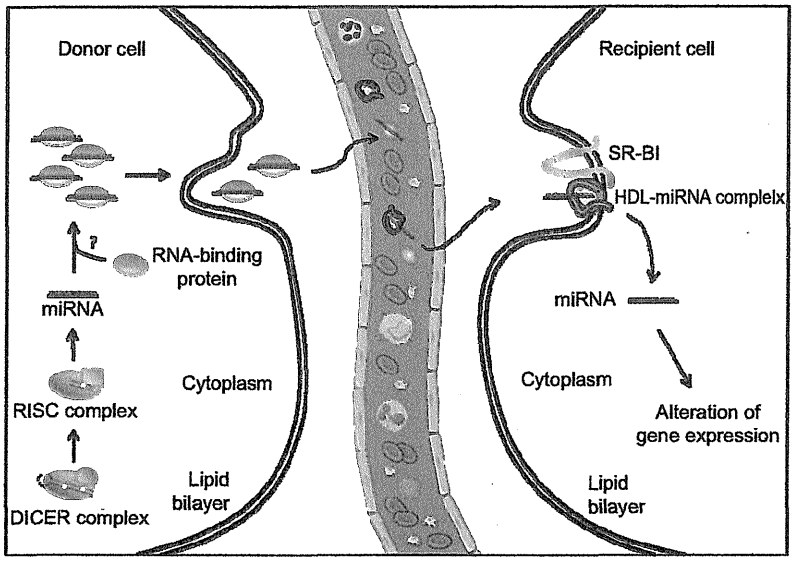

Figure 3. A model of miRNA intercellular communication mechanism involving high-density lipoprotein (HDL). Exported miRNA are carried and delivered to the recipient cells by HDL. The delivery process into the cell is dependent on the scavenger receptor class B type I protein (SR-BI). RISC, RNA-induced silencing complex.

HDL fractions. LDL and HDL have similar physico-chemical properties but differ in size, composition and biogenesis. The authors found the presence of miRNA in all the three separated fractions. Nevertheless, the miRNA-profile was different in HDL fraction compared to exosome and LDL fractions, with miR-223 highly expressed in HDL.

Vickers and colleagues demonstrated the capacity of HDL to load miRNA molecules *in vivo* by using wild-type and apolipoprotein E-null mutant mice. The authors also showed *in vitro* that native HDL could deliver functional miRNA to recipient cells, mediated by scavenger receptor class B type I (SR-BI). But, in contrast to miRNA-loading process into exosomes, inhibition of the nSMase2 increased the amount of miR-223 exported to HDL, suggesting likely common pathways but distinct mechanisms.

This was the first study to demonstrate a new role of HDL apart from its involvement in cholesterol dynamics. These findings open a perspective of use of HDL-miRNA complex as biomarker, therapeutic tool for disorders related to lipid metabolism or as delivery tool for other diseases.

3. EXTRACELLULAR miRNAs AS POTENTIAL BIOMARKERS IN CANCER

The origin of extracellular miRNAs has been explored here above and the different transporters identified to date are summarized in Fig. 4. Until now, we described only the miRNAs secreted in the bloodstream. However, recent studies have also demonstrated the presence of miRNAs in most of the other body fluids (urine, saliva, etc.), increasing the potential of the circulating miRNAs as non-invasive bioclinical tools. Through various techniques and samples, numerous groups have shown that cancers can have specific altered miRNA profiles in those body fluids (Table 1).

Lawrie and colleagues were the first to demonstrate the presence of miRNAs (miR-21, miR-155 and miR-210) at higher levels in the serum of patients suffering from diffuse large B-cell lymphoma (DLBCL) (Lawrie et al. 2008) than in control subjects. The authors also found that miR-21, highly expressed in DLBCL, was correlated with relapse-free survival. Mitchell and coworkers investigated the miRNAs that are usually expressed in prostate cancer cells and discovered that, in serum samples, the expression of miR-141 could differentiate patients from healthy controls (Mitchell et al. 2008). In this study, the group also demonstrated that miRNAs originating from human prostate cancer xenografts entered the circulation, were detected in the plasma and could distinguish xenografted mice from controls. In the breast cancer, blood circulating miR-195 and let-7a were found to be increased in patients, compared to the healthy controls, and decreased following tumor resection (Heneghan et al. 2010). Moreover, this group correlated the expression of let-7a and miR-21 to clinicopathological

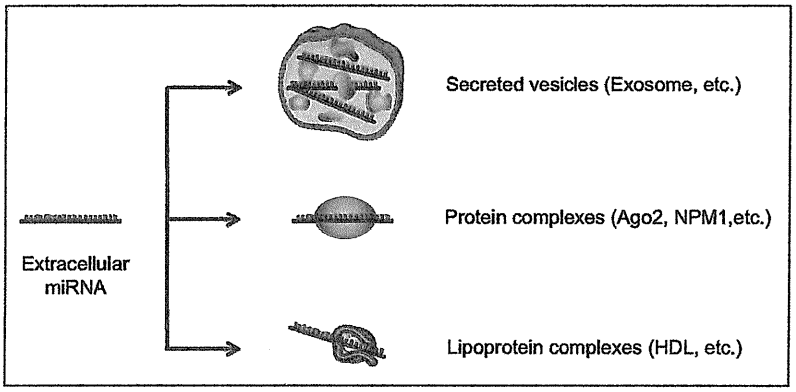

Figure 4. Transporters of extracellular miRNAs. Extracellular miRNAs are carried in secreted vesicles, protein complexes, and lipoprotein complexes. Ago2, argonaute 2 protein; NPM1, nucleophosmin 1; HDL, high-density lipoprotein.

Table 1. Circulating miRNA as cancer biomarkers.

Type of cancer	Body fluid	Potential miRNA biomarker	Justification	Usefulness	References
Bladder cancer	Urina	miR-126 and miR-182	Increased	Diagnosis	Hanke et al. 2010
		miR-96 and miR-183	Increased, Correlated with tumor grade, Decreased after surgical treatment	Diagnosis Prognosis	Yamada et al. 2011
Breast cancer	Serum/plasma	miR-195 and let-7a, let-7a and miR-21	Increased, Decreased after surgical treatment, Correlated with nodal status and ER status	Diagnosis Prognosis	Heneghan et al. 2010
		miR-126, miR-199a, miR-335, miR-21, miR-106a and miR-155	Increased or decreased, Correlated with ER/PR status and tumor grade	Diagnosis Prognosis	Wang et al. 2010b
Colorectal cancer	Serum/plasma	miR-92	Increased, Decreased after surgical treatment	Diagnosis	Ng et al. 2009
		miR-29a and miR-92a	Increased	Diagnosis	Huang et al. 2010
		miR-141	Increased	Prognosis	Cheng et al. 2011
Diffuse large B cell lymphoma	Serum/plasma	miR-21, miR-155 and miR-210 miR-21	Increased Correlated with disease-free survival	Diagnosis Prognosis	Lawrie et al. 2008
Esophageal squamous cell carcinoma	Serum/plasma	miR-10a, miR-22, miR-100, miR-148b, miR-223, miR-133a, and miR-127-3p	Increased	Diagnosis	Zhang et al. 2010
		miR-31	Increased, correlated with tumor stage	Diagnosis Prognosis	Zhang et al. 2011
Gastric cancer	Serum/plasma	miR-17-5p, miR-21, miR-106a, miR-106b and let-7a	Increased or decreased, Decreased after surgical treatment	Diagnosis	Tsujiura et al. 2010

Table 1. contd....

Table 1. contd....

Type of cancer	Body fluid	Potential miRNA biomarker	Justification	Usefulness	References
Gastric cancer	Serum/plasma	miR-1, miR-20a, miR-27a, miR-34 and miR-423-5p	Increased, Correlated with tumor stage	Diagnosis Prognosis	Liu et al. 2011a
		miR-378	Increased	Diagnosis	Liu et al. 2011b
Glioblastoma	Serum/plasma	miR-21	Increased, Found in EV	Diagnosis	Skog et al. 2008
Hepatocellular carcinoma (HCC)	Serum/plasma	miR-500	Increased, Decreased after surgical treatment	Diagnosis	Yamamoto et al. 2009
		miR-92a	Decreased, Increased after surgical treatment,	Diagnosis	Shigoka et al. 2010
		miR-25, miR-375, and let-7f	Correlated with HBV-positive HCC and -infection	Diagnosis	Li et al. 2010a
		miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a and miR-801	Could discriminate HCC group from other liver diseases' group and healthy group in a large cohort	Diagnosis	Zhou et al. 2011
Leukemia	Serum/plasma	miR-92a; ratio miR-92a/miR-638	Decreased, Ratio well correlated with AL diagnosis	Diagnosis	Tanaka et al. 2009
		miR-150*, miR-195, miR-222, MiR-29a;	Could discriminate CLL group from other hematologic malignancies' group and healthy group	Diagnosis	Moussay et al. 2011
		miR-20a	Correlated with CLL-ZAP-70 expression status	Prognosis	
Lung cancer	Serum/plasma	miR-25 and miR-223	Increased	Diagnosis	Chen et al. 2008
		miR-20a, -24, -25, -145, -152, -199a-5p, -221, -222, -223 and -320	Increased, Correlated with early diagnosis	Diagnosis	Chen et al. 2011
		miRNA in exosomes	Correlated with tumor-derived miRNA patterns	Diagnosis	Rabinowits et al. 2009
		let-7f, miR-20b and miR-30e-3p	Decreased, Found in EV, Correlated with tumor stage, disease-free survival and overall survival	Prognosis	Silva et al. 2011

Lung cancer	Serum/plasma	miR-1, miR-30d, miR-486 and miR-499	Correlated with overall survival	Prognosis	Hu et al. 2010
		miR-21, miR-210 and miR-486-5p	Increased or decreased	Diagnosis	Shen et al. 2011
		miR-21	Increased, Correlated with metastasis, Correlated with poor diagnosis in association with tumor miR-200c	Diagnosis, Prognosis	Wang et al. 2011, Wei et al. 2011 and Liu et al. 2011c
		miR-96, miR-182 and miR-183	Increased, Correlated with overall survival	Diagnosis Prognosis	Zhu et al. 2011
		miR-126 and miR-183	Correlated with tumor grade	Prognosis	Lin et al. 2012
		miR-155, miR-182, miR-197	Increased, Correlated with chemotherapy responsiveness	Diagnosis Prognosis	Zheng et al. 2011
		miR-10b, miR-141, miR-155 and miR-34a	Increased	Diagnosis	Roth et al. 2011
		miR-1254 and miR-574-5p	Increased	Diagnosis	Foss et al. 2011
	Effusion	miR-24, miR-26a and miR-30d, miR-152	Potentially correlated with malignant effusion and with chemotherapy responsiveness	Diagnosis Prognosis	Xie et al. 2010
Melanoma	Serum/plasma	16 miRNA	Increased and decreased	Diagnosis	Leidinger et al. 2010
		miR-221	Increased, Decreased after surgical treatment	Diagnosis Prognosis	Kanemaru et al. 2011
Oral cancer	Saliva	miR-125a and miR-200a	Decreased, Ago2 detected in the saliva	Diagnosis	Park et al. 2009
		miR-375	Decreased	Diagnosis	Wiklund et al. 2011
	Serum/plasma	miR-31	Increased, Decreased after surgical treatment, Also increased in saliva	Diagnosis	Liu et al. 2010d
		miR-184	Increased, Decreased after surgical treatment	Diagnosis	Wong et al. 2008

Table 1. contd....

Table 1. contd....

Type of cancer	Body fluid	Potential miRNA biomarker	Justification	Usefulness	References
Ovarian cancer	Serum/plasma	miR-21, miR-141, miR-200a, miR-200b, miR-200c, miR-203, miR-205 and miR-214	Increased, Found in EV and correlated with tumor-derived miRNA patterns, Correlated with tumor grade	Diagnosis Prognosis	Taylor and Gercel-Taylor 2008
		miR-21, miR-29a, miR-92, miR-93 and miR-126; miR-99b, miR-127, miR-155	Increased or decreased	Diagnosis	Resnick et al. 2009
Pancreatic cancer (PC)	Serum/plasma	miR-210	Increased	Diagnosis	Ho et al. 2010
		miR-200a and miR-200b	Increased	Diagnosis	Li et al. 2010b
		miR-20a, miR-21, miR-24, miR-25, miR-99a, miR-185, miR-191	Increased, Correlated with tumor grade, Correlated with overall survival, Could discriminate PC group from chronic pancreatitis group in a large cohort	Diagnosis Prognosis	Liu et al. 2011e
		miR-16, miR-196a	Increased, Improved diagnosis in combination with CA19-9 assay	Diagnosis	Liu et al. 2011f
		miR-18a	Increased, Decreased after surgical treatment and increased at recurrence in contrast to CA19-9	Diagnosis	Morimura et al. 2011
		miR-21, let-7 family, miR-146a	Increased or decreased	Diagnosis	Ali et al. 2010
Prostate cancer	Serum/plasma	miR-141	Increased	Diagnosis	Mitchell et al. 2008
		miR-30c, -26b, -451, -223, -24, -874, -1274a, -1207-5p, -93 and -106a	Increased or decreased, Correlated with risk of disease progression	Diagnosis Prognosis	Moltzahn et al. 2011
		miR-375 and miR-141	Increased, Correlated with metastasis	Diagnosis	Brase et al. 2011
Rhabdomyosarcoma	Serum/plasma	miR-206	Increased	Diagnosis	Miyachi et al. 2010

Abbreviations: AL, acute leukemia (comprising myeloid and lymphoblastic); CA19-9, carbohydrate antigen 19-9; CLL, chronic lymphocytic leukemia; ER, estrogen receptor; EV, extracellular vesicles; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; PC, pancreatic cancer; PR, progesterone receptor; ZAP-70, zeta-chain-associated protein kinase 70

variables (nodal and estrogen receptor status). Interestingly, miR-195 has been previously correlated to estrogen receptor status in breast cancer biopsies (Mattie et al. 2006). This group therefore provided miRNAs with potential use in diagnosis, prognosis and surgical monitoring. Wang and colleagues increased the panel by finding miRNAs correlated with the progesterone receptor and the tumor grade (Wang et al. 2010b). Some studies even showed a link between miRNA-profile and the chemotherapy responsiveness. So, Zheng and coworkers demonstrated that the plasma levels of miR-155 and miR-197 were significantly decreased in the patients with lung cancer in the late phase of chemotherapy compared to the early phase (Zheng et al. 2011). The authors also found that the combination of three plasma miRNAs (miR-155, miR-197 and miR-182) yielded about 81% sensitivity and 87% specificity in discriminating lung cancer patients from controls. Similar approaches using blood samples were done on other cancers such as colorectal cancer (Ng et al. 2009), esophageal squamous cell carcinoma (Zhang et al. 2011), gastric cancer (Tsujiura et al. 2010 and Liu et al. 2011a), lung cancer (Hu et al. 2010) or bladder cancer using urine samples in this latter case (Yamada et al. 2011).

3.1 The Need for Specific Cancer Signature

In order to improve the diagnostic approach, several groups found a specific combination of miRNAs to distinguish a specific cancer from other diseases or malignancies. In the case of hepatocellular carcinoma (HCC), Zhou and colleagues constructed a receiver operating characteristic (ROC) curve on a large cohort of nearly 1000 subjects and used the area under the ROC curve (AUC) for diagnostic evaluation (Zhou et al. 2011). The group identified a 7 miRNA-panel that provided a high diagnostic accuracy of HCC and one that could differentiate HCC group from healthy (AUC = 0.941), chronic hepatitis B virus (HBV, AUC = 0.842) and cirrhosis group (AUC = 0.884), respectively. Similarly, Moussay and coworkers differentiated chronic lymphocytic leukemia (CLL) group from other hematologic malignancies and healthy groups (Moussay et al. 2011).

Another way to improve the detection of cancer is to combine existing routine assays with circulating miRNA profiles. In the case of pancreatic cancer, one of the routine diagnostic assays is the detection of the carbohydrate antigen 19-9 (CA19-9), an antigen released from the cell surface of pancreatic tumor cells. Liu and colleagues demonstrated that detection of plasma miR-16 and miR-196a, in combination with CA19-9 assay, improved the diagnosis, especially in early tumor screening (Liu et al. 2011f).

3.2 Extracellular miRNAs Carried or Not by EV?

Most of the current studies focused only on the detection of miRNAs in the body fluid and determined the altered expression and the potential use as biomarker, without distinction about the form of these circulating miRNAs. Nevertheless, some questioned this point. Park and colleagues were not only the first ones to investigate and demonstrate the presence of miRNAs in the saliva. The authors were also the first ones to hypothesize the association of extracellular miRNAs to RISC complex component Ago2 and to provide indirect evidence by performing Ago2-immunoblot analysis on the saliva samples (Park et al. 2009).

On the other hand, several reports demonstrated the presence of circulating EV that carry miRNA and that are related to tumor cells. Taylor and Gercel-Taylor were the first to demonstrate the presence of tumor-derived exosomes in the case of ovarian cancer (Taylor and Gercel-Taylor 2008). The authors showed that the levels of circulating exosomes increased in patients compared to healthy subjects and that the increase was parallel with the tumor grade. This group also found correlation between tumor-derived and circulating exosome-derived miRNA patterns. Rabinowits and colleagues reached similar conclusions for lung cancer (Rabinowits et al. 2009). Working on a wider cohort of patients, Silva and colleagues designed experiments that allowed identifying three miRNAs of potential interest (let-7f, miR-30e-3p and miR-20b) (Silva et al. 2011). The levels of these three miRNAs were lower in plasma vesicles of lung cancer patients. The expression levels of let-7f and miR-30e-3p differentiate two groups of patients for grade of disease. Moreover, miR-30-e-3p and let-7f correlated with disease-free survival and overall survival, respectively.

4. EXTRACELLULAR miRNAs AS THERAPEUTIC TARGETS AND THE EMERGENCE OF COMBINED EV AND RNAi AS NEW THERAPEUTIC TOOLS IN CANCER

The discovery of small non-coding RNA in cells and their crucial regulatory roles was a major breakthrough in the understanding of cell function. It was also at the origin of the short interfering RNA (siRNA) as new therapeutic agents, especially in the case of cancer. But the existence of extracellular miRNAs, in many body fluids, with functional impact on the intercellular communication and carried by EV, brought along tools potentially more powerful; it not only allows identification of new therapeutic targets to fight diseases, but it also provides more appropriate delivery systems.

4.1 siRNAs as Potential Therapeutic Agents

Similarly to miRNA, RNAi implicates sequence-specific gene silencing by Watson-Crick base pairing using small RNA called, in this case, siRNA (for a review see Carthew and Sontheimer 2009). MiRNA and siRNA have common pathways and partners including Dicer enzymes and Ago proteins. Nevertheless, their are two main differences between them. Firstly, miRNAs are endogenous whereas siRNAs are exogenous in origin, derived from transgenes incorporated in the cells. Secondly, the processing appears to occur from stem-loop precursors with incomplete double-stranded design for miRNA whereas it occurs from long, complete complementary double-stranded RNA for siRNA. Thanks to genetic engineering, synthetic siRNAs were thus developed and used to suppress specific gene expression, especially for the genes whose expression is altered in the case of cancer, viral infections or other diseases. Elbashir and colleagues were the first to show the siRNA-mediated gene silencing in mammalian cells (Elbashir et al. 2001). Many other studies followed the work of this group. For example, Scherr and colleagues designed siRNA to target the bcr-abl oncogene, responsible of chronic myeloid leukemia (CML) and bcr-abl-positive acute lymphoblastic leukemia (ALL) (Scherr et al. 2003). The authors found a reduction of bcr-abl mRNA up to 87% in bcr-abl-positive cell lines and in primary cells from CML patients. The reduction of mRNA was specific and affected the protein expression level of the encoded proteins as well. Moreover, by targeting the same bcr-abl mRNA, Wohlbold and coworkers increased the sensitivity to the selective tyrosine kinase inhibitor imatinib in leukemic cells expressing the imatinib-resistant form of Bcr-Abl protein (Wohlbold et al. 2003). Nieth and colleagues also succeeded to reverse a resistance-phenotype in tumor cells (Nieth et al. 2003). This group worked on the 'classical' multi-drug resistance (MDR) that is mediated by the adenosine triphosphate binding cassette (ABC)-transporter P-glycoprotein (MDR1/P-gp). The authors used siRNA duplexes to test the resistance to daunorubicin in both human pancreatic and gastric carcinoma cells. The specific inhibition decreased the resistance up to 89% and 58% in pancreatic and gastric cell lines, respectively. The *in vivo* experiment was then performed using MDR1/P-gp-xenograft mice and showed complete reversal of the MDR phenotype (Stein et al. 2008). The authors used the technology of jet-injection delivery to incorporate non-viral vectors expressing the short hairpin RNA (shRNA), a precursor of siRNA, directly into the tumor.

Despite the promising results of siRNA and the recent advances in delivery systems, one of the major issues remain that is the existence of clinically suitable, safe, effective and specific delivery vehicles.

4.2 EV as Potential Delivery Systems

As stated previously, EV are natural nuclease-resistant delivery carriers implicated in intercellular communication. Among these, exosomes are the best characterized to date. They are complex nano-sized particles composed of proteins, lipids, carbohydrates and nucleic acids (Théry et al. 2009). Exosomes can contain proteins involved in various cellular processes: T-cell stimulation (MHC class I and II), adhesion (integrins, tetraspanins), signalling pathways (syntenin, Gα), membrane transport and trafficking (ATPase channels, Rho GDI, annexins), cytoskeleton (actin, myosin, tubulin), MVB formation (Alix), chaperones (heat shock proteins), metabolism (phosphoglycerate kinase 1, α-enolase, ADP ribosylation factor) or transcription (histones). The lipid composition is usually rich in cholesterol, sphingomyelin and ceramide, which suggests, in correlation with the proteins identified in exosomes (flotillin 1), the existence of lipid rafts. Lipid rafts are microdomains in the lipid bilayer cellular membrane that are detergent-resistant and work as signalling and sorting platforms (Staubach and Hanish 2011). An on-line database named Exocarta was created to pool all the research findings about exosomes (www.exocarta.org).

Although physiopathological role of exosomes requires more investigation to improve our knowledge and comprehension, evidences point out the intimate relation between their functions and their cellular origins, origins that implicate specific exosome composition. Exosomes have been demonstrated to be secreted by many cells including reticulocytes (first description of exosomes) (Pan and Johnstone 1983), B cells (Raposo et al. 1996), T cells (Blanchard et al. 2002), mast cells (Skodos et al. 2001), platelets (Heijnen et al. 1999), intestinal epithelial cells (Van Niel et al. 2003), dendritic cells (Pêche et al. 2003) and tumor cells (Taylor and Gercel-Taylor 2008). The adhesion molecules on the exosome surface play an important role in the cell targeting. For example, the exosomes produced by the B cells present MHC class II that stimulate CD4+ T cells *in vitro* (Raposo et al. 1996). Exosomes have also been demonstrated to inhibit or promote the immune responses. The observed inhibition was correlated with tumor-derived exosomes and included induction of T-cell apoptosis (Huber et al. 2005) or reduction of the cytotoxicity of natural killer cells (Ashiru et al. 2010). Some pro-immune activities were also identified for tumor-derived exosomes but mainly restricted to stress-induced conditions (Gastpar et al. 2005). The promotion of immune responses was also observed in macrophages. When infected by various pathogens (*Mycobacterium* or *Toxoplasma*), these cells release exosomes containing pathogen-derived inflammatory molecules that induce the secretion of pro-inflammatory cytokines by the recipient macrophages (Bhatnagar and Schorey 2007).

Exosomes thus have a strong potential as suitable multi-functional vesicles that can carry and transfer safely and efficiently materials able to affect other cells.

4.3 Building on the Natural System: A Combined Approach of EV and RNAi as Therapeutic Tools

Skog and colleagues were among the first to suggest the use of EV as carriers to deliver nucleic acids. This group found that glioblastoma derived-EV contain mRNA, miRNA and angiogenic proteins (Skog et al. 2008). The authors reported that glioblastoma derived-EV could enter human brain microvascular endothelial cells (HBMVEC) in *in vitro* system and translate a reporter mRNA carried by these EV. Skog and coworkers also demonstrated that the tubule length of HBMVEC doubled within 16 h in presence of the EV, supporting a role of the latter in initiating angiogenesis in brain endothelial cells. The authors therefore concluded that tumor vesicles act as multicomponent delivery vehicle for mRNA, miRNA and proteins, to communicate genetic information and signalling proteins to the neighboring cells. On the other hand, Yang and colleagues showed that macrophages regulate the invasiveness of breast cancer cells through EV-mediated delivery of oncogenic miRNA (Yang et al. 2011). The authors used a transwell co-culture system of interleukin 4-activated macrophages and breast cancer cells and they tracked the signal of fluorescently-labeled exogenous miRNA originating from macrophages. This group were therefore able to verify the transport of miRNA without direct cell–cell contact from the macrophages to the breast cancer cells. In the EV, Yang and coworkers detected the presence of miR-223, a miRNA specific of the interleukin 4-activated macrophages that has been previously reported to be implicated in the progression of renal (Gottardo et al. 2007) and HCC cancers (Xu et al. 2011). By treating macrophages with a miR-223 anti-sense oligonucleotide, the authors could reduce the observed effects.

The group of Alvarez-Erviti was the first to develop and test *in vivo* delivery of siRNA to the mouse brain by systemic injection of targeted exosomes (Alvarez-Erviti et al. 2011). The authors initially selected dendritic cells with specific characteristics from the bone marrow of mice, cultured them and purified their exosomes. In order to target the purified EV to the desired tissues (muscle and brain), targeting peptides were fused to exosomal membrane protein. The group subsequently proceeded to the incorporation of specific siRNA into the modified vesicles before re-administrating them intravenously to the mice. In contrast with the mice to which naked siRNA was injected, exosome-encapsulated siRNA were resistant to nonspecific uptake by spleen, liver or kidney. Moreover,

Alvarez-Erviti and colleagues found the silencing of the targeted mRNA in several brain regions, demonstrating the efficiency of targeted exosomes. The authors also showed that multiple injections of these 'self' modified particles did not affect the delivery efficacy. Altogether, the conditions required of delivery systems for clinical applications were met. Finally, this group demonstrated for the first time the possibility to deliver therapeutic agents *in vivo* across the blood-brain barrier. This point is of great importance to address the actual issues in the treatment of neuronal diseases.

5. CONCLUSIONS

This review focused on the extracellular miRNAs, from their discovery to their potential clinical applications in cancer. Many studies highlighted the usefulness of these miRNAs as biomarkers in three different ways (Table 1): (i) differentiate normal from diseased states, (ii) help to make a prognosis by differentiating the tumor grade, and (iii) monitor the response to therapy. Nevertheless, the published data is still inconsistent, which is probably due to several reasons. Firstly, different detection and normalization methods (i.e. microarray *vs* single-gene PCR) have been used. Secondly, the origin of the tumor samples could vary (i.e. breast cancer, which can originate from different cells). Thirdly, great variability in the size of the cohort has been observed. Standardization is therefore required to obtain reliable non-invasive body fluid-based routine detection tests. Another improvement to be made is on the specificity. Indeed, some miRNAs such as miR-21 have been identified in numerous cancers. Specific miRNA-panel signature and/or combination with already existing tests should thus be found to allow the correlation with specific cancer.

The discovery of intracellular and extracellular miRNAs also led to the development of RNAi strategies. However, these strategies need a safe and efficient carrier in order to deliver the therapeutic oligonucleotides into the targeted tissues or cells. Several small size cargo-loading vehicles such as liposomes, polymers or virus-like particles have already been designed for *in vivo* targeting but they still encounter some issues (de Wolf et al. 2007, Azuma et al. 2010, Guo et al. 2009). The EV are naturally occurring vesicles that are very attractive tools in clinical use because they retain the advantages of the existing nanoscale drug delivery systems while adding *in vivo* safety and low immunogenicity. The first proof-of-concept was provided recently by Alvarez-Erviti and colleagues (see above). The optimization of EV as a drug delivery system requires a better understanding of their biology (production, functions, targeting mechanisms, etc.). However due to their ability to cross impermeable biological barriers such as the blood-brain barrier and the possibility to use patient-derived cells as a source of

tailored biocompatible therapeutic carriers, EV are seen to have enormous potential in the therapeutic research field. The next decade should see the start of human clinical applications of extracellular miRNAs as diagnostic, prognostic and therapeutic tools.

6. SUMMARY POINTS

- Altered expression levels of miRNA have been correlated with cancer. Recently, miRNAs have also been detected in many body fluids including plasma, serum, and urine.
- Due to their stability and their expression levels that differentiate diseased patients from healthy subjects, these circulating miRNAs have revealed great potential as novel diagnostic and prognostic markers.
- A substantial amount of extracellular miRNAs is carried by secreted extracellular vesicles and participates to intercellular genetic material exchange.
- Using EV as vehicle of drug delivery and RNAi to counteract the altered expression levels of targeted miRNA creates a new combined strategy that holds promise to overcome impediments in the field of therapeutics for cancer and other diseases.

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ABBREVIATIONS

ABC	:	ATP binding cassette
ADP	:	adenosine diphosphate
Ago	:	argonaute protein
ALL	:	acute lymphoblastic leukemia
ATP	:	adenosine triphosphate
AUC	:	area under the ROC curve
CA19-9	:	carbohydrate antigen 19-9
CD4	:	cluster of differentiation 4

CLL	:	chronic lymphocytic leukemia
CML	:	chronic myeloid leukemia
DLBCL	:	diffuse large B-cell lymphoma
EV	:	extracellular vesicles
HBMVEC	:	human brain microvascular endothelial cells
HBV	:	hepatitis B Virus
HCC	:	hepatocellular carcinoma
HDL	:	high-density lipoprotein
LDL	:	low-density lipoprotein
MDR	:	multi-drug resistance
MDR1/P-gp	:	ABC-transporter P-glycoprotein
MHC	:	major histocompatibility complex
miRNA	:	microRNA
MVB	:	multivesicular bodies
NPM1	:	nucleophosmin 1
nSMase2	:	neutral sphingomyelinase-2
RhoGDI	:	Rho guanosine 5′-diphosphate-dissociation inhibitor
RNAi	:	RNA interference
ROC	:	receiver operating characteristic
SR-BI	:	scavenger receptor class B type I
shRNA	:	short hairpin RNA
siRNA	:	short interfering RNA

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