oncol 2023;27:101594. https://doi.org/10.1016/j.tranon.2022.101594
67. Liang G, Meng W, Huang X, Zhu W, Yin C, Wang C, Fassan M, Yu Y, Kudo M, Xiao S, Zhao C, Zou P, Wang Y, Li X, Croce CM, Cui R. MiR-196b-5p-mediated downregulation of TSPAN12 and GATA6 promotes tumor progression in non-small cell lung cancer. Proc Natl Acad Sci U S A 2020;117:4347-4357. https://doi.org/10.1073/pnas.1917531117
68. Roush S, Slack FJ. The let-7 family of microRNAs. Trends Cell Biol 2008;18:505-516. https://doi.org/10.1016/j.tcb.2008.07.007
69. He XY, Chen JX, Zhang Z, Li CL, Peng Q Le, Peng HM. The let-7a microRNA protects from growth of lung carcinoma by suppression of k-Ras and c-Myc in nude mice. J Cancer Res Clin Oncol 2010;136:1023-1028. https://doi.org/10.1007/s00432-009-0747-5
70. Guo L, Chen C, Shi M, Wang F, Chen X, Diao D, Hu M, Yu M, Qian L, Guo N. Stat3-coordinated Lin-28-let-7-HMGA2 and miR-200-ZEB1 circuits initiate and maintain oncostatin M-driven epithelial-mesenchymal transition. Oncogene 2013;32:5272-5282. https://doi.org/10.1038/onc.2012.573
71. Yeh CT, Huang WC, Rao YK, Ye M, Lee WH, Wang LS, Tzeng DTW, Wu CH, Shieh YS, Huang CYF, Chen YJ, Hsiao M, Wu ATH, Yang Z, Tzeng YM. A sesquiterpene lactone antrocin from Antrodia camphorata negatively modulates JAK2/STAT3 signaling via microRNA let-7c and induces apoptosis in lung cancer cells. Carcinogenesis 2013;34:2918-2928. https://doi.org/10.1093/carcin/bgt255
72. Johnson CD, Esquela-Kerscher A, Stefani G, Byrom M, Kelnar K, Ovcharenko D, Wilson M, Wang X, Shelton J, Shingara J, Chin L, Brown D, Slack FJ. The let-7 MicroRNA Represses Cell Proliferation Pathways in Human Cells. Cancer Res 2007;67:7713-7722. https://doi.org/10.1158/0008-5472.CAN-07-1083
73. Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ. RAS Is Regulated by the let-7 MicroRNA Family. Cell 2005;120:635-647. https://doi.org/10.1016/j.cell.2005.01.014
74. Zhao B, Han H, Chen J, Zhang Z, Li S, Fang F, Zheng Q, Ma Y, Zhang J, Wu N, Yang Y. MicroRNA let-7c inhibits migration and invasion of human non-small cell lung cancer by targeting ITGB3 and MAP4K3. Cancer Lett 2014;342:43-51. https://doi.org/10.1016/j.canlet.2013.08.030

75. Jin X, Chen Y, Chen H, Fei S, Chen D, Cai X, Liu L, Lin B, Su H, Zhao L, Su M, Pan H, Shen L, Xie D, Xie C. Evaluation of tumor-derived exosomal miRNA as potential diagnostic biomarkers for early-stage non-small cell lung cancer using next-generation sequencing. Clin Cancer Res 2017;23:5311-5319. https://doi.org/10.1158/1078-0432.CCR-17-0577
76. Zhang Z, Tang Y, Song X, Xie L, Zhao S, Song X. Tumor-derived exosomal miRNAs as diagnostic biomarkers in non-small cell lung cancer. Front Oncol 2020;10:2236. https://doi.org/10.3389/fonc.2020.560025
77. Zhang Y, Zhang Y, Yin Y, Li S. Detection of circulating exosomal miR-17-5p serves as a novel non-invasive diagnostic marker for non-small cell lung cancer patients. Pathol - Res Pract 2019;215:152466. https://doi.org/10.1016/j.prp.2019.152466
78. Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, Yatabe Y, Kawahara K, Sekido Y, Takahashi T. A Polycistronic MicroRNA Cluster, miR-17-92, Is Overexpressed in Human Lung Cancers and Enhances Cell Proliferation. Cancer Res 2005;65:9628-32. https://doi.org/10.1158/0008-5472.CAN-05-2352
79. Taguchi A, Yanagisawa K, Tanaka M, Cao K, Matsuyama Y, Goto H, Takahashi T. Identification of Hypoxia-Inducible Factor-1α as a Novel Target for miR-17-92 MicroRNA Cluster. Cancer Res 2008;68:5540-5. https://doi.org/10.1158/0008-5472.CAN-07-6460
80. Grillari J, Hackl M, Grillari-Voglauer R. miR-17-92 cluster: Ups and downs in cancer and aging. Biogerontology 2010;11:501-506. https://doi.org/10.1007/s10522-010-9272-9
81. Chen Q, Si Q, Xiao S, Xie Q, Lin J, Wang C, Chen L, Chen Q, Wang L. Prognostic significance of serum miR-17-5p in lung cancer. Med Oncol 2013;30:1-6. https://doi.org/10.1007/s12032-012-0353-2
82. Lai X, Friedman A. Exosomal miRs in lung cancer: a mathematical model. PLoS One 2016;11:e0167706. https://doi.org/10.1371/journal.pone.0167706
83. Rabinowits G, Gerçel-Taylor C, Day JM, Taylor DD, Kloecker GH. Exosomal MicroRNA: A Diagnostic Marker for Lung Cancer. Clin Lung Cancer 2009;10:42-46. https://doi.org/10.3816/CLC.2009.n.006
84. Poroyko V, Mirzapoiazova T, Nam A, Mambetsariev I, Mambetsariev B, Wu X, Husain A, Vokes EE, Wheeler DL, Salgia R. Exosomal miRNAs species in the blood of small cell and non-small cell lung cancer patients. Oncotarget 2018;9:19793. https://doi.org/10.18632/oncotarget.24857
85. Zhang YK, Zhu WY, He JY, Chen DD, Huang YY, Le HB, Liu XG. MiRNAs expression profiling to distinguish lung squamous-cell carcinoma from adenocarcinoma subtypes. J Cancer Res Clin Oncol 2012;138:1641-1650. https://doi.org/10.1007/s00432-012-1240-0
86. Zhou X, Wen W, Shan X, Zhu W, Xu J, Guo R, Cheng W, Wang F, Qi LW, Chen Y, Huang Z, Wang T, Zhu D, Liu P, Shu Y. A six-microRNA panel in plasma was identified as a potential biomarker for lung adenocarcinoma diagnosis. Oncotarget 2017;8:6513. https://doi.org/10.18632/oncotarget.14311
87. Xu Z, Wang Z, Sun H, Xin H. Evaluation of Exosomal miRNA in Blood as a Potential Diagnostic Biomarker for Human Non-Small Cell Lung Cancer. Med Sci Monit Int Med J Exp Clin Res 2020;26. https://doi.org/10.12659/MSM.924721
88. Hossain MS, Karuniawati H, Jairoun AA, Urbi Z, Ooi DJ, John A, Lim YC, Kaderi Kibria KM, Mohiuddin AKM, Ming LC, Goh KW, Hadi MA. Colorectal Cancer: A Review of Carcinogenesis, Global Epidemiology, Current Challenges, Risk Factors, Preventive and Treatment Strategies. Cancers (Basel) 2022;14:1732. https://doi.org/10.3390/cancers14071732
89. Siegel RL, Miller KD, Sauer AG, Fedewa SA, Butterly LF, Anderson JC, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2020. CA Cancer J Clin 2020;70:145-164. https://doi.org/10.3322/caac.21601, https://doi.org/10.3322/caac.21590
90. Zhang Y, Guo L, Li Y, Feng GH, Teng F, Li W, Zhou Q. MicroRNA-494 promotes cancer progression and targets adenomatous polyposis coli in colorectal cancer. Mol Cancer 2018;17:1. https://doi.org/10.1186/s12943-017-0753-1
91. Li KP, Fang YP, Liao JQ, Duan JD, Feng LG, Luo XZ, Liang ZJ. Upregulation of miR-598 promotes cell proliferation and cell cycle progression in human colorectal carcinoma by suppressing INPP5E expression. Mol Med Rep 2018;17:2991-7. https://doi.org/10.3892/mmr.2017.8207
92. Lu D, Tang L, Zhuang Y, Zhao P. MiR-17-3P regulates the proliferation and survival of colon cancer cells by targeting Par4. Mol Med Rep 2018;17:618-623. https://doi.org/10.3892/mmr.2017.7863

93. Wang S, Zeng Y, Zhou JM, Nie SL, Peng Q, Gong J, Huo JR. MicroRNA-1246 promotes growth and metastasis of colorectal cancer cells involving CCNG2 reduction. Mol Med Rep 2016;13:273-280. https://doi.org/10.3892/mmr.2015.4557
94. Matsumura T, Sugimachi K, Iinuma H, Takahashi Y, Kurashige J, Sawada G, Ueda M, Uchi R, Ueo H, Takano Y, Shinden Y, Eguchi H, Yamamoto H, Doki Y, Mori M, Ochiya T, Mimori K. Exosomal microRNA in serum is a novel biomarker of recurrence in human colorectal cancer. Br J Cancer 2015;113:275-281. https://doi.org/10.1038/bjc.2015.201
95. Chen M, Xu R, Rai A, Suwakulsiri W, Izumikawa K, Ishikawa H, Greening DW, Takahashi N, Simpson RJ. Distinct shed microvesicle and exosome microRNA signatures reveal diagnostic markers for colorectal cancer. PLoS One 2019;14:e0210003. https://doi.org/10.1371/journal.pone.0210003
96. Slattery ML, Mullany LE, Sakoda L, Samowitz WS, Wolff RK, Stevens JR, Herrick JS. The NF-κB signalling pathway in colorectal cancer: associations between dysregulated gene and miRNA expression. J Cancer Res Clin Oncol 2018;144:269-283. https://doi.org/10.1007/s00432-017-2548-6
97. Shao Y, Chen T, Zheng X, Yang S, Xu K, Chen X, Xu F, Wang L, Shen Y, Wang T, Zhang M, Hu W, Ye C, Yu XF, Shao J, Zheng S. Colorectal cancer-derived small extracellular vesicles establish an inflammatory premetastatic niche in liver metastasis. Carcinogenesis 2018;39:1368-1379. https://doi.org/10.1093/carcin/bgy115
98. Uratani R, Toiyama Y, Kitajima T, Kawamura M, Hiro J, Kobayashi M, Tanaka K, Inoue Y, Mohri Y, Mori T, Kato T, Goel A, Kusunoki M. Diagnostic Potential of Cell-Free and Exosomal MicroRNAs in the Identification of Patients with High-Risk Colorectal Adenomas. PLoS One 2016;11:e0160722. https://doi.org/10.1371/journal.pone.0160722
99. Zou SL, Chen YL, Ge ZZ, Qu YY, Cao Y, Kang ZX. Downregulation of serum exosomal miR-150-5p is associated with poor prognosis in patients with colorectal cancer. Cancer Biomarkers 2019;26:69-77. https://doi.org/10.3233/CBM-190156
100. Ogata-Kawata H, Izumiya M, Kurioka D, Honma Y, Yamada Y, Furuta K, Gunji T, Ohta H, Okamoto H, Sonoda H, Watanabe M, Nakagama H, Yokota J, Kohno T, Tsuchiya N. Circulating Exosomal microRNAs as Biomarkers of Colon Cancer. PLoS One 2014;9:e92921. https://doi.org/10.1371/journal.pone.0092921
101. Dohmen J, Semaan A, Kobilay M, Zaleski M, Branchi V, Schlierf A, Hettwer K, Uhlig S, Hartmann G, Kalff JC, Matthaei H, Lingohr P, Holdenrieder S. Diagnostic Potential of Exosomal microRNAs in Colorectal Cancer. Diagnostics 2022;12:1413. https://doi.org/10.3390/diagnostics12061413
102. Min L, Zhu S, Chen L, Liu X, Wei R, Zhao L, Yang Y, Zhang Z, Kong G, Li P, Zhang S. Evaluation of circulating small extracellular vesicles derived miRNAs as biomarkers of early colon cancer: a comparison with plasma total miRNAs. J Extracell Vesicles 2019;8. https://doi.org/10.1080/20013078.2019.1643670
103. Yan S, Han B, Gao S, Wang X, Wang Z, Wang F, Zhang J, Xu D, Sun B. Exosome-encapsulated microRNAs as circulating biomarkers for colorectal cancer. Oncotarget 2017;8:60149. https://doi.org/10.18632/oncotarget.18557
104. Liu C, Eng C, Shen J, Lu Y, Yoko T, Mehdizadeh A, Chang GJ, Rodriguez-Bigas MA, Li Y, Chang P, Mao Y, Hassan MM, Wang F, Li D. Serum exosomal miR-4772-3p is a predictor of tumor recurrence in stage II and III colon cancer. Oncotarget 2016;7:76250. https://doi.org/10.18632/oncotarget.12841
105. Peng ZY, Gu RH, Yan B. Downregulation of exosome-encapsulated miR-548c-5p is associated with poor prognosis in colorectal cancer. J Cell Biochem 2019;120:1457-63. https://doi.org/10.1002/jcb.27291
106. Vu T, Datta PK. Regulation of EMT in Colorectal Cancer: A Culprit in Metastasis. Cancers (Basel) 2017;9:171. https://doi.org/10.3390/cancers9120171
107. Zhou JJ, Zheng S, Sun LF, Zheng L. MicroRNA regulation network in colorectal cancer metastasis. World J Biol Chem 2014;5:301. https://doi.org/10.4331/wjbc.v5.i3.301
108. Yang N, Zhu S, Lv X, Qiao Y, Liu YJ, Chen J. MicroRNAs: Pleiotropic regulators in the tumor microenvironment. Front Immunol 2018;9:2491. https://doi.org/10.3389/fimmu.2018.02491
109. De Greef K, Rolfo C, Russo A, Chapelle T, Bronte G, Passiglia F, Coelho A, Papadimitriou K, Peeters M. Multisciplinary management of patients with liver metastasis from colorectal cancer. World J Gastroenterol 2016;22:7215. https://doi.org/10.3748/wjg.v22.i32.7215

110. Hofsli E, Sjursen W, Prestvik WS, Johansen J, Rye M, Tranø G, Wasmuth HH, Hatlevoll I, Thommesen L. Identification of serum microRNA profiles in colon cancer. Br J Cancer 2013;108:1712-1719. https://doi.org/10.1038/bjc.2013.121
111. Tang Y, Zhao Y, Song X, Song X, Niu L, Xie L. Tumor-derived exosomal miRNA-320d as a biomarker for metastatic colorectal cancer. J Clin Lab Anal 2019;33:e23004. https://doi.org/10.1002/jcla.23004
112. Takano Y, Masuda T, Iinuma H, Yamaguchi R, Sato K, Tobo T, Hirata H, Kuroda Y, Nambara S, Hayashi N, Iguchi T, Ito S, Eguchi H, Ochiya T, Yanaga K, Miyano S, Mimori K. Circulating exosomal microRNA-203 is associated with metastasis possibly via inducing tumor-associated macrophages in colorectal cancer. Oncotarget 2017;8:78598. https://doi.org/10.18632/oncotarget.20009
113. Yan S, Jiang Y, Liang C, Cheng M, Jin C, Duan Q, Xu D, Yang L, Zhang X, Ren B, Jin P. Exosomal miR-6803-5p as potential diagnostic and prognostic marker in colorectal cancer. J Cell Biochem 2018;119:4113-4119. https://doi.org/10.1002/jcb.26609