Send Orders for Reprints to reprints@benthamscience.ae

Non-coding RNAs in Exosomes: New Players in Cancer Biology

Miguel Silva[a] and Sonia A. Melo[a,*]

[a]*Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal and Institute of Pathology and Molecular Immunology of the University of Porto (IPATIMUP), 4200 Porto, Portugal*

S. A. Melo

Abstract: Exosomes are lipid bilayer extracellular vesicles (EVs) of 50-150nm in size, which contain nucleic acids (mRNA, ncRNAs and DNA), proteins and lipids. They are secreted by all cells and circulate in all body fluids. Exosomes are key mediators of several processes in cancer that mediate tumor progression and metastasis. These nano-vesicles, when secreted from cancer cells, are enriched in non-coding RNAs (e.g. microRNAs) complexed with the RNA-Induced Silencing Complex (RISC), that mediate an efficient and rapid silencing of mRNAs at the recipient cell, reprogramming their transcriptome. MicroRNAs in circulation encapsulated in exosomes are protected from degradation by a lipid bilayer and might serve as potential non-invasive diagnostic and screening tools to detect early stage cancer, to facilitate treatment options and possible help in curative surgical therapy decisions. Additionally, engineered exosomes can be used as therapy vehicles for targeted delivery of RNAi molecules, escaping the immune system detection.

Keywords: Exosomes, Extracellular vesicles, microRNAs, microRNA biogenesis, Non-coding RNAs.

1. INTRODUCTION

The conceptual idea postulated by scientists [1] that relies on RNA as the main responsible for biology to emerge from chemistry in a primitive time of the Earth, points to the existence of a dynamic yet heterogeneous set of players with different functional and biological purposes in the different organisms.

1.1. Non-Coding RNAs

Protein coding genes are the most well studied genomic sequences, however exons account for only ~2% of the genome if one considers untranslated regions (UTRs) [2]. It has become increasingly evident that this significant non-protein coding portion (98%) harbors crucial importance in terms of development, homeostasis and disease [3].

This is largely supported by fact that the biological complexity of organisms is often proportional to the non-protein coding portion of the genome, coupled with evidences regarding the transcription of this large portion of genome into non-coding RNAs (nc-RNA) [1].

The term non-coding RNA (ncRNA) most commonly refers to RNA that does not encode a specific protein in the cell. Although they were initially thought to arise as a result of low polymerase fidelity, the discovery that many genomic sequences are transcribed in accordance to the developmental state of the organism and tissue location prompted the characterization of these molecules in complex organisms [4]. There is no clear distinction between the different types of ncRNAs however they can be divided according to its size in short ncRNAs which comprises molecules ranging from ~17bp to ~32bp (miRNAs, piRNAs and tiRNAs), mid-size ncRNAs with sizes between 60bp and <200bp (snoRNAs, PASRs, TSSs-RNAs and PROMPTs) and long ncRNAs with >200bp (lincRNA, T-UCRs and other long ncRNAs [3, 5]. Also their functions are highly heterogeneous with implication in many cellular processes such as ribosomal RNA (rRNA) modifications [6], messenger RNA (mRNA) targeting [7], chromatin modification and transcriptional regulation [4]. Of all ncRNAs, the microRNAs (miRNAs) are the most widely studied ones since the first small ncRNA *lin-4* was described in *C. elegans* [8].

MicroRNAs are small (19-24bp) endogenous ncRNA that regulate gene expression at the posttranscriptional level, by targeting mRNA transcripts. The action of miRNAs is mediated by its binding to the 3-untranslated region (3'UTR) of the target mRNAs thus regulating targeted mRNAs stability and protein synthesis [9, 10]. Regarding its biogenesis, miRNAs are transcribed in the nucleus either from transcripts of specific genes or introns of protein-coding genes, by RNA polymerase II (RNAP II) into a primary miRNA (pri-miRNA) [11]. Next the pri-miRNA is enzymatically modeled in a two-step process catalyzed by two members of RNase I family Drosha and Dicer, in association with the dsRNA-binding proteins (dsRBPs) DiGeorge Syndrome Crtical Region Gene 8 (DGCR8) and transactivation-responsive RNA-binding protein (TRBP). The first step occurs in the nucleus and comprises the recognition and cleavage of the stem-loop of the pri-miRNA, by the Drosha-DGCR8 complex (also called microprocessor complex), into a ~70 nucleotide precursor hairpin (pre-miRNA). It is then transported to the cytoplasm by the Exportin-5 (XPO5)-Ran-GTP complex for the dicing step in which the pre-miRNA is cleaved by the Dicer-TRBP complex into a double stranded miRNA. After strand separation, the functional strand of the

*Address correspondence to this author at the Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Rua Dr Roberto Frias, s/n, 4200-465 Porto, Portugal; Tel: (00351)-2255-70700; E-mail: smelo@ipatimup.pt

1875-5488/15 $58.00+.00 **©2015 Bentham Science Publishers**

mature miRNA is loaded together with Argonaute (AGO2) proteins into the RNA-induced silencing complex (RISC). This complex exerts its function by silencing the target mRNA through cleavage, translational repression or deadenylation. The other strand, called passenger strand is degraded (Fig. **1**) [11, 12].

MiRNAs are estimated to regulate the translation of nearly 60% of protein-coding genes as they are involved in the regulation of many processes including differentiation, proliferation, apoptosis and development. Deregulation of certain miRNAs' expression in the cell was consistently observed during various pathologies including cancers [13]. Every miRNA has a unique nucleotide sequence and unique expression pattern in a certain cell type [13, 14], however different miRNAs can cooperatively regulate the same target [3]. There have been more than 2000 different human miRNA species discovered so far and this amount is increasing [15]. MicroRNAs are very stable small RNAs that are most often bound to Argonaute (AGO) proteins. Interestingly, miRNAs have been found in circulation in the serum of healthy individuals as well as in patients with several different pathologies [16].

2. CIRCULATING NON-CODING RNAs

The detection of miRNAs in biological fluids including blood plasma, urine, tears, breast milk, amniotic fluid, cerebrospinal fluid, saliva, and semen, evidence the stability of these molecules in an adverse environment [15]. These extracellular circulating miRNAs survive under unfavorable physiological conditions such as extreme variations in pH, which contrast to common RNA species like mRNA, rRNA, and tRNA that are degraded within seconds after being placed in a nuclease rich extracellular environment [15].

In 2008 Hunter *et al.*, proposed that extracellular miRNAs were protected by encapsulation into membrane-vesicles, after miRNAs detection in peripheral blood microvesicles [17]. These results combined with the previous

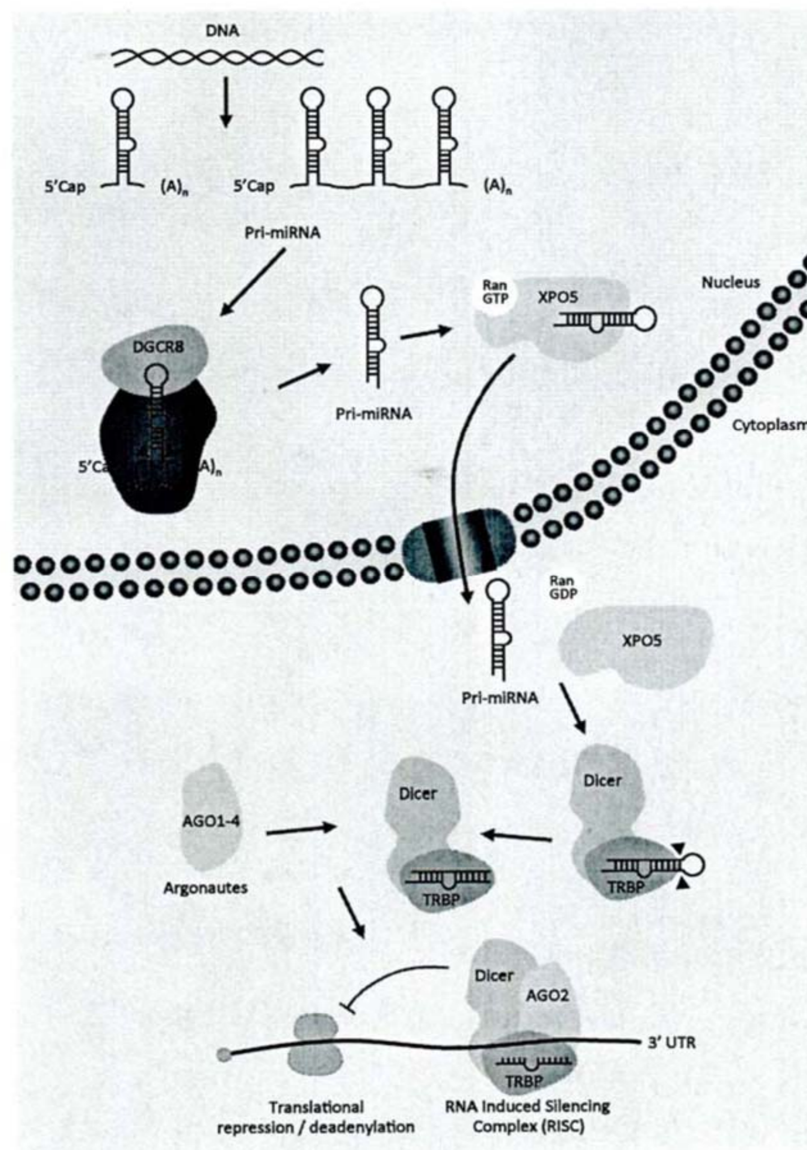

Fig. (1). MiRNAs biogenesis pathway.

findings that cells in culture transport intracellular miRNAs into the extracellular environment by exosomes [18] led to hypothesize the existence of an intercellular and inter-organ communication system in the body mediated by Extracellular Vesicles (EVs) encapsulated miRNAs. However, four years later two studies suggested that 90–99% of extracellular miRNA are indeed outside EVs and associated with proteins of the AGO family both in blood plasma/serum and cell culture media [16, 19]. Turchinovich *et al*., (2011) also showed the stability of AGO2 protein in protease rich environment which explains the stability and resistance of miRNAs in biological fluids [16] thus unraveling the two mechanisms by which miRNA can be shielded from RNase activity when in circulation: 1)EVs encapsulation and 2)AGO proteins association. Whilst circulating miRNAs bound solely by AGO proteins are apparently non-specific remnants resulting from physiological activity of the cells and cell death, there is increasing evidence that cells selectively encapsulate miRNAs into EVs and secrete them outside the cell. The mechanisms behind this statement remains, however, to be elucidated [15].

3. EXTRACELLULAR VESICLES

The concept of intercellular communication has gained insight in recent years. Cell-cell contact and the transfer of secreted molecules are the best studied mechanisms accounting for this process, however a third mechanism has emerged which involves the intercellular transfer of EVs [20]. EVs are small membrane vesicles that contain different types of RNAs [21], proteins [22] and more recently demonstrated, DNA [23] enclosed by a phospholipid bilayer released by all eukaryotic and prokaryotic cells. The diameter of EVs typically ranges from 30 nm to 1 μm, the smallest of which are 100-fold smaller than the smallest cells [24]. The origin and nomenclature of EVs is still very elusive and is mainly because current available extraction and purification methods are not clear enough to precisely discriminate between different types of vesicles, as exossomes and microvesicles (MVs) [20]. As such, many names have been proposed referring to important criteria for classification such as size, density, morphology, lipid composition, protein composition, and subcellular origin [24, 25]. The terms ectosome, shedding vesicle, microparticle and MV generally refer to 150-1000 nm vesicles released by budding from the plasma membrane [26]. The term exosomes on the other hand was initially used for vesicles ranging from 40-1000 nm that are released by a variety of cultured cells [27], and later for 40-100nm vesicles resulting from multivesicular endosome fusion with the plasma membrane during the process of reticulocyte differentiation [28]. Finally a range of 40-150 nm in diameter of vesicles coined the term exosomes [25]. Later, B lymphocytes and dendritic cells were shown to release similar vesicles of endosomal origin [29]. Several studies demonstrated the release of both exosomes and MVs by a single cell type [30-32]. One of the main criteria for assessing the differences between exosomes and MVs resides on the shedding process of these vesicles. While larger vesicles are directly shed from the plasma membrane, exosomes derive from the intercellular endosomal compartment [22].

Starting from an early endosome (Fig. **2A**), exosomes biogenesis involves the production of intraluminal vesicles (ILVs) within multivesicular bodies (MVBs) of the membrane and the release of the vesicle to its lumen (Fig. **2B**). This is a well studied process with its known intervenients, such as the endosomal sorting complex required for transport (ESCRT). The ESCRT-0, -I and –II complexes are responsible for the recognition and sequestration of ubiquitinated proteins in the endosomal membrane while ESCRT-III is responsible for the inward budding of the membrane [25, 33]. MVBs containing the ILVs then fuse with cytoplasmic membrane (Fig. **2C**). Further secretion of exosomes (Fig. **2D**) is promoted by the RabGTPases27a and 27b which belong to a small family of proteins involved in cellular trafficking [22]. Recent studies provided evidence that exosomes biogenesis could also occur via an ESCRT-independent mechanism dependent on sphingomyelinase, an enzyme that produces ceramide [34]. These observations are consistent with the presence of high concentrations of ceramide and its derivatives in exosomes [34, 35]. Importantly, the composition of an exosome is not a mere reflection of the donor cell, and it has been shown that the profiles of exosomal cargo can be substantially different from the originating cell, which indicates the existence of a highly controlled sorting process [36].

Currently, exosomes are defined as 40-150 nm diameter bilayered membrane vesicles of endocytic origin, with a cup-shaped morphology with densities ranging between 1.13 - 1.19 g/mL [24]. Because they fall below the resolution limit of optical microscopy, transmission electron microscopy and atomic force microscopy have been the preferred techniques for the direct observation of exosomes' size and morphology, so far [25]. Yet, recently a device allowing Nanoparticle Tracking Analysis (NTA) which tracks the movement of laser-illuminated individual particles under Brownian motion, has been developed, enabling a fast and simple way of analyzing large numbers of particles at the same time [37]. Exosomes are present in almost all biological fluids including blood, urine, ascites, cerebrospinal fuid [38-41] serum and plasma [7], and in the culture medium of cell cultures [42]. In terms of biochemical composition, the membrane of exosomes contains high levels of cholesterol, sphingomyelin, ceramide and detergent-resistant membrane domains [43, 44]. One important feature of exosomes is that due to its biogenesis, the proteins on the surface of its membrane have the same orientation as the cell's. Initial proteomic studies indicated that exosomes contain a particular set of proteins depending on the cell that secretes them, while others are found in most exosomes independent on the cell type [25]. Proteins from endosomes, plasma membrane and cytosol refer to the second group, whereas proteins from the mitochondria, endoplasmic reticulum and Golgi complex are not usually present in exosomes. A clear distinction between MVs and exosomes in terms of protein characterization is hampered by the presence of ubiquitous proteins including ALIX, CHMP4B, RAB11A, RAB5 [25]. Exosomes however, are characterized by the presence of proteins involved in membrane transport and fusion processes such as Rab, GTPases, annexins and flotilin, components of the endosomal sorting complex required for transport (ESCRT) complex, tumor susceptibility gene 101 (TSG101), heat shock proteins (HSPs), integrins and tetraspanins including CD81, CD63 and CD82 [20, 25, 45]. Furthermore, exosomes also contain proteins that are involved in specific cell functions. For in-

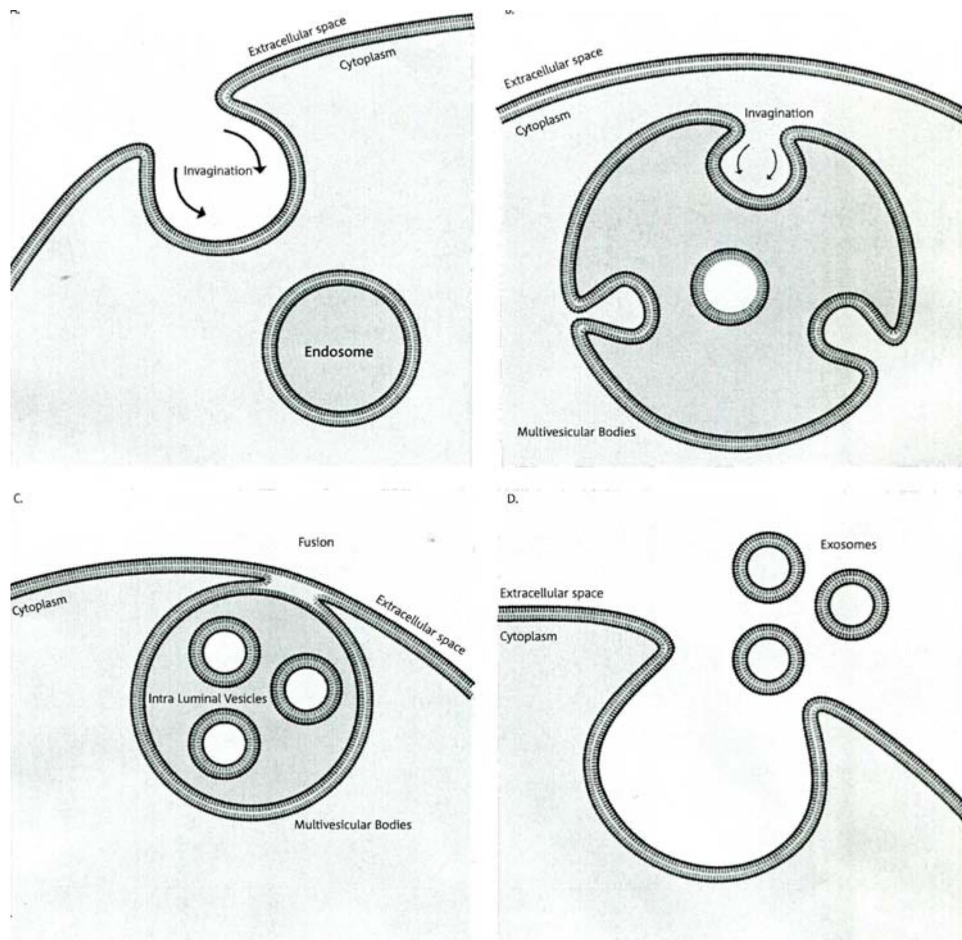

Fig. (2). Schematic representation of exosomes biogenesis. Following endocytosis into early endosomes (**A**) ILVs are formed through an inward budding of the membrane (**B**). MVBs can then fuse with the plasma membrane (**C**), resulting in the release of the ILVs as exosomes into the extracellular space (**D**).

stance, MHC class II molecules were shown to be abundant in exosomes from all antigen presenting cells (APCs) that express MHC class II [46]. Additionally epithelial tumor cells secrete exosomes carrying the epithelial cell adhesion molecule (EpCAM) [47] and gastric [48], breast [49], and pancreas cancer [50] derived exosomes express members of the human epidermal receptor (HER) family. Although not thoroughly studied, enrichment of sphingomyelin, phosphatidylserine, cholesterol, saturated fatty acids, ceramide and its derivatives were observed as lipid components of the membranes of exosomes (Fig. **3**) [34, 51, 52].

Although some models of exosomes uptake in target cells have been proposed, there is not a consensus regarding the definition of the mechanisms. Exosomal communication may occur through the direct interaction of membrane proteins with receptors in a target cell, and activate intracellular signaling processes (Fig. **4A**). The second proposed mechanism resides in the cleavage of exosomal membrane proteins by proteases in the extracellular space resulting in fragments with different sizes. These fragments can then act as a ligand that binds to the target cell protein receptor (Fig. **4B**). Exosomes can also fuse with the target cell membrane and release its content directly onto the recipient cell (Fig. **4C**). Lastly exosomes are phagocyted in an actin-cytoskeletal and phsphatidylinositol 3-kinase-dependent manner (Fig. **4D**) [22, 44].

Exosomes have recently emerged as important mediators in cell communication due to their enriched content in genetic material like mRNAs and non-coding RNAs [18]. These were shown to be functional in recipient cells [18]. Nonetheless several questions remained to be addressed, amongst them how can exosomes carry stoichiometric amounts of miRNAs to affect gene expression post-transcriptionally in the recipient cell?

4. EXOSOMES AND CANCER

Although the discovery of exosomes dates decades ago, only recently its study has gained serious insight as they are continuously being implicated in important disease mechanisms such as cancer. In fact exosomes can help the cancer to progress and disseminate by manipulating the local and distant biological environment. On the other hand, exosomes can also program the immune system in order to evoke an anti-tumor response by the organism [22]. The duality of roles made clear that the network of interactions created by exosomes is complex and of utmost importance for a better understanding of the carcinogenic process.

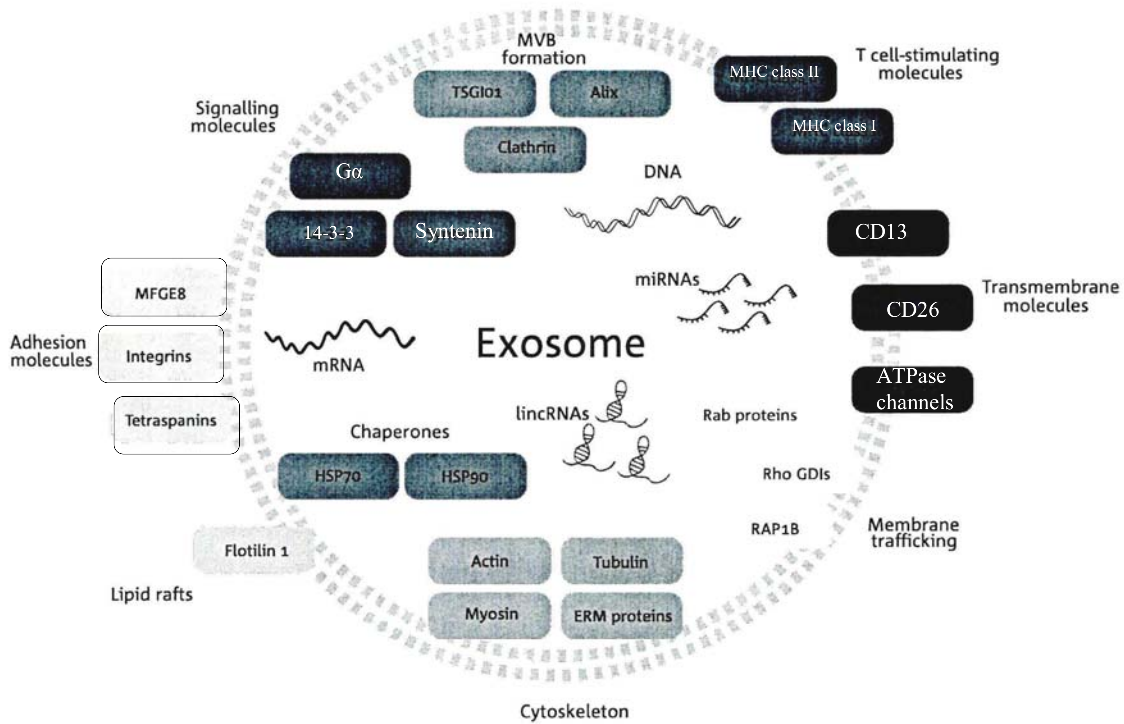

Fig. (3). Overall composition of exosomes.

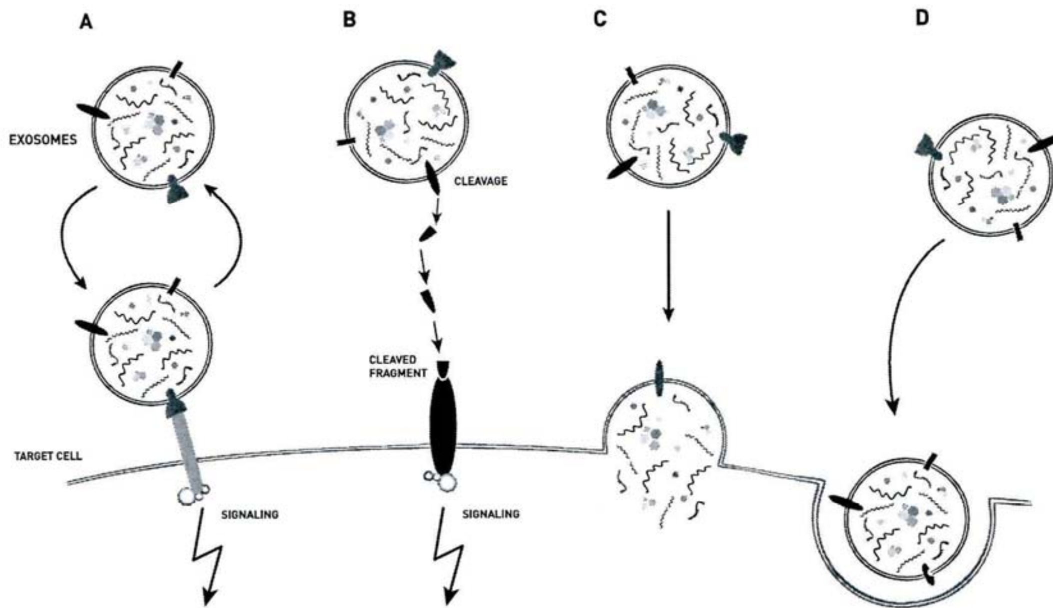

Fig. (4). Proposed mechanisms of intercellular communication between exosomes and target cells: **A**) Direct interaction with receptors in the target cell and activation of intracellular signaling; **B**) Interaction with the target cell through ligation of previous cleaved fragments released from exosomes; **C**) Fusion of the exosomes with the membrane of target cell; **D**) Exosomes are phagocyted by the target cell.

Tumor cells exchange oncogenic proteins between them or with normal surrounding cells, via exosomes [53]. Although the purpose of this cross-talk is not yet known, some examples have confirmed this level of communication. For instance, the protein EGFRvIII can be delivered intercellularly through exosomes from glioma cells to nearby cells lacking this mutant form, which in turn leads to activation of transforming signaling pathways [53]. Furthermore, exosomes extracted from a *KRAS* mutant cell line containing mutant KRAS protein enhanced cell growth and tumorigenicity in a wild-type KRAS-expressing non-transformed cells, upon transfer [54]. *In vitro* experiments showed that

exosomes containing TGF-B1 can trigger the differentiation of fibroblasts to myofibroblasts through SMAD-dependent signaling [55]. Since myofibroblasts are key producers of proteins involved in the remodeling of the matrix of the tumor microenvironment and actively participate in angiogenesis, the role of exosomes in the recruitment of fibroblasts could enhance angiogenesis [22]. In fact, exosomes were shown to participate in the formation of the pre-metastatic niche in an *in vivo* pancreas cancer model [56]. Another example depicting the tumorigenic role of exosomes is the study by Peinado and colleagues (2012) where they demonstrate in mice, that exosomes from metastatic melanoma cells can enhance tumorigenesis by recruiting bone marrow derived cells to initiate a pre-metastatic niche [57].

Exosomes are reported to predominantly contain different kinds of RNA and protein. Two previous studies have shown the presence of mitochondrial DNA [58], single stranded DNA and transposable elements [59] in exosomes. However, only recently, evidences were found that exosomes carry fragments of double-stranded DNA in a study where exosomes from pancreas cancer cells and serum from patients were used [23]. Furthermore, mutations in *KRAS* and *p53* were detected in the genomic DNA of these exosomes.

MiRNAs play important roles in several cellular processes by regulating the expression of hundreds of genes. Studies reported evidences that transfer of exosomes associated miRNAs to recipient cells occurred, which results in altered gene expression and functional effects [18, 60-63]. In 2012 Chiba *et al*., demonstrated that the exosomes derived from three different colorectal cancer cell lines contained mRNAs, miRNAs, and natural antisense RNAs, and were delivered into recipient cells [63]. In addition, some of these reports have demonstrated that the transferred exosomal content can be functional in the receptor cells [64]. Yang *et al.* (2011) reported the migration of SKBR3 and MDA-MB-231 breast cancer cells in a transwell invasion assay after treating macrophages with IL-4 secreted exosomes containing the miRNA miR-223. Conversely blocking miR-223 prevented the increased invasion capacity previously observed. Furthermore the mRNA target level of that specific miR-223 was reduced in the recipient cells after exosome treatment [64].

The modulating features of exosomes were assessed in a recent study in which exosomes from normal bone marrow cells containing miR-15 can have a tumor suppressor effect upon transfer to multiple myeloma cells, where the expression of this miRNA is low [65]. Also, after infecting B-lymphoblastoid cells with Epstein-Barr virus, Pegtel and colleagues, (2010) showed that exosomes secreted the virus specific miRNAs and that these affected the expression of the target gene, thus revealing the ability of exosomes to facilitate viral infection though miRNAs [61]. More recently, exosomes were implicated in the metastatic process by a study of Valencia and colleagues (2014). Using an *in vivo* murine model they demonstrated that the miR-192 was specifically enriched in exosomes and that these markedly appeased the metastatic burden and tumor colonization in the bone [66]. The work from Kosaka and colleagues (2012) showed the tumor suppressor effect of the exosomal miR-143 derived from normal protstate cells through inhibition of the growth of target cancer cells *in vivo* and *in vitro* [67].

Intercellular communication through exosomes has also been proposed as a possible mechanism of spread of resistance or sensitivity of cancer cells to a specific therapy. Although the precise mechanism(s) by which it occurs is still elusive, Xiao *et al*, (2014) demonstrated that exosomes released by cells that were exposed to chemotherapy could in turn influence the resistance of target cells to that specific agent [68].

5. BIOMARKERS

Since they are readily accessible in nearly all bodily fluids, exosomes can provide great diagnostic opportunities to profile cancer subtypes (virtually a *liquid biopsy*), unraveling new therapeutic targets and predicting therapeutic responses. The mere fact that exosomes production is increased in cancer, allows for exosomes analysis to be useful for early cancer detection and assessment of disease progression, without the need for tumor biopsy [69].

MicroRNAs in exosomes have been recently described as good biomarkers easily accessible in circulation of cancer patients [60, 70-72]. Based on the levels of eight exosomal miRNAs, Taylor and colleagues (2008) demonstrated that malignant ovarian cancers could be distinguished from benign disease [70]. Other studies have further demonstrated an association of specific miRNAs with a cancer type, for instance miR-107, miR-574-3p, miR-1290 and miR-375 in prostate cancer [71, 73], miR-141 and miR- 195 in breast cancer [72] and the serum-derived miR-21 in glioblastoma [60]. In 2014 Rodríguez and colleagues demonstrated the potential use of exosomal miRNAs as biomarkers, which were enriched in plasma of lung cancer patients compared to the bronchoalveolar lavage of these patients [74]. In another report, the authors suggested a distinction between normal and lung adenocarcinoma samples could be achieved based on the expression of the exosomal miRNAs miR-378a, miR-379, miR-139-5p and miR-200-5p [75].

It is not only in serum or plasma that exosomes exert their potential as cancer biomarkers. Recently, a miRNA panel extracted from bile-derived exosomes from cholangiocarcinoma patients was proposed to be of relevance in terms of disease diagnosis [76]. Also results from the work of Liu and colleagues (2014) showed high expression levels of miR-21 and miR-146a in exosomes derived from cervico-vaginal fluid compared to those from HPV positive and HPV negative normal samples [77].

6. PRECURSOR microRNAs PROCESSING IN CANCER EXOSOMES

Several reports suggest that miRNAs contained in exosomes can influence gene expression in recipient cells [18, 61, 67, 78]. Nonetheless, single-stranded miRNAs by themselves incorporate the RNA-Induced Silencing Complex (RISC) very poorly and therefore, cannot be efficiently directed to the target mRNAs for post-transcriptional regulation [79, 80]. We have recently described a mechanism by which cancer-derived exosomes are able to incorporate precursor miRNAs (pre-miRNAs) in complex with Dicer,

TRBP and AGO2 proteins, allowing for their processing in a cell-independent fashion [42]. Therefore, after their processing, miRNAs are already integral part of the RISC complex that will guide the miRNA in the recipient cell to its mRNA target more efficiently. This process allows for cancer cell-derived exosomes to more efficiently regulate gene expression post-transcriptionally at the recipient cell. This was the first study to report a cell-autonomous process occurring in exosomes upon their secretion to the extracellular space [42]. This study opens a new perspective on exosomes biology, as vesicles that might have cell-independent processes occurring in the extracellular space.

7. NON-CODING RNAs IN EXOSOMES AND THERAPY

The intrinsic ability of exosomes to efficiently shuttle small molecules as perfect non-immunogenic carriers of therapeutic agents to target cells makes them an extremely promising therapeutic tool for numerous diseases, including cancer. Thus intercellular transfer by exosomes can be used as ncRNAs carriers for instance to restore miRNA expression in target cells where they might play a therapeutic role as tumor suppressor factors [81]. As Ohno and colleagues (2013) elegantly demonstrated, engineered exosomes expressing the transmembrane domain of the PDGF fused to the GE11 peptide can accurately deliver the let-7a miRNA after injection to EGFR-expressing xenograft breast cancer tissue in immunodeficient mice [82]. It is important to note however that miRNAs do not require full binding to their target mRNA sequences for inhibiting effect. This allows them to act synergistically on various molecules within signaling pathways lowering its target specificity [83]. The use of synthetic siRNA has been exploited as an alternative, more selective tool. Exosomes loaded with exogenous siRNA to GAPDH were injected in mice and the siRNA delivered specifically to neurons, microglia, oligodendrocytes in the brain resulting in a selective gene knockdown [84]. Additionally, the delivery of a siRNA leads to a selective gene silencing of *MAPK1* in monocytes and lymphocytes in a study by Wahlgren and colleagues (2012) [85].

8. CONCLUSION

The prospective of accessibility of exosomes in almost all biofluids, such as plasma, lymph, cerebrospinal fluid, urine, or malignant ascites brings to the fore some truly unprecedented diagnostic opportunities. The identification of the non-coding RNAs in circulation during tumor progression and therapy, may provide a unique, remote, non-invasive, and virtually continuous access to the changing molecular make up of cancer cells (virtually a liquid biopsy), with significant clinical implications. Finally, the understanding of their role and selective packaging in cancer exosomes will unravel novel functions of these non-coding RNAs in cancer progression.

LIST OF ABBREVIATIONS

snoRNAs = Small nucleolar RNAs

PASRs = Promoter-associated short RNAs

TSSs-RNA = Transcription start site RNA

PROMPT = Promoter Upstream Transcript

lincRNA = Long interspersed ncRNA

T-UCR = Transcribed ultraconserved ncRNA

3' UTR = 3' Untranslated Region

ESCRT = Endosomal sorting complex required for transport

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Higgs, P.G.; Lehman, N. The RNA World: molecular cooperation at the origins of life. *Nat. Rev. Genet.*, **2015**, *16*(1), 7-17.

[2] Mattick, J.S.; Makunin, I.V. Non-coding RNA. *Hum. Mol. Genet.*, **2006**, *15* Spec No 1, R17-29.

[3] Esteller, M. Non-coding RNAs in human disease. *Nat. Rev. Genet.*, **2011**, *12*(12), 861-74.

[4] Mercer, T.R.; Dinger, M.E.; Mattick, J.S. Long non-coding RNAs: insights into functions. *Nat. Rev. Genet.*, **2009**, *10*(3), 155-9.

[5] Taft, R.J.; Pang, K.C.; Mercer, T.R.; Dinger, M.; Mattick, J.S. Non-coding RNAs: regulators of disease. *J Pathol.*, **2010**, *220*(2), 126-39.

[6] Kiss-Laszlo, Z.; Henry, Y.; Bachellerie, J.P.; Caizergues-Ferrer, M.; Kiss, T. Site-specific ribose methylation of preribosomal RNA: a novel function for small nucleolar RNAs. *Cell*, **1996**, *85*(7), 1077-88.

[7] Melo, S.A.; Moutinho, C.; Ropero, S.; Calin, G.A.; Rossi, S.; Spizzo, R.; Fernandez, A.F.; Davalos, V.; Villanueva, A.; Montoya, G.; Yamamoto, H.; Schwartz, S. Jr.; Esteller, M. A genetic defect in exportin-5 traps precursor microRNAs in the nucleus of cancer cells. *Cancer Cell*, **2010**, *18*(4), 303-15.

[8] Lee, R.C.; Feinbaum, R.L.; Ambros, V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell*, **1993**, *75*(5), 843-54.

[9] Ambros, V. The functions of animal microRNAs. *Nature*, **2004**, *431*(7006), 350-5.

[10] Bartel, D.P. MicroRNAs: target recognition and regulatory functions. *Cell*, **2009**, *136*(2), 215-33.

[11] Melo, S.A.; Esteller, M. Disruption of microRNA nuclear transport in human cancer. *Semin. Cancer Biol.*, **2014**, *27*, 46-51.

[12] Winter, J.; Jung, S.; Keller, S.; Gregory, R.I.; Diederichs, S. Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat. Cell Biol.*, **2009**, *11*(3), 228-34.

[13] Lu, J.; Getz, G.; Miska, E.A.; Alvarez-Saavedra, E.; Lamb, J.; Peck, D.; Sweet-Cordero, A.; Ebert, B.L.; Mak, R.H.; Ferrando, A.A.; Downing, J.R.; Jacks, T.; Horvitz, H.R.; Golub, T.R. MicroRNA expression profiles classify human cancers. *Nature*, **2005**, *435*(7043), 834-8.

[14] Landgraf, P.; Rusu, M.; Sheridan, R.; Sewer, A.; Iovino, N.; Aravin, A.; Pfeffer, S.; Rice, A.; Kamphorst, A.O.; Landthaler, M., Lin, C.; Socci, N.D.; Hermida, L.; Fulci, V.; Chiaretti, S.; Foa, R.; Schliwka, J.; Fuchs, U.; Novosel, A.; Muller, R.U.; Schermer, B.; Bissels, U.; Inman, J.; Phan, Q.; Chien, M.; Weir, D.B.; Choksi, R.; De Vita, G.; Frezzetti, D.; Trompeter, H.I.; Hornung, V.; Teng, G.; Hartmann, G.; Palkovits, M.; Di Lauro, R.; Wernet, P.; Macino, G.; Rogler, C.E.; Nagle, J.W.; Ju, J.; Papavasiliou, F.N.; Benzing, T.; Lichter, P.; Tam, W.; Brownstein, M.J.; Bosio, A.; Borkhardt, A.; Russo, J.J.; Sander, C.; Zavolan, M.; Tuschl, T. A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell*, **2007**, *129*(7), 1401-14.

[15] Turchinovich, A.; Samatov, T.R.; Tonevitsky, A.G.; Burwinkel, B. Circulating miRNAs: cell-cell communication function? *Front. Genet.*, **2013**, *4*, 119.

[16] Turchinovich, A.; Weiz, L.; Langheinz, A.; Burwinkel, B. Charac-

terization of extracellular circulating microRNA. *Nucleic Acids Res.*, **2011**, *39*(16), 7223-33.

[17] Hunter, M.P.; Ismail, N.; Zhang, X.; Aguda, B.D.; Lee, E.J.; Yu, L.; Xiao, T.; Schafer, J.; Lee, M.L.; Schmittgen, T.D.; Nana-Sinkam, S.P.; Jarjoura, D.; Marsh, C.B. Detection of microRNA expression in human peripheral blood microvesicles. *PLoS One*, **2008**, *3*(11), e3694.

[18] Valadi, H.; Ekstrom, K.; Bossios, A.; Sjostrand, M.; Lee, J.J.; Lotvall, J.O. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell Biol.*, **2007**, *9*(6), 654-9.

[19] Arroyo, J.D.; Chevillet, J.R.; Kroh, E.M.; Ruf, I.K.; Pritchard, C.C.; Gibson, D.F.; Mitchell, P.S.; Bennett, C.F.; Pogosova-Agadjanyan, E.L.; Stirewalt, D.L.; Tait, J.F.; Tewari, M. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc. Natl. Acad. Sci. U.S.A.*, **2011**, *108*(12), 5003-8.

[20] Raposo, G.; Stoorvogel, W. Extracellular vesicles: exosomes, microvesicles, and friends. *J. Cell Biol.*, **2013**, *200*(4), 373-83.

[21] Crescitelli, R.; Lasser C.; Szabo T.G.; Kittel A.; Eldh M.; Dianzani, I.; Buzas, E.I.; Lotvall, J. Distinct RNA profiles in subpopulations of extracellular vesicles: apoptotic bodies, microvesicles and exosomes. *J. Extracell. Vesicles*, **2013**, *2*.

[22] Kahlert, C.; Kalluri, R. Exosomes in tumor microenvironment influence cancer progression and metastasis. *J. Mol. Med. (Berl)*, **2013**, *91*(4), 431-7.

[23] Kahlert, C.; Melo, S.A.; Protopopov, A.; Tang, J.; Seth, S.; Koch, M.; Zhang, J.; Weitz, J.; Chin, L.; Futreal, A.; Kalluri, R. Identification of double-stranded genomic DNA spanning all chromosomes with mutated KRAS and p53 DNA in the serum exosomes of patients with pancreatic cancer. *J. Biol. Chem.*, **2014**, *289*(7), 3869-75.

[24] van der Pol, E.; Boing, A.N.; Harrison, P.; Sturk, A.; Nieuwland, R. Classification, functions, and clinical relevance of extracellular vesicles. *Pharmacol. Rev.*, **2012**, *64*(3), 676-705.

[25] Colombo, M.; Raposo, G.; Thery, C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu. Rev. Cell. Dev. Biol.*, **2014**, *30*, 255-89.

[26] Gould, S.J.; Raposo, G. As we wait: coping with an imperfect nomenclature for extracellular vesicles. *J. Extracell. Vesicles*, **2013**, *2*.

[27] Trams, E.G.; Lauter, C.J.; Salem, N. Jr.; Heine, U. Exfoliation of membrane ecto-enzymes in the form of micro-vesicles. *Biochim. Biophys. Acta*, **1981**, *645*(1), 63-70.

[28] Harding, C.; Heuser, J.; Stahl, P. Endocytosis and intracellular processing of transferrin and colloidal gold-transferrin in rat reticulocytes: demonstration of a pathway for receptor shedding. *Eur. J. Cell Biol.*, **1984**, *35*(2), 256-63.

[29] Raposo, G.; Nijman, H.W.; Stoorvogel, W.; Liejendekker, R.; Harding, C.V.; Melief, C.J.; Geuze, H.J. B lymphocytes secrete antigen-presenting vesicles. *J. Exp. Med.*, **1996**, *183*(3), 1161-72.

[30] Heijnen, H.F.; Schiel, A.E.; Fijnheer, R.; Geuze, H.J.; Sixma, J.J. Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. *Blood*, **1999**, *94*(11), 3791-9.

[31] Deregibus, M.C.; Cantaluppi, V.; Calogero, R.; Lo Iacono, M.; Tetta, C.; Biancone, L.; Bruno, S.; Bussolati, B.; Camussi, G. Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. *Blood*, **2007**, *110*(7), 2440-8.

[32] Muralidharan-Chari, V.; Clancy, J.; Plou, C.; Romao, M.; Chavrier, P.; Raposo, G.; D'Souza-Schorey, C. ARF6-regulated shedding of tumor cell-derived plasma membrane microvesicles. *Curr. Biol.*, **2009**, *19*(22), 1875-85.

[33] Simons, M.; Raposo, G. Exosomes--vesicular carriers for intercellular communication. *Curr. Opin. Cell Biol.*, **2009**, *21*(4), 575-81.

[34] Trajkovic, K.; Hsu, C.; Chiantia, S.; Rajendran, L.; Wenzel, D.; Wieland, F.; Schwille, P.; Brugger, B.; Simons, M. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science*, **2008**, *319*(5867), 1244-7.

[35] Brouwers, J.F.; Aalberts, M.; Jansen, J.W.; van Niel, G.; Wauben. M.H.; Stout, T.A.; Helms, J.B.; Stoorvogel, W. Distinct lipid compositions of two types of human prostasomes. *Proteomics*, **2013**, *13*(10-11), 1660-6.

[36] Villarroya-Beltri, C.; Baixauli, F.; Gutierrez-Vazquez C.; Sanchez-Madrid, F.; Mittelbrunn, M. Sorting it out: regulation of exosome loading. *Semin. Cancer Biol.*, **2014**, *28*, 3-13.

[37] Dragovic, R.A.; Gardiner, C.; Brooks, A.S.; Tannetta, D.S.; Ferguson, D.J.; Hole, P.; Carr, B.; Redman, C.W.; Harris, A.L.; Dobson, P.J.; Harrison, P.; Sargent I.L. Sizing and phenotyping of cellular vesicles using Nanoparticle Tracking Analysis. *Nanomedicine*, **2011**, *7*(6), 780-8.

[38] Pisitkun, T.; Shen, R.F.; Knepper, M.A. Identification and proteomic profiling of exosomes in human urine. *Proc. Natl. Acad. Sci. U.S.A.*, **2004**, *101*(36), 13368-73.

[39] Caby, M.P.; Lankar. D.; Vincendeau-Scherrer. C.; Raposo, G.; Bonnerot, C. Exosomal-like vesicles are present in human blood plasma. *Int. Immunol.*, **2005**, *17*(7), 879-87.

[40] Keller, S.; Sanderson M.P.; Stoeck, A.; Altevogt, P. Exosomes: from biogenesis and secretion to biological function. *Immunol. Lett.*, **2006**, *107*(2), 102-8.

[41] Vella, L.J.; Greenwood, D.L.; Cappai, R.; Scheerlinck, J.P.; Hill, A.F. Enrichment of prion protein in exosomes derived from ovine cerebral spinal fluid. *Vet. Immunol. Immunopathol.,* **2008**, *124*(3-4), 385-93.

[42] Melo, S.A.; Sugimoto, H.; O'Connell, J.T.; Kato, N.; Villanueva, A.; Vidal, A.; Qiu, L.; Vitkin, E.; Perelman, L.T.; Melo, C.A.; Lucci, A.; Ivan, C.; Calin, G.A.; Kalluri, R. Cancer exosomes perform cell-independent microRNA biogenesis and promote tumorigenesis. *Cancer Cell*, **2014**, *26*(5), 707-21.

[43] Thery, C.; Ostrowski, M.; Segura, E. Membrane vesicles as conveyors of immune responses. *Nat. Rev. Immunol.*, **2009**, *9*(8), 581-93.

[44] Mathivanan, S.; Ji, H.; Simpson, R.J. Exosomes: extracellular organelles important in intercellular communication. *J. Proteomics*, **2010**, *73*(10), 1907-20.

[45] Record, M.; Subra, C.; Silvente-Poirot, S.; Poirot, M. Exosomes as intercellular signalosomes and pharmacological effectors. *Biochem. Pharmacol.*, **2011**, *81*(10), 1171-82.

[46] Thery, C.; Zitvogel, L.; Amigorena, S. Exosomes: composition, biogenesis and function. *Nat. Rev. Immunol.*, **2002**, *2*(8), 569-79.

[47] Runz, S.; Keller, S.; Rupp, C.; Stoeck, A.; Issa, Y.; Koensgen, D.; Mustea, A.; Sehouli, J.; Kristiansen, G.; Altevogt, P. Malignant ascites-derived exosomes of ovarian carcinoma patients contain CD24 and EpCAM. *Gynecol. Oncol.*, **2007**, *107*(3), 563-71.

[48] Baran, J.; Baj-Krzyworzeka, M.; Weglarczyk, K.; Szatanek, R.; Zembala, M.; Barbasz, J.; Czupryna, A.; Szczepanik, A.; Zembala, M. Circulating tumour-derived microvesicles in plasma of gastric cancer patients. *Cancer Immunol. Immunother.*, **2010**, *59*(6), 841-50.

[49] Ciravolo, V.; Huber, V.; Ghedini, G.C.; Venturelli, E.; Bianchi, F.; Campiglio, M.; Morelli, D.; Villa, A.; Della Mina, P.; Menard, S.; Filipazzi, P.; Rivoltini, L.; Tagliabue, E.; Pupa, S.M. Potential role of HER2-overexpressing exosomes in countering trastuzumab-based therapy. *J. Cell Physiol.*, **2012**, *227*(2), 658-67.

[50] Adamczyk, K.A.; Klein-Scory, S.; Tehrani, M.M.; Warnken, U.; Schmiegel, W.; Schnolzer, M.; Schwarte-Waldhoff, I. Characterization of soluble and exosomal forms of the EGFR released from pancreatic cancer cells. *Life Sci.*, **2011**, *89*(9-10), 304-12.

[51] Llorente, A.; Skotland, T.; Sylvanne, T.; Kauhanen, D.; Rog, T.; Orlowski, A.; Vattulainen, I.; Ekroos, K.; Sandvig, K. Molecular lipidomics of exosomes released by PC-3 prostate cancer cells. *Biochim. Biophys. Acta*, **2013**, *1831*(7), 1302-9.

[52] Laulagnier, K.; Motta, C.; Hamdi, S.; Roy, S.; Fauvelle, F.; Pageaux, J.F.; Kobayashi, T.; Salles, J.P.; Perret, B.; Bonnerot, C.; Record, M. Mast cell- and dendritic cell-derived exosomes display a specific lipid composition and an unusual membrane organization. *Biochem. J.*, **2004**, *380*(Pt 1), 161-71.

[53] Al-Nedawi, K.; Meehan, B.; Micallef, J.; Lhotak, V.; May, L.; Guha, A.; Rak, J. Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. *Nat. Cell Biol.*, **2008**, *10*(5), 619-24.

[54] Demory Beckler, M.; Higginbotham, J.N.; Franklin, J.L.; Ham, A.J.; Halvey, P.J.; Imasuen, I.E.; Whitwell, C.; Li, M.; Liebler, D.C.; Coffey, R.J. C. Proteomic analysis of exosomes from mutant KRAS colon cancer cells identifies intercellular transfer of mutant KRAS. *Mol. Cell. Proteomics*, **2013**, *12*(2), 343-55.

[55] Webber, J.; Steadman, R.; Mason, M.D.; Tabi, Z.; Clayton, A. Cancer exosomes trigger fibroblast to myofibroblast differentiation. *Cancer Res.*, **2010**, *70*(23), 9621-30.

[56] Jung, T.; Castellana, D.; Klingbeil, P.; Cuesta Hernandez, I.; Vita-

colonna, M.; Orlicky, D.J.; Roffler, S.R.; Brodt, P.; Zoller, M. CD44v6 dependence of premetastatic niche preparation by exosomes. *Neoplasia*, **2009**, *11*(10), 1093-105.

[57] Peinado, H.; Aleckovic, M.; Lavotshkin, S.; Matei, I.; Costa-Silva, B.; Moreno-Bueno, G.; Hergueta-Redondo, M.; Williams, C.; Garcia-Santos, G.; Ghajar, C.; Nitadori-Hoshino, A.; Hoffman, C.; Badal, K.; Garcia. B.A.; Callahan, M.K.; Yuan, J.; Martins, V.R.; Skog, J.; Kaplan, R.N.; Brady, M.S.; Wolchok, J.D.; Chapman, P.B.; Kang, Y.; Bromberg, J.; Lyden, D. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat. Med.*, **2012**, *18*(6), 883-91.

[58] Guescini, M.; Guidolin, D.; Vallorani, L.; Casadei, L.; Gioacchini, A.M.; Tibollo, P.; Battistelli, M.; Falcieri, E.; Battistin, L.; Agnati, L.F.; Stocchi, V. C2C12 myoblasts release micro-vesicles containing mtDNA and proteins involved in signal transduction. *Exp. Cell Res.*, **2010**, *316*(12), 1977-84.

[59] Balaj, L.; Lessard, R.; Dai, L.; Cho, Y.J.; Pomeroy, S.L.; Breakefield, X.O.; Skog, J. Tumour microvesicles contain retrotransposon elements and amplified oncogene sequences. *Nat. Commun.*, **2011**, *2*, 180.

[60] Skog, J.; Wurdinger, T.; van Rijn, S.; Meijer, D.H.; Gainche, L.; Sena-Esteves, M.; Curry, W.T.; Carter, B.S.; Krichevsky, A.M.; Breakefield, X.O. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat. Cell Biol.*, **2008**, *10*(12), 1470-6.

[61] Pegtel, D.M.; Cosmopoulos, K.; Thorley-Lawson, D.A.; van Eijndhoven, M.A.; Hopmans, E.S.; Lindenberg, J.L.; de Gruijl, T.D.; Wurdinger, T.; Middeldorp, J.M. Functional delivery of viral miRNAs via exosomes. *Proc. Natl. Acad. Sci. U.S.A.*, **2010**, *107*(14), 6328-33.

[62] Montecalvo, A.; Larregina, A.T.; Shufesky, W.J.; Stolz, D.B.; Sullivan, M.L.; Karlsson, J.M.; Baty, C.J.; Gibson, G.A.; Erdos, G.; Wang, Z.; Milosevic, J.; Tkacheva, O.A.; Divito, S.J.; Jordan, R.; Lyons-Weiler, J.; Watkins, S.C.; Morelli, A.E. Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes. *Blood*, **2012**, *119*(3), 756-66.

[63] Chiba, M.; Kimura, M.; Asari, S. Exosomes secreted from human colorectal cancer cell lines contain mRNAs, microRNAs and natural antisense RNAs, that can transfer into the human hepatoma HepG2 and lung cancer A549 cell lines. *Oncol. Rep.*, **2012**, *28*(5), 1551-8.

[64] Yang, M.; Chen, J.; Su, F.; Yu, B.; Su, F.; Lin, L.; Liu, Y.; Huang, J.D.; Song, E. Microvesicles secreted by macrophages shuttle invasion-potentiating microRNAs into breast cancer cells. *Mol. Cancer*, **2011**, *10*, 117.

[65] Roccaro, A.M.; Sacco, A.; Maiso, P.; Azab, A.K.; Tai, Y.T.; Reagan, M.; Azab, F.; Flores, L.M.; Campigotto, F.; Weller, E.; Anderson, K.C.; Scadden, D.T.; Ghobrial, I.M. BM mesenchymal stromal cell-derived exosomes facilitate multiple myeloma progression. *J. Clin. Invest.*, **2013**, *123*(4), 1542-55.

[66] Valencia, K.; Luis-Ravelo, D.; Bovy, N.; Anton, I.; Martinez-Canarias, S.; Zandueta, C.; Ormazabal, C.; Struman, I.; Tabruyn, S.; Rebmann, V.; De Las Rivas, J.; Guruceaga, E.; Bandres, E.; Lecanda, F. miRNA cargo within exosome-like vesicle transfer influences metastatic bone colonization. *Mol. Oncol.*, **2014**, *8*(3), 689-703.

[67] Kosaka, N.; Iguchi, H.; Yoshioka, Y.; Hagiwara, K.; Takeshita, F.; Ochiya, T. Competitive interactions of cancer cells and normal cells via secretory microRNAs. *J. Biol. Chem.*, **2012**, *287*(2), 1397-405.

[68] Xiao, X.; Yu, S.; Li, S.; Wu, J.; Ma, R.; Cao, H.; Zhu, Y.; Feng, J. Exosomes: decreased sensitivity of lung cancer A549 cells to cisplatin. *PLoS One*, **2014**, *9*(2), e89534.

[69] Brinton, L.T.; Sloane, H.S.; Kester, M.; Kelly, K.A. Formation and role of exosomes in cancer. *Cell. Mol. Life Sci.*, **2015**, *72*(4), 659-71.

[70] Taylor, D.D.; Gercel-Taylor, C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecol. Oncol.*, **2008**, *110*(1), 13-21.

[71] Bryant, R.J.; Pawlowski, T.; Catto, J.W.; Marsden, G.; Vessella, R.L.; Rhees, B.; Kuslich, C.; Visakorpi, T.; Hamdy, F.C. Changes in circulating microRNA levels associated with prostate cancer. *Br. J. Cancer*, **2012**, *106*(4), 768-74.

[72] Corcoran, C.; Friel, A.M.; Duffy, M.J.; Crown, J.; O'Driscoll, L. Intracellular and extracellular microRNAs in breast cancer. *Clin. Chem.*, **2011**, *57*(1), 18-32.

[73] Huang, X.; Yuan, T.; Liang, M.; Du, M.; Xia, S.; Dittmar, R.; Wang, D.; See, W.; Costello, B.A.; Quevedo, F.; Tan, W.; Nandy, D.; Bevan, G.H.; Longenbach, S.; Sun, Z.; Lu, Y.; Wang, T.; Thibodeau, S.N.; Boardman, L.; Kohli, M.; Wang, L. Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer. *Eur. Urol.*, **2015**, *67*(1), 33-41.

[74] Rodriguez, M.; Silva, J.; Lopez-Alfonso, A.; Lopez-Muniz, M.B.; Pena, C.; Dominguez, G.; Garcia, J.M.; Lopez-Gonzalez, A.; Mendez, M.; Provencio, M.; Garcia, V.; Bonilla, F. Different exosome cargo from plasma/bronchoalveolar lavage in non-small-cell lung cancer. *Genes Chromosomes Cancer*, **2014**, *53*(9), 713-24.

[75] Cazzoli, R.; Buttitta, F.; Di Nicola, M.; Malatesta, S.; Marchetti, A.; Rom, W.N.; Pass, H.I. microRNAs derived from circulating exosomes as noninvasive biomarkers for screening and diagnosing lung cancer. *J. Thorac. Oncol.*, **2013**, *8*(9), 1156-62.

[76] Li, L.; Masica, D.; Ishida, M.; Tomuleasa, C.; Umegaki, S.; Kalloo, A.N.; Georgiades, C.; Singh, V.K.; Khashab, M.; Amateau, S.; Li, Z.; Okolo, P.; Lennon, A.M.; Saxena, P.; Geschwind, J.F.; Schlachter, T.; Hong, K.; Pawlik, T.M.; Canto, M.; Law, J.; Sharaiha, R.; Weiss, C.R.; Thuluvath, P.; Goggins, M.; Shin, E.J.; Peng, H.; Kumbhari, V.; Hutfless, S.; Zhou, L.; Mezey, E.; Meltzer, S.J.; Karchin, R.; Selaru, F.M. Human bile contains microRNA-laden extracellular vesicles that can be used for cholangiocarcinoma diagnosis. *Hepatology*, **2014**, *60*(3), 896-907.

[77] Liu, J.; Sun, H.; Wang, X.; Yu, Q.; Li, S.; Yu, X.; Gong, W. Increased exosomal microRNA-21 and microRNA-146a levels in the cervicovaginal lavage specimens of patients with cervical cancer. *Int. J. Mol. Sci.*, **2014**, *15*(1), 758-73.

[78] Ismail, N.; Wang, Y.; Dakhlallah, D.; Moldovan, L.; Agarwal, K.; Batte, K.; Shah, P.; Wisler, J.; Eubank, T.D.; Tridandapani, S.; Paulaitis, M.E.; Piper, M.G.; Marsh, C.B. Macrophage microvesicles induce macrophage differentiation and miR-223 transfer. *Blood*, **2013**, *121*(6), 984-95.

[79] Tang, G. siRNA and miRNA: an insight into RISCs. .*Trends Biochem. Sci*., **2005**, *30*(2), 106-14.

[80] Thomson, D.W.; Bracken, C.P.; Szubert, J.M.; Goodall, G.J. On measuring miRNAs after transient transfection of mimics or antisense inhibitors. *PLoS One*, **2013**, *8*(1), e55214.

[81] Kooijmans, S.A.; Vader, P.; van Dommelen, S.M.; van Solinge, W.W.; Schiffelers, R.M. Exosome mimetics: a novel class of drug delivery systems. *Int. J. Nanomedicine*, **2012**, 7, 1525-41.

[82] Ohno, S.; Takanashi, M.; Sudo, K.; Ueda, S.; Ishikawa, A.; Matsuyama, N.; Fujita, K.; Mizutani, T.; Ohgi, T.; Ochiya, T.; Gotoh, N.; Kuroda, M. Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells. *Mol. Ther.*, **2013**, *21*(1), 185-91.

[83] Sato-Kuwabara, Y.; Melo, S.A.; Soares, F.A.; Calin, G.A. The fusion of two worlds: non-coding RNAs and extracellular vesicles--diagnostic and therapeutic implications (Review). *Int. J. Oncol.*, **2015**, *46*(1), 17-27.

[84] Alvarez-Erviti, L.; Seow, Y.; Yin, H.; Betts, C.; Lakhal, S.; Wood, M.J. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat. Biotechnol.*, **2011**, *29*(4), 341-5.

[85] Wahlgren, J.; De, L.K.T.; Brisslert, M.; Vaziri Sani, F.; Telemo, E.; Sunnerhagen, P.; Valadi, H. Plasma exosomes can deliver exogenous short interfering RNA to monocytes and lymphocytes. *Nucleic Acids Res.*, **2012**, *40*(17), e130.

Received on: March 20, 2015 **Revised on: April 16, 2015** **Accepted on: April 18, 2015**