World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.4291/wjgp.v4.i4.74

World J Gastrointest Pathophysiol 2013 November 15; 4(4): 74-90
ISSN 2150-5330 (online)

REVIEW

Pancreatic cancer diagnosis by free and exosomal miRNA

Margot Zöller

Margot Zöller, Department of Tumor Cell Biology, University Hospital of Surgery, D 69120 Heidelberg, Germany
Author contributions: Zöller M solely contributed to this paper.
Supported by The Wilhelm Sander Stiftung
Correspondence to: Dr. Margot Zöller, Department of Tumor Cell Biology, University Hospital of Surgery, Im Neuenheimer Feld 365, D 69120 Heidelberg, Germany. margot.zoeller@uni-heidelberg.de
Telephone: +49-6221-565146 Fax: +49-6221-565199
Received: May 25, 2013 Revised: August 1, 2013
Accepted: September 18, 2013
Published online: November 15, 2013

Abstract

Patients with pancreatic adenocarcinoma (PaCa) have a dismal prognosis. This is in part due to late diagnosis prohibiting surgical intervention, which provides the only curative option as PaCa are mostly chemo- and radiation resistance. Hope is raised on a reliable non-invasive/minimally invasive diagnosis that is still missing. Recently two diagnostic options are discussed, serum MicroRNA (miRNA) and serum exosomes. Serum miRNA can be free or vesicle-, particularly, exosomes-enclosed. This review will provide an overview on the current state of the diagnostic trials on free serum miRNA and proceed with an introduction of exosomes that use as a diagnostic tool in serum and other body fluids has not received sufficient attention, although serum exosome miRNA in combination with protein marker expression likely will increase the diagnostic and prognostic power. By their crosstalk with host cells, which includes binding-initiated signal transduction, as well as reprogramming target cells *via* the transfer of proteins, mRNA and miRNA exosomes are suggested to become a most powerful therapeutics. I will discuss which hurdles have still to be taken as well as the different modalities, which can be envisaged to make therapeutic use of exosomes. PaCa are known to most intensely crosstalk with the host as apparent by desmoplasia and frequent paraneoplastic syndromes. Thus, there is hope that the therapeutic application of exosomes brings about a major breakthrough.

Key words: Pancreatic cancer; Exosomes; MicroRNA; Diagnosis; Serum

Core tip: Patients with pancreatic adenocarcinoma have a dismal prognosis due to late diagnosis prohibiting surgical intervention, which is further burdened by chemo- and radiation resistance. Hope is raised on a minimally invasive diagnosis that is still missing. Recently two diagnostic options are discussed, serum microRNA (miRNA) and serum exosomes. Serum miRNA can be free or vesicle-, particularly, exosomes-enclosed. This review presents an overview on the current state on miRNA as a cancer diagnostics and discusses arguments in favor of tumor exosomes as a diagnostic tool that additionally could provide a powerful therapeutic option in the near future.

Zöller M. Pancreatic cancer diagnosis by free and exosomal miRNA. *World J Gastrointest Pathophysiol* 2013; 4(4): 74-90 Available from: URL: http://www.wjgnet.com/2150-5330/full/v4/i4/74.htm DOI: http://dx.doi.org/10.4291/wjgp.v4.i4.74

INTRODUCTION

Pancreatic adenocarcinoma (PaCa) ranks fourth in mortality among cancer-related deaths. With an overall 5-year survival rate of below 1% and a mean survival time of 4-6 mo it is the deadliest cancer[1,2]. There has been considerable progress in the treatment of patients with early stage PaCa. But late initial diagnosis that prohibits resection, chemotherapy and radiation resistance and the early metastatic spread of PaCa account for the non-satisfactory progress in therapy[3,4]. Thus, research has focused on defining a reliable non-invasive or minimally invasive diagnosis. So far, serum markers allowing for a non-invasive diagnosis and follow up studies are rare.

CA19-9 is still the most reliable diagnostic serum marker, but should be used in conjunction with other diagnostic tools. Additional markers are carcino-embryonic antigen (CEA), CA125 and CA242, their specificity and particularly sensitivity being below that of CA19-9[5-7]. However, recently, two non-invasive diagnostic tools have come into focus. First, serum microRNA (miRNA) was repeatedly described to allow for differential diagnosis of cancer, where PaCa patients' serum miRNA might allow differentiating between benign and malignant tumors as well as inflammation[8,9]. Second, tumor-derived exosomes are readily detected in body fluids. Their protein, mRNA and miRNA profiles might well serve as diagnostic tools[10]. In addition, exosomes are hotly debated as potent therapeutics[11-13].

TUMOR DIAGNOSIS AND miRNA

Recovery of non-coding RNA in body fluids

A new class of small noncoding RNA known as miRNA endogenously regulates gene expression at the posttranscriptional level[14]. miRNA range in size from 19 to 25 nucleotides. They regulate translation and degradation of mRNA through base pairing to complementary sites mostly in the untranslated region[15]. MiR constitute only 1%-3% of the human genome, but control about 30% of the coding genes[16], most miR controlling multiple mRNA[17]. miR biogenesis is a multistep process, where a long primary transcript (pri-miR) is processed into a 70-100 nt hairpin precursor pre-miR. The pre-miR is translocated to the cytoplasm, where it is cleaved by the ribonuclease Dicer into a mature miR duplex, which is incorporated into the RNA-induced silencing complex (RISC) resulting in degradation of the duplex and binding to target mRNA by complementary base pairing at the 3'-untranslated region[14]. Seed sequence complementarity of about 7 base pairs enables miRNA to bind the target mRNA, which results in inhibition of translation or a reduction in mRNA stability[18]. miRNA in the serum may derive from necrosis, apoptosis[19] or be actively released in microvesicles[20]. Free extracellular miRNA is associated with argonaute proteins (Ago) The Ago2-miRNA complex accounts for the stability of the free miRNA[21,22].

In advance of discussing serum miRNA as a potential diagnostic tool, it should be stated that data normalization is an important factor and that due to any fluctuation, epigenetic factors or others, like age, gender, diurnal changes and many more, cohort sizes should be large. Also due to these variabilities, it is very unlikely that a set of reference housekeeping miRNA with universal applicability can be identified[23,24]. Furthermore, it has to be kept in mind that most miRNA regulate more than one mRNA. Thus, in turn, a given miRNA may be deregulated in multiple diseases, including different types of cancer[25,26].

miRNA and cancer

The increased knowledge on miRNA greatly fostered progress in oncology, where miRNA could be linked to prognosis, disease progression, local recurrence and metastasis[24,27-29]. As summarized in a recent review[30] miRNA plays an important role in epithelial-mesenchymal transition (EMT), maintenance of cancer stem cells as well as tumor invasion and migration. EMT is regulated by the mir-200 family, miR-141, miR-429 and miR-205. The expression level of miR-200 negatively correlates with znc finger E-box-binding homeobox (ZEB)1 and 2, which inhibit E-cadherin expression[31]. In PaCa, down-regulation of miR-30 correlates with EMT, targets being vimentin and snail-1[32]. Examples for the involvement of miR in cancer stem cell (CSC) control, including pancreatic cancer, are the tumor suppressor miR-34 that regulates Notch and Bcl2[33,34] and miR-21 that correlates with chemoresistance[35]. Instead, miR-9, regulating E-cadherin expression, is suggested to be of major importance for metastasis-associated mobility and invasiveness[36,37]. miR-34a overexpression can inhibit metastasis by regulating CD44[38] and miR-340 suppresses invasion and metastasis by regulating c-Met and *via* c-Met MMP2 and MMP9[39,40].

For PaCa Jamieson *et al*[41] performed microarray analysis on resected PaCa tissue on a cohort of 48 and 24 patients. They describe associations with lymph node involvement, tumor grading and overall survival, where high expression of miR-21 and low expression of miR-34a significantly correlated with poor survival. Additional studies on PaCa tissue, non-transformed pancreatic ductal cells, CP samples and on PaCa culture lines by array or RT-PCR[42-46] have been summarized by Li *et al*[47], which also provides an overview on their function as tumor suppressors (miR-15a, miR-34a, miR-96, miR-375) or oncogenes (miR-27a, miR-132, miR-155, miR-194, miR-200b, miR-220c, miR-429, miR-212, miR-214, miR-301a, miR-421, miR-483-3p) and potential molecular targets, which include besides others WNT3A, p53, K-Ras, Akt, 14-3-3zeta and Smad4[43,48-56].

Taken together, there is increasing evidence that miRNA plays a central role in carcinogenesis and tumor progression, where the recovery of miRNA in body fluids may, additionally, provide a minimally invasive diagnostic tool. This has created hope particularly for most deadly PaCa, late diagnosis considerably contributing to the poor prognosis.

Serum miRNA as a diagnostic tool in pancreatic cancer

The stability of free miRNA in serum and other body fluids has fostered the hope for a minimally invasive diagnostic tool that may also be of prognostic value[57-59], which meanwhile has been experimentally supported for different types of cancer[60-62] including PaCa, where it will be particularly important as late diagnosis prohibits a curative intervention.

In an earlier study 4 miRNA, miR-21, miR-210, miR-155 and miR-196a have been found to differentiate PaCa patients' serum from that of healthy controls, where miR-155 is a biomarker of early PaCa and miR-196a cor-

relates with progression[63]. Evaluating a combination of CA19-9 with plasma miRNA in PaCa revealed 4 miRNA, miR-155, miR-181a, miR-181b and miR-196a, to differ significantly from healthy donors' miRNA, where only miR-16 and miR-196a allowed for discrimination from chronic pancreatitis (CP). Including CA19-9 increased sensitivity and specificity of the analysis, 85.2% of PaCa samples being positive even at stage 1[64]. An elegant recent study on serum miRNA in PaCa based on sequencing of pooled samples, a selection phase based on quantitative reverse transcriptase PCR (qRT-PCR) followed by a testing phase revealed upregulation of miR-20a, miR-21, miR-24, miR-25, miR-99a, miR-185 and miR-191 in the serum of PaCa patients as compared to healthy controls. The authors also confirmed that these 7 miRNA allowed for differentiation towards CP, where expression in CP did not differ significantly from that of healthy donors[9]. Additional studies mostly confirmed abundance of miR-21, miR-155, miR-196a, miR-210 and miR-16 in PaCa patients' sera[65-69]. A statistical meta-analysis, which includes 9 studies, from which 5 were performed with tissue and 4 with serum or plasma[9,63,64] suggests a potential role for miRNA assays in screening for and confirming PaCa diagnosis[70]. However, the authors also point out that none of these miRNA is selective for PaCa. An additional concern should be mentioned. A differential analysis of free versus vesicular, particularly, exosomal miRNA in the serum of PaCa patients appears to be missing. An exosomal miRNA analysis may well be advantageous as exosomal miRNA derives from living cells, whereas free miRNA may mostly derive from dead cells and thus could significantly change particularly during therapy or in late stage PaCa[19,71,72]. Serum exosome screening could have an additional advantage. Membrane integrated PaCa markers will be recovered on exosomes, thus allowing for a concomitant screening of miRNA and proteins.

EXOSOMES AS A DIAGNOSTIC TOOL

Exosomes are small 40-100 nm vesicles, which derive from the fusion of the intraluminal vesicles of multivesicular bodies (MVB) with the plasma membrane[10,73]. Their homogeneous size is one of the major criteria to differentiate exosomes from apoptotic blebs, microparticles and microvesicles, which vary in size[74]. Exosomes are composed of a lipid bilayer; they contain selected proteins, mRNA and miRNA[75]. Exosomes are secreted by many cells and abundantly by tumor cells[76] and are found in all body fluids[77]. Due to their presence in all body fluids and the expression of selected markers, exosomes are suggested to be optimal candidates for non-invasive diagnosis[78,79]. Exosomal proteins, mRNA and miRNA being functionally active[80,81] and transferred into target cells[13,81-85], exosomes are the most important intercellular communicators[75] and are suggested to become a very powerful therapeutic tool[12,86,87]. To reach the goals of exosomes as diagnostics and therapeutics great efforts are taken to elaborate the prerequisites, such as exosome assembly and exosomal message transfer.

Exosome assembly and secretion

It is well known that the relative abundance of proteins, mRNA and miRNAs differs between exosomes and donor cells, which implies active sorting into MVB. Indeed, the sorting of proteins into exosomes is a highly regulated process, where monoubiquitinylation as well as the endosomal sorting complex required for transport (ESCRT) play a role, some components of ESCRT, like Tsg101 and Alix being recovered in exosomes. The ESCRT machinery consists of 3 complexes, ESCRT Ⅰ, Ⅱ and Ⅲ, where Tsg1 in the ESCRT complex I binds ubiquitinated protein and recruits ESCRT Ⅱ. ESCRT Ⅲ becomes recruited *via* ESCRT Ⅱ or Alix. ESCRT Ⅲ recruits a deubiquitinating enzyme that removes the ubiquitin tag from the cargo proteins prior to sorting into MVB[88,89]. However, not all proteins require the ESCRT complex for incorporation into exosomes. Alternatively, proteins in detergent resistant membrane complexes can become incorporated into MVB like MHC Ⅱ molecules in dendritic cells[90]. Lipid affinity also can account for MVB incorporation[91]. Tetraspanins and other proteins with high affinity for cholesterol and sphingolipids are partitioned into membrane domains which according to their physical properties are prone for internalization[92-95]. Proteins also may become recruited by associated proteins such as integrins associated with tetraspanins or the transferin receptor (TfR), which associates with heat shock proteins (HSP)[94]. In particular for tetraspanin-associated molecules it has been described that protein complexes rather than singular molecules are recruited into exosomes. This complex binding severely influences exosome targeting and the crosstalk with target structures[96-98]. Besides members of the tetraspanin family (CD9, CD63, CD81, CD82, CD151, Tspan8), where tetraspanins are constitutive components of exosomes[91,99] and are frequently used to differentiate exosomes from other extracellular vesicles[75,91], additional molecules most abundantly recovered in exosomes are HSP[100,101], proteases[102,103], MHC molecules, cytoskeletal proteins and signal transduction molecules[104], where engulfment of cytosolic proteins involves proteins located close to the outer membrane of MVB by automicrophagy[105].

Interest in exosomes has steeply increased, when it was reported that exosomes contain mRNA and microRNA that will be transferred into target cells[106]. Exosomal mRNA and miRNA also differs from that in the donor cell. mRNA recruitment can be guided by a zip code in the 3'-UTR[107]. Exosomal mRNA is less abundant than exosomal miRNA. Exosomal mRNA are mostly involved in cell cycle progression, angiogenesis, migration, or histone modification[98,108,109]. Exosomes also contain selected miRNA. miRNA recruitment is facilitated by coupling of RISCs (RNA-induced silencing complexes) to components of the sorting complex[110,111], the release

of miRNA being controlled through ceramide-dependent machinery associated with exosome secretion[112]. Exosomes contain > 120 miRNA from a selected number of genes. Network based analysis of exosomal miRNA points towards an involvement in stem cell differentiation (let-7), organogenesis (miR-1), hematopoiesis (miR-181) tumorigenesis (miR-17, miR-18, miR-19a, miR-20, miR-19b-1, miR-93-1)[13,14] and metastasis[105].

As exosomes are found in all body fluids[77], the selective enrichment of "marker" proteins as well as of miRNA makes exosomes a very attractive means for non-invasive diagnosis[104,113].

Tumor diagnosis by serum exosomes

Exosomes are separated by sequential centrifugation steps followed or preceded by 0.2 μm filtering. For pre-evaluation exosomes should be further purified by sucrose density gradient centrifugation[114,115]. This, however may not be possible for large sample number evaluation and also may not be feasible with the amount of available serum. According to our experience and in line with literature reports, 1 mL of serum will be sufficient for screening of a limited number of proteins and miRNA. Particularly for miRNA screening, recently a thorough comparative evaluation of mRNA preparation has been published[116], which should be taken into account as in dependence of the exosome source minor differences may lead to a pronounced loss of miRNA. Besides these "home made" exosomes, several commercially available exosome purification kits are available that were described to reveal comparable results. In addition, there are special diagnostic kits on the market, which will be helpful, if a clearly defined question is to be answered, *e.g.*, searching for one marker or a few selected miRNA. As far as one is interested to find out the protein marker or miRNA profile of exosomes of a tumor entity that has not yet already been analyzed, it may be preferable to start open minded without any preselection. In concern of the readout system, I strongly recommend for miRNA the protocol of Liu *et al*[9] described above for free serum miRNA, starting with a microarray of pooled serum exosomes from patients and control donors. According to our unpublished experience the ten most abundant miRNA are with high likeliness recovered in exosome pools of different patients. As the serum contains much more exosomes that are not tumor-derived, taking into account that only platelets account for roughly 50% of serum exosomes[117] , the comparison to healthy donors' exosomes provides already a good means to select out non-tumor exosomal miRNA. As an additional control, I would recommend exosomes from culture supernatant of tumor lines from the same cancer type.

It also should be remembered that exosome collect a limited number of mRNA and miRNA that does not correlate to the mRNA or miRNA profile of the cell, which we confirmed for a rat pancreatic cancer line and exosomes derived thereof[109]. Our unpublished study on human PaCa serum exosomes confirms this inasmuch as the miRNA profile of serum exosomes and of culture supernatant exosomes show abundance of the same miRNA. In addition, the absence of a miRNA that is recovered in serum exosomes from healthy donors and PaCa patients provides a strong hint towards this miRNA being not derived from tumor exosomes. Having selected for miRNA abundant in pools of PaCa patients serum exosomes, one can proceed with verification by qRT-PCR.

In concern of serum exosome marker profiles one should also start with pooled healthy donors serum exosomes and select for markers that are undetectable on healthy donors' exosomes. Antibodies against constitutive exosome markers may serve as controls. After this screening one can either proceed with enzyme-linked immunosorbent assay (ELISA)[118] or flow cytometry, where latex beads can be coated with antibody in advance or latex beads are loaded with exosomes and marker expression is evaluated by incubation with antibodies after blocking free binding sites of the latex beads[114,119,120]. Both procedures have advantages and disadvantages and it depends on the individual question, which to prefer. For diagnostic purposes several kits are commercially available.

So far, at least to my knowledge studies being concerned about serum diagnosis or diagnosis in other body fluids, like the urine, by miRNA have rarely taken into account the particular profile of exosomes. To give a few examples. In glioblastoma serum exosomes miR-21 was 40-fold increased[108]. In serum exosomes from ovarian cancer patients, 8 miRNA were significantly increased[121] and in prostate cancer urine exosomal miR-107 and miR-574-3p are upregulated[122].

In concern of the comparably rare trials on serum or other body fluids exosomes as diagnostic tool, I want to stress again that only exosomal miRNA is delivered by live cells[19,71,72]. Thus, this miRNA can be expected to be particularly selected for functional relevance. In addition, CSC/migrating tumor cells are suggested to be enriched in the serum[123,124] and could well contribute to the serum exosome pool and to its diagnostic validity, cancer progression relying on the small population of CSC, which account for drug resistance, metastasis and late recurrence[125-127]. Finally, exosomes being delivered by live tumor cells, the amount of exosomal miRNA may change with the size of the tumor, but the miRNA profile most likely will be stable.

Serum exosomes as a diagnostic tool have an additional advantage, as besides tumor miRNA, membrane bound tumor markers can be searched for. Thus, in ovarian cancer, $CD24^+$ and $EpCAM^+$ exosomes were recovered in ascites of tumor patients and in serum $CD24^+$ exosomes were detected, the absence of $EpCAM^+$ exosomes in serum being due to cleavage by exosomal ADAM10[128,129]. Also in ovarian cancer claudin4 was upregulated in 32 of 63 patients' serum exosomes, but only in 1 of 50 control serum exosomes[130]. In plasma exo-

somes of prostate cancer patients' survivin is upregulated compared to controls and benign prostate hyperplasia[131]. In urine exosomes of prostate cancer also PCA3 and TMPRSS2: ERG, deriving from a chromosomal rearrangement were detected, verifying body fluid exosomes as diagnostic marker[132], though in another study on prostate cancer urinary exosomes PSA and PSMA were detected, but exosomes in urine showed great variability[133]. Also in plasma exosomes from melanoma patients caveolin-1 and CD63 were consistently elevated[134] and tumor exosomes could be efficiently isolated with anti-HER2/neu from ascites of cancer patients[135]. Last, not least, the tumor-specific epidermal growth factor receptor VIII (EGFRVIII) was detected in 7 out of 25 glioblastoma patients serum exosomes[108] and our ongoing study on pancreatic cancer serum exosomes confirms recovery of exosomes carrying PaCa stem cell markers[124].

Taken together, comparably few studies on cancer patients serum/plasma or urinary exosomes confirmed the suggestion that exosomes in body fluids can serve as a diagnostic tool. Unfortunately, at least according to my stage of knowledge, PaCa serum exosomes have not yet been evaluated, where I strongly recommend to take into account that exosomes offer the possibility to evaluate both miRNA and protein markers. Our ongoing studies strongly suggest that combining the analysis of these two parameters most likely will bring about a considerable improvement in early PaCa diagnosis.

EXOSOMES AS A THERAPEUTIC TOOL

Exosomes are hotly debated as the most potent gen therapeutic option of the futur4[12]. In advance of discussing this option, I should briefly introduce what is known so far about the interaction between exosomes and target cells. I will first discuss exosome binding and uptake and proceed giving a brief overview on exosome binding and uptake-induced target modulation.

Exosome binding and uptake

In advance of considering options for the therapeutic use of exosomes, it is a conditio sine qua non to be aware, which cells in the body are potentially targeted by exosomes. Though it is well appreciated that exosomes only interact with selected targets[97,98,136], the mode of selection requires further clarification. Several options, which are mutually not exclusive are discussed, receptor-ligand interactions, attachment, fusion with the target cell membrane, or internalization[136-138].

Due to inward budding of endosomes into MVB, the outer membrane of exosomes is characterized by phosphatidylserine (PS), which can trigger exosome uptake by binding to scavenger receptors, integrins, complement receptors and PS receptors (TIM), particularly TIM-4[139,140]. In line with this, macrophages (Mϕ) very rapidly bind exosomes, binding being efficiently blocked by anti-CD11b[141]. However, *in vivo* studies did not provide evidence that exosome uptake is dictated by scavenger receptors. Furthermore, the selectivity of exosome uptake argues for PS facilitating binding, but not for being involved in exosome uptake[97,141,142].

Instead, already in 2004 evidence was presented that exosome uptake by dendritic cells (DC), Kupffer cells and some macrophages (Mϕ) involves, besides PS, milk fat globulin-E8, CD11a, CD54, CD9 and CD81 on exosomes and requires αvβ3, CD11a and CD54 as ligands on DC[143] suggesting exosome binding and uptake to involve receptor-ligand interactions that may vary depending on the protein pattern on exosomes and target cells[144]. Notably, this early study also pointed towards a later on confirmed contribution of tetraspanins[97,145,146]. We additionally unraveled that target cell ligands are also located in internalization prone protein clusters, which include annexins, chaperons, molecules involved in vesicular transport, tetraspanins and tetraspanin-associated molecules[97]. Thus, internalization by donor cells and the exosome uptake by target cells use similar fusion/fission machineries, maintenance of internalization complexes and re-use of these complexes for exosome uptake apparently being a common theme[146-148]. Furthermore, antibody blocking of CD91, a common receptor for several HSP interferes with exosome activity[149]. Of note, exosomes also bind with high avidity several matrix proteins[102], where matrix protein binding is selective and requires defined tetraspanin-adhesion molecule complexes[103]. Less is known about the discussed mechanism allowing for fusion of exosomes with their target cell. However, it has been shown that exosome fusion is facilitated or requires an acid pH[150].

Thus, exosomes display target cell selectivity, which at least partly builds on the engagement of protein complexes in internalization prone membrane domains.

Target modulation by exosomes

First to note, exosomal proteins, mRNA and miRNA are function competent[112,145]. Accordingly, there are several modes, whereby exosomes can modulate their targets. Binding-induced target modulation mostly relies on activation of exosome ligands and protein cleavage by exosomal proteases. Exosome uptake-initiated changes can be brought about by transferred proteins, mRNA and miRNA. These distinct activities of exosomes are far from being comprehensively understood, but all have exemplary been confirmed. I will mention some examples, as I feel it is important to be aware of this ongoing research to understand the potential power of an exosome based therapy.

Exosome-binding induced target modulation

Exosomes are rich in proteases[102], which modulate the exosomes protein profile as well the ECM and target cells.

A tumor creates its own matrix, but also influences the host matrix to generate surroundings promoting tumor cell migration and survival. The phenomenon is poorly understood and the impact of tumor exosomes is largely unexplored. First to note, exosome proteases

modulate the exosome protein profile, described for L1 and CD44 shedding by ADAM10 and for EpCAM, CD46, TNFR1 by unknown metalloproteinases[151-153]. Exosomal proteases also modulate the ECM, where exosomal tetraspanins due to their association with proteases and integrins become important[152,154-156]. The collagenolytic and laminin-degrading activity of exosomes facilitates angiogenesis and metastasis[142,157-162], degradation of aggrecan increases invasiveness[163,164] and exosomal MMP2, MMP9, MMP14 and cathepsinB correlate with invasiveness[160,165]. Focalizing exosomal matrix degrading enzymes allows for paving the path of metastasizing CSC towards the premetastatic niche, which we confirmed for a rat metastasizing pancreatic adenocarcinoma[103,166]. As the ECM also is a storage of bioactive compounds[167], modulation of the ECM by exosomal proteases[168] can account for cytokine/chemokine and protease liberation and generation of cleavage products that promote motility, angiogenesis and stroma cell activation[102]. Thus, the modulation of the ECM by exosomal proteases creates a path for migrating cells, favors a tumor growth promoting microenvironment, angiogenesis and premetastatic niche establishment.

Exosome-initiated signal transduction: Exosome-initiated signal transduction can be promoted by exosome binding and exosome uptake, which in most instances is experimentally difficult to decipher. Nonetheless, the impact of tumor exosome binding-initiated signal transduction on tumor immunity, angiogenesis, tumor growth/metastasis has been convincingly demonstrated.

DC-exosomes are one of the best explored examples for exosome binding-initiated signal transduction. DC-exosomes can replace DC in immune response induction and exosome-based therapy was first explored using DC-exosomes as a cancer vaccine. DC also take up exosomes secreted by other cells, including tumor cells, which they internalize and process for presentation. Thus, DC use exosomes as a source of antigen and produce exosomes that suffice for T cell activation, both features expanding the operational range of DC[143,169-171].

Tumor exosomes also affect the immune system[172]. Tumor exosomes inhibit $CD4^+$ T cell proliferation, which is accompanied by up-regulation and stronger suppressive activity of regulatory T cells (Treg) due to exosome-associated transforming growth factor beta 1 (TGF-β1)[168]. NK activity also becomes impaired *via* tumor exosome inhibiting activation of Stat5, Jak3, cyclinD3 expression and perforin release[173] or due to blocking NK cells *via* NKG2D binding[174]. Induction of myeloid-derived suppressor cell (MDSC) is promoted by exosomal TGFβ and PGE2[175]. *Via* stimulating TGFβ1 secretion by Mϕ, tumor exosomes suppress anti-tumor immune responses allowing for tumor growth and metastasis formation in allogeneic mice[176] and by high ICAM1 expression, tumor exosomes block the interaction between T cells and endothelial cells, thereby decreasing T cell recruitment[177]. On the other hand. high level HSP expression on tumor exosomes-HSP functioning as an endogenous danger signal-promotes NK activation and tumor cell lysis[178,179] and supports T cell activation and effector functions[180] as well as induction of costimulatory molecule expression in DC[181,182]. Tumor exosomal chemokines attract and activate DC and T cells, such that intratumoral injection efficiently inhibits tumor growth[183]. Tumor exosomes also can be an efficient antigen source, which induce a potent Th, CTL and B cell response, even where lysates of the same tumor are non-immunogenic[141,184].

Taken together, there is an intense crosstalk between tumor exosomes and the immune system that may be due predominantly to exosome binding-initiated signal transduction. Depending on the individual tumor's exosome composition, immune responses are suppressed, but also can be strengthened and in combination with DC tumor exosomes could well contribute to cancer immunotherapy.

Angiogenesis induction being one of the hallmarks of cancer, intense efforts have been taken to elaborate the contribution of tumor exosomes. Tumor exosomes containing tumor necrosis factor alpha (TNF-α), IL1β, TGFβ and TNFR1 recruit endothelial cell (EC) progenitors, promote angiogenesis[107] and stimulate EC by paracrine signaling[185]. Delta-like4 bearing tumor exosomes confer a tip cell phenotype to EC with filipodia formation, enhanced vessel density and branching[186], which involves activation of PPARα and NFκB activation[187]. In a feedback, prostate cancer exosomes lead to activation of fibroblasts, which then shed exosomes that increase tumor cell migration *via* CX3C-CX3CR1[188].

Another elegant examples of tumor exosome-mediated signal transduction describes overexpression of CD9 or CD82 promoting formation and secretion of exosomes that contain β-catenin, thereby reducing its cellular content and impairing Wnt signaling, which proceeds *via* tetraspanin-associated E-cadherin[189]. Besides indicating that the cargo of exosomes differs depending on ESCRT- or tetraspanin-initiated internalization, this study demonstrates that by depletion of inhibitors or stimulators tumor exosomes can opposingly affect signal transduction[190]. Also, tumor exosome-promoted tumor growth may vary for individual tumors. Thus, a deficit in Rab27a leading to reduced exosome production affected growth of a tumor line that required recruitment of neutrophils, but not of another neutrophil-independent line[191].

Briefly, binding of tumor exosomes to hematopoietic cells, EC and stroma cells can severely affect the target cell, which may become activated or suppressed. Additionally, the export of proteins into tumor exosomes affects the tumor cell itself. It also has to be kept in mind that tumor exosome-initiated signaling varies with the origin and composition of tumor exosomes. Last and importantly, the strength of tumor exosome initiated signaling relies on their accessibility throughout the body.

Exosome uptake promoted target cell modulation: Early reports on the information transfer *via* exosomes

showed that embryonic stem cell exosomes transfer messages into hematopoietic progenitor cells that promoted survival and expression of early pluripotency markers[20]. Adult tissue exosomes, too, had the capacity to alter the phenotype of their target such that upon coculture bone marrow cells (BMC) express markers found on the exosome donor cell[192], where uptake of exosome proteins, mRNA and miRNA are contributing. These findings also account for tumor exosomes, which transfer receptor and oncoproteins or miRNA[20,193].

One of the first evidences to support tumor exosome-uptake plays a critical role in autocrine stimulation of tumor growth revealed that the intercellular transfer of the oncogenic receptor EGFRVIII *via* tumor exosomes to glioma cells, lacking this receptor, causes transformation of indolent glioma cells[194] and reprograms growth factor pathways in EC[126]. Other oncogenes, like Ras, Myc, SV40T also induce signaling and gene expression[195-197], where *e.g.*, exosomal amphiregulin, an EGFR ligand, increased tumor invasiveness 5-fold compared to the recombinant protein[198].

Tumor exosome uptake-induced changes in recipient non-tumor cells can be transient, but also suffice to drive tumor growth as described for tissue transglutaminase and fibronectin[199] or high level c-Met uptake by BMC, which leads to their re-education to support premetastatic niche formation for melanoma cells, where in melanoma patients, too, circulating BM-derived cells express Met[200]. Tumor exosomes also transport apoptosis inhibitory proteins[201] and present TGFβ. This drives differentiation of fibroblasts towards myofibroblasts, which support tumor growth[202]. Adipose-tissue derived mesenchymal stem cells (MSC) also can be driven into myofibroblasts by tumor exosomes[203]. Lung cancer tumor exosome uptake stimulates IL8, VEGF, LIF, oncostatin and MMP secretion, which promotes tumor growth[204]. Instead, uptake of tumor suppressor genes from non-transformed cells can mitigate cancer cell aggressiveness[12,205].

An involvement of exosomes in metastasis was first described for platelet-derived exosomes, which transferred the αIIb integrin chain to lung cancer cells, stimulated the MAPK pathway and increased expression of MT1-MMP, cyclin D2 and angiogenic factors and enhanced adhesion to fibrinogen and human umbilical vein EC[206]. We explored that exosomes from a PaCa together with a soluble tumor matrix facilitated recruitment of hematopoietic progenitors from the BM as well as activation of stroma cells and leukocytes in premetastatic lymph nodes such that a non-metastatic tumor line settled and formed metastases[207]. The recruitment of tumor cells also becomes facilitated by exosomal HSP90, a complex of exosomal HSP90 with MMP2 and tissue plasminogen activator promoting together with exosomal annexin II plasmin activation tumor cell motility[208]. As already mentioned, the transfer of c-Met contributes to premetastatic niche formation mostly *via* bone marrow cell modulation[200]. Thus, tumor exosomes enhance migration and homing of tumor cells in sentinel lymph nodes due to stroma and hematopoietic cell as well as matrix modulation[77,108,200,207]. Finally, uptake of exosomes from non-transformed cells in the tumor surrounding can affect tumor cells such that fibroblast-exosomes promote breast cancer motility *via* Wnt planar polarity signaling[209].

Tumor exosome uptake also accounts for EC modulation. Colorectal cancer exosomes, enriched in cell cycle-related mRNA, promote EC proliferation[210]. Glioblastoma-exosome-induced angiogenesis relies on the transfer of exosomal proteins and mRNA[108]. Uptake of EGFR-positive tumor exosomes by EC elicit EGFR-dependent responses including activation of the MAPK and Akt pathway and VEGFR2 expression[211]. Transfer of exosomal Notch-ligand-delta-like-4 increases angiogenesis[183] and tumor exosomes expressing a complex of Tspan8 with CD49d preferentially are taken up by EC and EC progenitors, which initiates progenitor maturation and EC activation including VEGFR transcription[60]. Chronic myeloid leukemia (CML)-exosomes induce angiogenic activity in EC, where a Src inhibitor affects exosome production as well as vascular differentiation[212].

As mentioned tumor exosome uptake-induced target cell modulation frequently represent the net result of protein transfer-initiated signal transduction, transferred mRNA translation and mRNA silencing by miRNA. Though a separation between these activities appears somewhat artificial, a few reports describing preferential activities of mRNA and miRNA should be mentioned.

By the transfer of miR-150 in AML-exosomes to hematopoietic progenitors CXCR4 expression becomes reduced and HSC migration is impaired[213]. $CD105^+$ renal cell CSC exosomes carry proangiogenic mRNA and miRNA, which trigger the angiogenic switch[158]. mRNA and miRNA of exosomes from a metastasizing PaCa are recovered in lymph node stroma and lung fibroblasts, and transferred miRNA significantly affects mRNA translation, which was exemplified for abundant exosomal miR-494 and miR-542-3p, which target cadherin17. Concomitantly, MMP transcription, accompanying cadherin17 downregulation, was up-regulated in lymph node stroma cells transfected with miR-494 or miR-542-3p or co-cultured with tumor exosomes. Thus, tumor exosome miRNA uptake affected premetastatic organ stroma cells towards supporting tumor cell hosting[109]. Exosomes from virus transfected cells transfer viral miRNA[214,215]. Leukemia cell exosomes contain miR-92a that is transferred into EC, downregulates CD49e and increases migration and tube formation[216]. In lung cancer exosomes miR-21 and miR-29a act as a ligand of mouse TLR7 or human TLR8, functioning as agonist and leading to NFκB activation and IL6 and TNFα secretion, which promotes metastasis[217]. Hepatocellular carcinoma exosomes abundantly contain miR-584, miR-517c, one of the potential targets, TGFβ activated kinase 1, activates JNK and MAPK pathway and NFκB, where transfer of exosomal miRNA in coculture promoted anchorage-

independent growth and apoptosis resistance[218].

Stroma cells also release exosomes, whose miRNA can influence tumor cells. BM stroma cell exosomes inhibit the growth of multiple myeloma, but those derived from patients with multiple myeloma force multiple myeloma progression, the latter exosomes showing a lower content of tumor suppressor miR-15a, but high levels of oncogenic proteins, cytokines and adhesion molecules[219]. Tumor-associated Mϕ secret exosomes with high miR-223, that binds Mef2c, causing nuclear accumulation of β-catenin[220]. Monocyte exosomal miR-150, when transferred to EC, promotes migration[221].

Taken together, transferred exosomal miRNA can reprogram target cells, the linkage between exosomal miRNA and the targeted mRNA remaining to be elaborated in detail in many instances. In concern of the described impact of transferred proteins and mRNA, the question on long-lasting *in vivo* efficacy awaits clarification. Exosomes being a most powerful means of intercellular communication that function across long distance, it is utmost important to answer these open questions. Nonetheless, therapeutic exploitation of exosomes appears promising.

EXOSOMES AS THERAPEUTICS

Exosomes are discussed as most potent gene delivery system, as they are easy to manipulate and efficiently transfer proteins and genes. This could offer a means to interfere with tumor exosome promoted angiogenesis and metastasis, two major targets in cancer therapy[91,222]. In addition, exosomes are discussed as cancer vaccine[172]. Nonetheless, in advance of discussing the possibilities to interfere with tumor growth and progression *via* exosomes, I want to stress three points. First, uptake by selective target cells needs to be most thoroughly controlled. Second, the pathway whereby exosomes affect a selected target cells has to be well defined. Besides the still open question, whether transferred proteins, mRNA and miRNA or a combination account for observed effects, the multiple targets of individual miRNA could create problems such that side effects at the present state of knowledge can not be excluded. Third, it should be mentioned that the indispensability of exosome transfer in human cancer remains questionable. In A431 PS blocking inhibits uptake of exosomes by EC, but the antiangiogenic effect was only transient[194]. Also a blockade of cellular vesiculation (TSAP6, acidic sphingomyelinase) did not prevent tumorigenesis[223,224]. Furthermore, blocking of Rab27a involved in exosome biogenesis exerts distinct effects on primary versus metastatic tumor growth and also differs between tumors[200,225]. These findings should not be taken to discourage attempts to translate experimental studies on the power of exosomes into therapeutic settings, but should foster the point that clinical translation in many instances essentially awaits progress in elaborating the mode of exosome activities. These clauses account particularly for active interference with tumor exosomes. Instead, DC exosomes are already used as a vaccine[226,227].

Exosomes to substitute or support dendritic cells

Exosome research became highly stimulated, when it was noted that antigen presenting cells release exosomes derived from MVB of the MHC class Ⅱ compartment, which can stimulate T cells *in vitro* and *in vivo*[228]. Several studies report that DC-exosomes were well tolerated, induced an antigen-specific response and or NK recovery and that the disease-free survival time was mostly prolonged. For the therapeutic translation it is also beneficial that exosomes can be stored at -80 ℃ and that recovery is high. Limitation were mostly restricted to the requirement of large amounts of DC-exosomes[229-231].

Though tumor exosomes can be immunosuppressive, this does not affect their use for loading DC. Several groups report that exosomes delivered from DC after coculture with tumor exosomes might be superior to exosomes derived from peptide-pulsed DC. DC pulsed with exosomes of an AML line provoked a strong anti-leukemia response[232]. In line with this, directing tumor-associated, non-mutated antigens like CEA and HER2 to exosomes by coupling to lactadherin increased their immunogenicity[233]. Targeting prostate-specific antigen or prostatic acid phosphatase *via* lactadherin to exosomes also induced a superior immune response[234]. Furthermore, anticancer drug force the release of HSP-bearing exosomes, which efficiently activate NK cells[235]. Taking this into account, tumor exosomes should be particularly helpful as antigen source, when immunogenic entities of a tumor are unknown.

Competing with tumor exosomes

Even taking into account that an individual tumor may not essentially depend on exosomes for survival and progression, tumor exosomes doubtless support the tumor by modulating the host. Thus, competing with tumor exosomes might be a means to retard metastasis formation.

Blocking of exosome uptake could be performed at the exosome or the target cell level[229], where PS blocking of tumor exosomes only transiently inhibited angiogenesis[194]. Instead, in a rat PaCa, where exosomes expressing the tetraspanin Tspan8 induced a lethal systemic consumption coagulopathy, blocking exosomes by a Tspan8-specific antibody completely prevented undue angiogenesis, although primary tumor growth was not impaired[236,237]. Based on this finding and our ongoing studies that exosomes bind *via* tetraspanin-complexes to ligands also located in internalization prone membrane domains[59], we speculate that a scrutinized analysis of an individual tumors' exosome-binding complex should provide the information for hampering undue tumor exosome-initiated angiogenesis and premetastatic niche formation, where exosomes from non-transformed cells modulated to express the tumor exosome-binding complex will be most promising[59]. As an alternative

approach, tumor exosomes can be removed by affinity plasmapheresis known as Aethlon ADAPT™[238]. Blocking of tumor exosomes also can affect drug and radiation resistance due to enhanced release of export transporter MRP2, ATP7A and ATP7B or Annexin A3[239,240].

Tailored exosomes for drug delivery

Greatest hope in exosome therapy is based on the discovery of horizontal transfer of mRNA and miRNA[106,241], which can be translated or mediate RNA silencing[20,73,242].

As exosomes are natural products, are small and flexible, which allows them to cross biological membranes and to protect their cargo from degradation by a lipid bilayer[138], they are discussed as ideal and possibly the most potent gene delivery system[73,86,138,241,243]. Notably, exosome electroporation efficiently transfers siRNA into exosomes[112]. Furthermore, special devices can be developed, *e.g.*, to cross the blood-brain barrier, which was explored for the delivery of BACE1 siRNA, where mast cell exosomes were equipped with a brain penetrating peptide fused to the vesicular membrane protein Lamp2[244,245]. Also, curcumin or Stat3 inhibitor delivery confirmed exosomes to be well suited for drug delivery[246,247], where chemotherapeutic drug efficacy was increased by lowering the pH of exosomes[150,248]. Adenoviral vectors associated with exosomes displayed higher transduction efficacy than purified AAV vectors[249]. As exosomes from non-tumor cells contain tumor-suppressive miRNA, it was suggested to use exosomes loaded with those miRNA, which was exemplified for miR-143 as a therapeutic strategy in cancer[213]. In a mouse hepatoma, systemic administration of miR-26a, inducing cell cycle arrest, exerted a dramatic protective effect without toxicity[250]. Additional approaches like miRNA inhibitors (miRNA sponges), antagomirs, locked-nucleic-acid-modified oligonucleotides are reviewed in[23].

At the present state of knowledge miRNA based therapies have to be considered as double-edged sword as most miRNA have a multitude of targets. However, as soon as the above mentioned hurdles are solved, rapid progress in clinical translation can be expected[251,252].

CONCLUSION

The recovery of tumor-associated miRNA and of tumor exosomes in serum and other body fluids has created hope for non/minimally invasive diagnostics, where our own, unpublished data indicate that an exosome-based screening may be advantageous as it offers the possibility to search concomitantly for tumor-related protein markers as well as tumor-associated miRNA. Taking into account that the poor prognosis of PaCa patients despite considerable progress in surgical treatment is mostly due to late diagnosis, a reliable serum-based diagnosis at early stages could already significantly contribute improving the rate of curative treatment.

Beyond diagnosis, the discovery of exosomes as intercellular communicators throughout the body fostered reconsideration of many aspects of tumor biology and is hoped to bring a major breakthrough in therapy. The power of exosomes is due to their ubiquitous presence, their particular protein profile and their equipment with mRNA and miRNA as well as their most efficient transfer in target cells. Together with the ease of transfecting exosomes, there should be hardly any limits in the use of exosomes as therapeutics. The therapeutic use of exosomes from non-transformed cells to compete, to induce an immune response or to silence immunosuppression should not become a danger for the patient's organism. Instead, therapeutic approaches based on tailored tumor exosomes still awaits answers to the targeting receptors and their ligands, which most likely will offer modalities to further restrict the panel of potential targets of natural tumor exosomes and a precise knowledge on miRNA targets and consequences on release from repression. Answering these questions will take time, but is not an insurmountable hurdle.

PaCa are burdened by desmoplasia and early metastatic spread. Both features essentially depend on the crosstalk with the host, which has been convincingly demonstrated to be to a considerably degree mediated by tumor exosomes. Thus, it is my personal opinion that PaCa treatment/rognosis will particularly profit from unraveling the option of exosome-based therapy.

REFERENCES

1 **Jemal A**, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin* 2010; **60**: 277-300 [PMID: 20610543 DOI: 10.3322/caac.20073]

2 **Loos M**, Kleeff J, Friess H, Büchler MW. Surgical treatment of pancreatic cancer. *Ann N Y Acad Sci* 2008; **1138**: 169-180 [PMID: 18837898 DOI: 10.1196/annals.1414.024]

3 **Wang Z**, Li Y, Ahmad A, Banerjee S, Azmi AS, Kong D, Sarkar FH. Pancreatic cancer: understanding and overcoming chemoresistance. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 27-33 [PMID: 21102532 DOI: 10.1038/nrgastro.2010.188]

4 **Paulson AS**, Tran Cao HS, Tempero MA, Lowy AM. Therapeutic advances in pancreatic cancer. *Gastroenterology* 2013; **144**: 1316-1326 [PMID: 23622141 DOI: 10.1053/j.gastro.2013.01.078]

5 **Guo J**, Wang W, Liao P, Lou W, Ji Y, Zhang C, Wu J, Zhang S. Identification of serum biomarkers for pancreatic adenocarcinoma by proteomic analysis. *Cancer Sci* 2009; **100**: 2292-2301 [PMID: 19775290 DOI: 10.1111/j.1349-7006.2009.01324]

6 **Giovinazzo F**, Turri G, Zanini S, Butturini G, Scarpa A, Bassi C. Clinical implications of biological markers in Pancreatic Ductal Adenocarcinoma. *Surg Oncol* 2012; **21**: e171-e182 [PMID: 22981281 DOI: 10.1016/j.suronc.2012.07.004]

7 **Kaur S**, Baine MJ, Jain M, Sasson AR, Batra SK. Early diagnosis of pancreatic cancer: challenges and new developments. *Biomark Med* 2012; **6**: 597-612 [PMID: 23075238 DOI: 10.2217/bmm.12.69]

8 **Bhat K**, Wang F, Ma Q, Li Q, Mallik S, Hsieh TC, Wu E. Advances in biomarker research for pancreatic cancer. *Curr Pharm Des* 2012; **18**: 2439-2451 [PMID: 22372502]

9 **Liu R**, Chen X, Du Y, Yao W, Shen L, Wang C, Hu Z, Zhuang R, Ning G, Zhang C, Yuan Y, Li Z, Zen K, Ba Y, Zhang CY. Serum microRNA expression profile as a biomarker in the diagnosis and prognosis of pancreatic cancer. *Clin Chem* 2012; **58**: 610-618 [PMID: 22194634 DOI: 10.1373/clinchem.2011.172767]

10 **György B**, Szabó TG, Pásztói M, Pál Z, Misják P, Aradi B, László V, Pállinger E, Pap E, Kittel A, Nagy G, Falus A,

Buzás EI. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. *Cell Mol Life Sci* 2011; **68**: 2667-2688 [PMID: 21560073 DOI: 10.1007/s00018-011-0689-3]
11 **O'Loughlin AJ**, Woffindale CA, Wood MJ. Exosomes and the emerging field of exosome-based gene therapy. *Curr Gene Ther* 2012; **12**: 262-274 [PMID: 22856601]
12 **Lee Y**, El Andaloussi S, Wood MJ. Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. *Hum Mol Genet* 2012; **21**: R125-R134 [PMID: 22872698]
13 **Corrado C**, Raimondo S, Chiesi A, Ciccia F, De Leo G, Alessandro R. Exosomes as intercellular signaling organelles involved in health and disease: basic science and clinical applications. *Int J Mol Sci* 2013; **14**: 5338-5366 [PMID: 23466882 DOI: 10.3390/ijms14035338]
14 **Bartel DP**. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281-297 [PMID: 14744438]
15 **Calin GA**, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer* 2006; **6**: 857-866 [PMID: 17060945]
16 **Filipowicz W**, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* 2008; **9**: 102-114 [PMID: 18197166 DOI: 10.1038/nrg2290]
17 **Lim LP**, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* 2005; **433**: 769-773 [PMID: 15685193]
18 **Bartel DP**. MicroRNAs: target recognition and regulatory functions. *Cell* 2009; **136**: 215-233 [PMID: 19167326 DOI: 10.1016/j.cell.2009.01.002]
19 **Tzimagiorgis G**, Michailidou EZ, Kritis A, Markopoulos AK, Kouidou S. Recovering circulating extracellular or cell-free RNA from bodily fluids. *Cancer Epidemiol* 2011; **35**: 580-589 [PMID: 21514265 DOI: 10.1016/j.canep.2011.02.016]
20 **Ratajczak J**, Miekus K, Kucia M, Zhang J, Reca R, Dvorak P, Ratajczak MZ. Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. *Leukemia* 2006; **20**: 847-856 [PMID: 16453000]
21 **Turchinovich A**, Weiz L, Langheinz A, Burwinkel B. Characterization of extracellular circulating microRNA. *Nucleic Acids Res* 2011; **39**: 7223-7233 [PMID: 21609964 DOI: 10.1093/nar/gkr254]
22 **Johnston M**, Hutvagner G. Posttranslational modification of Argonautes and their role in small RNA-mediated gene regulation. *Silence* 2011; **2**: 5 [PMID: 21943311 DOI: 10.1186/1758-907X-2-5]
23 **Ajit SK**. Circulating microRNAs as biomarkers, therapeutic targets, and signaling molecules. *Sensors* (Basel) 2012; **12**: 3359-3369 [PMID: 22737013 DOI: 10.3390/s120303359]
24 **Orlova IA**, Alexander GM, Qureshi RA, Sacan A, Graziano A, Barrett JE, Schwartzman RJ, Ajit SK. MicroRNA modulation in complex regional pain syndrome. *J Transl Med* 2011; **9**: 195 [PMID: 22074333 DOI: 10.1186/1479-5876-9-195]
25 **White NM**, Fatoohi E, Metias M, Jung K, Stephan C, Yousef GM. Metastamirs: a stepping stone towards improved cancer management. *Nat Rev Clin Oncol* 2011; **8**: 75-84 [PMID: 21045789 DOI: 10.1038/nrclinonc.2010.173]
26 **Reid G**, Kirschner MB, van Zandwijk N. Circulating microRNAs: Association with disease and potential use as biomarkers. *Crit Rev Oncol Hematol* 2011; **80**: 193-208 [PMID: 21145252 DOI: 10.1016/j.critrevonc.2010.11.004]
27 **Allen KE**, Weiss GJ. Resistance may not be futile: microRNA biomarkers for chemoresistance and potential therapeutics. *Mol Cancer Ther* 2010; **9**: 3126-3136 [PMID: 20940321 DOI: 10.1158/1535-7163.MCT-10-0397]
28 **Heneghan HM**, Miller N, Kerin MJ. Circulating microRNAs: promising breast cancer Biomarkers. *Breast Cancer Res* 2011; **13**: 402; author reply 403 [PMID: 21345257 DOI: 10.1186/bcr2798]
29 **Cortez MA**, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA. MicroRNAs in body fluids--the mix of hormones and biomarkers. *Nat Rev Clin Oncol* 2011; **8**: 467-477 [PMID: 21647195 DOI: 10.1038/nrclinonc.2011.76]
30 **Zhao L**, Chen X, Cao Y. New role of microRNA: carcinogenesis and clinical application in cancer. *Acta Biochim Biophys Sin* (Shanghai) 2011; **43**: 831-839 [PMID: 21908856 DOI: 10.1093/abbs/gmr080]
31 **Park SM**, Gaur AB, Lengyel E, Peter ME. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. *Genes Dev* 2008; **22**: 894-907 [PMID: 18381893 DOI: 10.1101/gad.1640608]
32 **Joglekar MV**, Patil D, Joglekar VM, Rao GV, Reddy DN, Mitnala S, Shouche Y, Hardikar AA. The miR-30 family microRNAs confer epithelial phenotype to human pancreatic cells. *Islets* 2009; **1**: 137-147 [PMID: 21099261 DOI: 10.4161/isl.1.2.9578]
33 **Wang Z**, Zhang Y, Li Y, Banerjee S, Liao J, Sarkar FH. Down-regulation of Notch-1 contributes to cell growth inhibition and apoptosis in pancreatic cancer cells. *Mol Cancer Ther* 2006; **5**: 483-493 [PMID: 16546962]
34 **Ji Q**, Hao X, Zhang M, Tang W, Yang M, Li L, Xiang D, Desano JT, Bommer GT, Fan D, Fearon ER, Lawrence TS, Xu L. MicroRNA miR-34 inhibits human pancreatic cancer tumor-initiating cells. *PLoS One* 2009; **4**: e6816 [PMID: 19714243 DOI: 10.1371/journal.pone.0006816]
35 **Misawa A**, Katayama R, Koike S, Tomida A, Watanabe T, Fujita N. AP-1-Dependent miR-21 expression contributes to chemoresistance in cancer stem cell-like SP cells. *Oncol Res* 2010; **19**: 23-33 [PMID: 21141738]
36 **Khew-Goodall Y**, Goodall GJ. Stromal miR-320 keeps an oncogenic secretome in check. *Nat Cell Biol* 2012; **14**: 124-125 [PMID: 22298040 DOI: 10.1038/ncb2431]
37 **Uchida N**. MicroRNA-9 controls a migratory mechanism in human neural progenitor cells. *Cell Stem Cell* 2010; **6**: 294-296 [PMID: 20362531 DOI: 10.1016/j.stem.2010.03.010]
38 **Liu C**, Kelnar K, Liu B, Chen X, Calhoun-Davis T, Li H, Patrawala L, Yan H, Jeter C, Honorio S, Wiggins JF, Bader AG, Fagin R, Brown D, Tang DG. The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. *Nat Med* 2011; **17**: 211-215 [PMID: 21240262 DOI: 10.1038/nm.2284]
39 **Wu ZS**, Wu Q, Wang CQ, Wang XN, Huang J, Zhao JJ, Mao SS, Zhang GH, Xu XC, Zhang N. miR-340 inhibition of breast cancer cell migration and invasion through targeting of oncoprotein c-Met. *Cancer* 2011; **117**: 2842-2852 [PMID: 21692045 DOI: 10.1002/cncr.25860]
40 **Le XF**, Merchant O, Bast RC, Calin GA. The Roles of MicroRNAs in the Cancer Invasion-Metastasis Cascade. *Cancer Microenviron* 2010; **3**: 137-147 [PMID: 21209780 DOI: 10.1007/s12307-010-0037-4]
41 **Jamieson NB**, Morran DC, Morton JP, Ali A, Dickson EJ, Carter CR, Sansom OJ, Evans TR, McKay CJ, Oien KA. MicroRNA molecular profiles associated with diagnosis, clinicopathologic criteria, and overall survival in patients with resectable pancreatic ductal adenocarcinoma. *Clin Cancer Res* 2012; **18**: 534-545 [PMID: 22114136 DOI: 10.1158/1078-0432.CCR-11-0679]
42 **Lee EJ**, Gusev Y, Jiang J, Nuovo GJ, Lerner MR, Frankel WL, Morgan DL, Postier RG, Brackett DJ, Schmittgen TD. Expression profiling identifies microRNA signature in pancreatic cancer. *Int J Cancer* 2007; **120**: 1046-1054 [PMID: 17149698]
43 **Kent OA**, Mullendore M, Wentzel EA, López-Romero P, Tan AC, Alvarez H, West K, Ochs MF, Hidalgo M, Arking DE, Maitra A, Mendell JT. A resource for analysis of microRNA expression and function in pancreatic ductal adenocarcinoma cells. *Cancer Biol Ther* 2009; **8**: 2013-2024

[PMID: 20037478]
44 **Zhang Y**, Li M, Wang H, Fisher WE, Lin PH, Yao Q, Chen C. Profiling of 95 microRNAs in pancreatic cancer cell lines and surgical specimens by real-time PCR analysis. *World J Surg* 2009; **33**: 698-709 [PMID: 19030927 DOI: 10.1007/s00268-008-9833-0]
45 **Bloomston M**, Frankel WL, Petrocca F, Volinia S, Alder H, Hagan JP, Liu CG, Bhatt D, Taccioli C, Croce CM. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. *JAMA* 2007; **297**: 1901-1908 [PMID: 17473300]
46 **Szafranska AE**, Davison TS, John J, Cannon T, Sipos B, Maghnouj A, Labourier E, Hahn SA. MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma. *Oncogene* 2007; **26**: 4442-4452 [PMID: 17237814]
47 **Li W**, Lebrun DG, Li M. The expression and functions of microRNAs in pancreatic adenocarcinoma and hepatocellular carcinoma. *Chin J Cancer* 2011; **30**: 540-550 [PMID: 21801602 DOI: 10.5732/cjc.011.10197]
48 **Gironella M**, Seux M, Xie MJ, Cano C, Tomasini R, Gommeaux J, Garcia S, Nowak J, Yeung ML, Jeang KT, Chaix A, Fazli L, Motoo Y, Wang Q, Rocchi P, Russo A, Gleave M, Dagorn JC, Iovanna JL, Carrier A, Pébusque MJ, Dusetti NJ. Tumor protein 53-induced nuclear protein 1 expression is repressed by miR-155, and its restoration inhibits pancreatic tumor development. *Proc Natl Acad Sci USA* 2007; **104**: 16170-16175 [PMID: 17911264]
49 **Mees ST**, Mardin WA, Wendel C, Baeumer N, Willscher E, Senninger N, Schleicher C, Colombo-Benkmann M, Haier J. EP300--a miRNA-regulated metastasis suppressor gene in ductal adenocarcinomas of the pancreas. *Int J Cancer* 2010; **126**: 114-124 [PMID: 19569050 DOI: 10.1002/ijc.24695]
50 **Hao J**, Zhang S, Zhou Y, Liu C, Hu X, Shao C. MicroRNA 421 suppresses DPC4/Smad4 in pancreatic cancer. *Biochem Biophys Res Commun* 2011; **406**: 552-557 [PMID: 21352803 DOI: 10.1016/j.bbrc.2011.02.086]
51 **Hao J**, Zhang S, Zhou Y, Hu X, Shao C. MicroRNA 483-3p suppresses the expression of DPC4/Smad4 in pancreatic cancer. *FEBS Lett* 2011; **585**: 207-213 [PMID: 21112326 DOI: 10.1016/j.febslet.2010.11.039]
52 **Park JK**, Henry JC, Jiang J, Esau C, Gusev Y, Lerner MR, Postier RG, Brackett DJ, Schmittgen TD. miR-132 and miR-212 are increased in pancreatic cancer and target the retinoblastoma tumor suppressor. *Biochem Biophys Res Commun* 2011; **406**: 518-523 [PMID: 21329664 DOI: 10.1016/j.bbrc.2011.02.065]
53 **Ma Y**, Yu S, Zhao W, Lu Z, Chen J. miR-27a regulates the growth, colony formation and migration of pancreatic cancer cells by targeting Sprouty2. *Cancer Lett* 2010; **298**: 150-158 [PMID: 20638779 DOI: 10.1016/j.canlet.2010.06.012]
54 **Lu Z**, Li Y, Takwi A, Li B, Zhang J, Conklin DJ, Young KH, Martin R, Li Y. miR-301a as an NF-κB activator in pancreatic cancer cells. *EMBO J* 2011; **30**: 57-67 [PMID: 21113131 DOI: 10.1038/emboj.2010.296]
55 **Zhang XJ**, Ye H, Zeng CW, He B, Zhang H, Chen YQ. Dysregulation of miR-15a and miR-214 in human pancreatic cancer. *J Hematol Oncol* 2010; **3**: 46 [PMID: 21106054 DOI: 10.1186/1756-8722-3-46]
56 **Yu S**, Lu Z, Liu C, Meng Y, Ma Y, Zhao W, Liu J, Yu J, Chen J. miRNA-96 suppresses KRAS and functions as a tumor suppressor gene in pancreatic cancer. *Cancer Res* 2010; **70**: 6015-6025 [PMID: 20610624 DOI: 10.1158/0008-5472.CAN-09-4531]
57 **Cortez MA**, Calin GA. MicroRNA identification in plasma and serum: a new tool to diagnose and monitor diseases. *Expert Opin Biol Ther* 2009; **9**: 703-711 [PMID: 19426115 DOI: 10.1517/14712590902932889]
58 **Metias SM**, Lianidou E, Yousef GM. MicroRNAs in clinical oncology: at the crossroads between promises and problems. *J Clin Pathol* 2009; **62**: 771-776 [PMID: 19734473 DOI: 10.1136/jcp.2009.064717]
59 **Ferracin M**, Veronese A, Negrini M. Micromarkers: miRNAs in cancer diagnosis and prognosis. *Expert Rev Mol Diagn* 2010; **10**: 297-308 [PMID: 20370587 DOI: 10.1586/erm.10.11]
60 **Allegra A**, Alonci A, Campo S, Penna G, Petrungaro A, Gerace D, Musolino C. Circulating microRNAs: new biomarkers in diagnosis, prognosis and treatment of cancer (review). *Int J Oncol* 2012; **41**: 1897-1912 [PMID: 23026890 DOI: 10.3892/ijo.2012.1647]
61 **Zen K**, Zhang CY. Circulating microRNAs: a novel class of biomarkers to diagnose and monitor human cancers. *Med Res Rev* 2012; **32**: 326-348 [PMID: 22383180 DOI: 10.1002/med.20215]
62 **Qu H**, Xu W, Huang Y, Yang S. Circulating miRNAs: promising biomarkers of human cancer. *Asian Pac J Cancer Prev* 2011; **12**: 1117-1125 [PMID: 21875254]
63 **Wang J**, Chen J, Chang P, LeBlanc A, Li D, Abbruzzesse JL, Frazier ML, Killary AM, Sen S. MicroRNAs in plasma of pancreatic ductal adenocarcinoma patients as novel blood-based biomarkers of disease. *Cancer Prev Res* (Phila) 2009; **2**: 807-813 [PMID: 19723895 DOI: 10.1158/1940-6207.CAPR-09-0094]
64 **Liu J**, Gao J, Du Y, Li Z, Ren Y, Gu J, Wang X, Gong Y, Wang W, Kong X. Combination of plasma microRNAs with serum CA19-9 for early detection of pancreatic cancer. *Int J Cancer* 2012; **131**: 683-691 [PMID: 21913185 DOI: 10.1002/ijc.26422]
65 **Bauer AS**, Keller A, Costello E, Greenhalf W, Bier M, Borries A, Beier M, Neoptolemos J, Büchler M, Werner J, Giese N, Hoheisel JD. Diagnosis of pancreatic ductal adenocarcinoma and chronic pancreatitis by measurement of microRNA abundance in blood and tissue. *PLoS One* 2012; **7**: e34151 [PMID: 22511932 DOI: 10.1371/journal.pone.0034151]
66 **Tavano F**, di Mola FF, Piepoli A, Panza A, Copetti M, Burbaci FP, Latiano T, Pellegrini F, Maiello E, Andriulli A, di Sebastiano P. Changes in miR-143 and miR-21 expression and clinicopathological correlations in pancreatic cancers. *Pancreas* 2012; **41**: 1280-1284 [PMID: 22836856 DOI: 10.1097/MPA.0b013e31824c11f4]
67 **LaConti JJ**, Shivapurkar N, Preet A, Deslattes Mays A, Peran I, Kim SE, Marshall JL, Riegel AT, Wellstein A. Tissue and serum microRNAs in the Kras(G12D) transgenic animal model and in patients with pancreatic cancer. *PLoS One* 2011; **6**: e20687 [PMID: 21738581 DOI: 10.1371/journal.pone.0020687]
68 **Morimura R**, Komatsu S, Ichikawa D, Takeshita H, Tsujiura M, Nagata H, Konishi H, Shiozaki A, Ikoma H, Okamoto K, Ochiai T, Taniguchi H, Otsuji E. Novel diagnostic value of circulating miR-18a in plasma of patients with pancreatic cancer. *Br J Cancer* 2011; **105**: 1733-1740 [PMID: 22045190 DOI: 10.1038/bjc.2011.453]
69 **Kawaguchi T**, Komatsu S, Ichikawa D, Morimura R, Tsujiura M, Konishi H, Takeshita H, Nagata H, Arita T, Hirajima S, Shiozaki A, Ikoma H, Okamoto K, Ochiai T, Taniguchi H, Otsuji E. Clinical impact of circulating miR-221 in plasma of patients with pancreatic cancer. *Br J Cancer* 2013; **108**: 361-369 [PMID: 23329235 DOI: 10.1038/bjc.2012.546]
70 **Wan C**, Shen Y, Yang T, Wang T, Chen L, Wen F. Diagnostic value of microRNA for pancreatic cancer: a meta-analysis. *Arch Med Sci* 2012; **8**: 749-755 [PMID: 23185182 DOI: 10.5114/aoms.2012.31609]
71 **Mo MH**, Chen L, Fu Y, Wang W, Fu SW. Cell-free Circulating miRNA Biomarkers in Cancer. *J Cancer* 2012; **3**: 432-448 [PMID: 23074383 DOI: 10.7150/jca.4919]
72 **Gallo A**, Tandon M, Alevizos I, Illei GG. The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. *PLoS One* 2012; **7**: e30679 [PMID: 22427800 DOI: 10.1371/journal.pone.0030679]
73 **Simons M**, Raposo G. Exosomes--vesicular carriers for

intercellular communication. *Curr Opin Cell Biol* 2009; **21**: 575-581 [PMID: 19442504 DOI: 10.1016/j.ceb.2009.03.007]
74 **Vlassov AV**, Magdaleno S, Setterquist R, Conrad R. Exosomes: current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. *Biochim Biophys Acta* 2012; **1820**: 940-948 [PMID: 22503788 DOI: 10.1016/j.bbagen.2012.03.017]
75 **Mathivanan S**, Ji H, Simpson RJ. Exosomes: extracellular organelles important in intercellular communication. *J Proteomics* 2010; **73**: 1907-1920 [PMID: 20601276 DOI: 10.1016/j.jprot.2010.06.006]
76 **Kharaziha P**, Ceder S, Li Q, Panaretakis T. Tumor cell-derived exosomes: a message in a bottle. *Biochim Biophys Acta* 2012; **1826**: 103-111 [PMID: 22503823 DOI: 10.1016/j.bbcan.2012.03.006]
77 **Lee TH**, D'Asti E, Magnus N, Al-Nedawi K, Meehan B, Rak J. Microvesicles as mediators of intercellular communication in cancer--the emerging science of cellular 'debris'. *Semin Immunopathol* 2011; **33**: 455-467 [PMID: 21318413 DOI: 10.1007/s00281-011-0250-3]
78 **Simpson RJ**, Jensen SS, Lim JW. Proteomic profiling of exosomes: current perspectives. *Proteomics* 2008; **8**: 4083-4099 [PMID: 18780348 DOI: 10.1002/pmic.200800109]
79 **Simpson RJ**, Lim JW, Moritz RL, Mathivanan S. Exosomes: proteomic insights and diagnostic potential. *Expert Rev Proteomics* 2009; **6**: 267-283 [PMID: 19489699 DOI: 10.1586/epr.09.17]
80 **Rak J**. Microparticles in cancer. *Semin Thromb Hemost* 2010; **36**: 888-906 [PMID: 21049390 DOI: 10.1055/s-0030-1267043]
81 **Record M**, Subra C, Silvente-Poirot S, Poirot M. Exosomes as intercellular signalosomes and pharmacological effectors. *Biochem Pharmacol* 2011; **81**: 1171-1182 [PMID: 21371441 DOI: 10.1016/j.bcp.2011.02.011]
82 **Ramachandran S**, Palanisamy V. Horizontal transfer of RNAs: exosomes as mediators of intercellular communication. *Wiley Interdiscip Rev RNA* 2012; **3**: 286-293 [PMID: 22012863 DOI: 10.1002/wrna.115]
83 **Martins VR**, Dias MS, Hainaut P. Tumor-cell-derived microvesicles as carriers of molecular information in cancer. *Curr Opin Oncol* 2013; **25**: 66-75 [PMID: 23165142 DOI: 10.1097/CCO.0b013e32835b7c81]
84 **Gusachenko ON**, Zenkova MA, Vlassov VV. Nucleic acids in exosomes: disease markers and intercellular communication molecules. *Biochemistry* (Mosc) 2013; **78**: 1-7 [PMID: 23379554 DOI: 10.1134/S000629791301001X]
85 **Boon RA**, Vickers KC. Intercellular transport of microRNAs. *Arterioscler Thromb Vasc Biol* 2013; **33**: 186-192 [PMID: 23325475 DOI: 10.1161/ATVBAHA.112.300139]
86 **Lässer C**. Exosomal RNA as biomarkers and the therapeutic potential of exosome vectors. *Expert Opin Biol Ther* 2012; **12** Suppl 1: S189-S197 [PMID: 22506888 DOI: 10.1517/14712598.2012.680018]
87 **Raposo G**, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol* 2013; **200**: 373-383 [PMID: 23420871 DOI: 10.1083/jcb.201211138]
88 **Katzmann DJ**, Odorizzi G, Emr SD. Receptor downregulation and multivesicular-body sorting. *Nat Rev Mol Cell Biol* 2002; **3**: 893-905 [PMID: 12461556]
89 **Février B**, Raposo G. Exosomes: endosomal-derived vesicles shipping extracellular messages. *Curr Opin Cell Biol* 2004; **16**: 415-421 [PMID: 15261674]
90 **Buschow SI**, Nolte-'t Hoen EN, van Niel G, Pols MS, ten Broeke T, Lauwen M, Ossendorp F, Melief CJ, Raposo G, Wubbolts R, Wauben MH, Stoorvogel W. MHC Ⅱ in dendritic cells is targeted to lysosomes or T cell-induced exosomes via distinct multivesicular body pathways. *Traffic* 2009; **10**: 1528-1542 [PMID: 19682328 DOI: 10.1111/j.1600-0854.2009.00963.x]
91 **Zöller M**. Tetraspanins: push and pull in suppressing and promoting metastasis. *Nat Rev Cancer* 2009; **9**: 40-55 [PMID: 19078974 DOI: 10.1038/nrc2543]
92 **Blanc L**, Vidal M. Reticulocyte membrane remodeling: contribution of the exosome pathway. *Curr Opin Hematol* 2010; **17**: 177-183 [PMID: 20173636 DOI: 10.1097/MOH.0b013e328337b4e3]
93 **Hurley JH**, Emr SD. The ESCRT complexes: structure and mechanism of a membrane-trafficking network. *Annu Rev Biophys Biomol Struct* 2006; **35**: 277-298 [PMID: 16689637]
94 **Trajkovic K**, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, Schwille P, Brügger B, Simons M. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science* 2008; **319**: 1244-1247 [PMID: 18309083 DOI: 10.1126/science.1153124]
95 **Fang Y**, Wu N, Gan X, Yan W, Morrell JC, Gould SJ. Higher-order oligomerization targets plasma membrane proteins and HIV gag to exosomes. *PLoS Biol* 2007; **5**: e158 [PMID: 17550307]
96 **Rana S**, Claas C, Kretz CC, Nazarenko I, Zoeller M. Activation-induced internalization differs for the tetraspanins CD9 and Tspan8: Impact on tumor cell motility. *Int J Biochem Cell Biol* 2011; **43**: 106-119 [PMID: 20937409 DOI: 10.1016/j.biocel.2010.10.002]
97 **Rana S**, Yue S, Stadel D, Zöller M. Toward tailored exosomes: the exosomal tetraspanin web contributes to target cell selection. *Int J Biochem Cell Biol* 2012; **44**: 1574-1584 [PMID: 22728313 DOI: 10.1016/j.biocel.2012.06.018]
98 **Nazarenko I**, Rana S, Baumann A, McAlear J, Hellwig A, Trendelenburg M, Lochnit G, Preissner KT, Zöller M. Cell surface tetraspanin Tspan8 contributes to molecular pathways of exosome-induced endothelial cell activation. *Cancer Res* 2010; **70**: 1668-1678 [PMID: 20124479 DOI: 10.1158/0008-5472.CAN-09-2470]
99 **Pols MS**, Klumperman J. Trafficking and function of the tetraspanin CD63. *Exp Cell Res* 2009; **315**: 1584-1592 [PMID: 18930046 DOI: 10.1016/j.yexcr.2008.09.020]
100 **Cho JA**, Lee YS, Kim SH, Ko JK, Kim CW. MHC independent anti-tumor immune responses induced by Hsp70-enriched exosomes generate tumor regression in murine models. *Cancer Lett* 2009; **275**: 256-265 [PMID: 19036499 DOI: 10.1016/j.canlet.2008.10.021]
101 **Xie Y**, Bai O, Zhang H, Yuan J, Zong S, Chibbar R, Slattery K, Qureshi M, Wei Y, Deng Y, Xiang J. Membrane-bound HSP70-engineered myeloma cell-derived exosomes stimulate more efficient CD8(+) CTL- and NK-mediated antitumour immunity than exosomes released from heat-shocked tumour cells expressing cytoplasmic HSP70. *J Cell Mol Med* 2010; **14**: 2655-2666 [PMID: 19627400 DOI: 10.1111/j.1582-4934.2009.00851.x]
102 **Shimoda M**, Khokha R. Proteolytic factors in exosomes. *Proteomics* 2013; **13**: 1624-1636 [PMID: 23526769 DOI: 10.1002/pmic.201200458]
103 **Mu W**, Rana S, Zöller M. Host matrix modulation by tumor exosomes promotes motility and invasiveness. *Neoplasia* 2013; **15**: 875-877 [PMID: 23908589]
104 **Henderson MC**, Azorsa DO. High-throughput RNAi screening for the identification of novel targets. *Methods Mol Biol* 2013; **986**: 89-95 [PMID: 23436407 DOI: 10.3389/fonc.2012.00038]
105 **Sahu R**, Kaushik S, Clement CC, Cannizzo ES, Scharf B, Follenzi A, Potolicchio I, Nieves E, Cuervo AM, Santambrogio L. Microautophagy of cytosolic proteins by late endosomes. *Dev Cell* 2011; **20**: 131-139 [PMID: 21238931 DOI: 10.1016/j.devcel.2010.12.003]
106 **Valadi H**, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 2007; **9**: 654-659 [PMID: 17486113]
107 **Pant S**, Hilton H, Burczynski ME. The multifaceted exosome: biogenesis, role in normal and aberrant cellular function, and frontiers for pharmacological and biomarker opportunities. *Biochem Pharmacol* 2012; **83**: 1484-1494 [PMID: 22230477 DOI: 10.1016/j.bcp.2011.12.037]
108 **Skog J**, Würdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, Curry WT, Carter BS, Krichevsky AM,

Breakefield XO. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol* 2008; **10**: 1470-1476 [PMID: 19011622 DOI: 10.1038/ncb1800]
109 **Rana S**, Malinowska K, Zöller M. Exosomal tumor microRNA modulates premetastatic organ cells. *Neoplasia* 2013; **15**: 281-295 [PMID: 23479506]
110 **Gibbings DJ**, Ciaudo C, Erhardt M, Voinnet O. Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. *Nat Cell Biol* 2009; **11**: 1143-1149 [PMID: 19684575 DOI: 10.1038/ncb1929]
111 **Lee YS**, Pressman S, Andress AP, Kim K, White JL, Cassidy JJ, Li X, Lubell K, Lim do H, Cho IS, Nakahara K, Preall JB, Bellare P, Sontheimer EJ, Carthew RW. Silencing by small RNAs is linked to endosomal trafficking. *Nat Cell Biol* 2009; **11**: 1150-1156 [PMID: 19684574 DOI: 10.1038/ncb1930]
112 **Kosaka N**, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. *J Biol Chem* 2010; **285**: 17442-17452 [PMID: 20353945 DOI: 10.1074/jbc.M110.107821]
113 **Wittmann J**, Jäck HM. Serum microRNAs as powerful cancer biomarkers. *Biochim Biophys Acta* 2010; **1806**: 200-207 [PMID: 20637263 DOI: 10.1016/j.bbcan.2010.07.002]
114 **Tauro BJ**, Greening DW, Mathias RA, Ji H, Mathivanan S, Scott AM, Simpson RJ. Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes. *Methods* 2012; **56**: 293-304 [PMID: 22285593 DOI: 10.1016/j.ymeth.2012.01.002]
115 **Cantin R**, Diou J, Bélanger D, Tremblay AM, Gilbert C. Discrimination between exosomes and HIV-1: purification of both vesicles from cell-free supernatants. *J Immunol Methods* 2008; **338**: 21-30 [PMID: 18675270 DOI: 10.1016/j.jim.2008.07.007]
116 **Lässer C**, Eldh M, Lötvall J. Isolation and characterization of RNA-containing exosomes. *J Vis Exp* 2012; **9**: e3037 [PMID: 22257828 DOI: 10.3791/3037]
117 **Burger D**, Schock S, Thompson CS, Montezano AC, Hakim AM, Touyz RM. Microparticles: biomarkers and beyond. *Clin Sci* (Lond) 2013; **124**: 423-441 [PMID: 23249271 DOI: 10.1042/CS20120309]
118 **Chen CL**, Lai YF, Tang P, Chien KY, Yu JS, Tsai CH, Chen HW, Wu CC, Chung T, Hsu CW, Chen CD, Chang YS, Chang PL, Chen YT. Comparative and targeted proteomic analyses of urinary microparticles from bladder cancer and hernia patients. *J Proteome Res* 2012; **11**: 5611-5629 [PMID: 23082778 DOI: 10.1021/pr3008732]
119 **Orozco AF**, Lewis DE. Flow cytometric analysis of circulating microparticles in plasma. *Cytometry A* 2010; **77**: 502-514 [PMID: 20235276 DOI: 10.1002/cyto.a.20886]
120 **Kim G**, Yoo CE, Kim M, Kang HJ, Park D, Lee M, Huh N. Noble polymeric surface conjugated with zwitterionic moieties and antibodies for the isolation of exosomes from human serum. *Bioconjug Chem* 2012; **23**: 2114-2120 [PMID: 23025585 DOI: 10.1021/bc300339b]
121 **Taylor DD**, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecol Oncol* 2008; **110**: 13-21 [PMID: 18589210 DOI: 10.1016/j.ygyno.2008.04.033]
122 **Bryant RJ**, Pawlowski T, Catto JW, Marsden G, Vessella RL, Rhees B, Kuslich C, Visakorpi T, Hamdy FC. Changes in circulating microRNA levels associated with prostate cancer. *Br J Cancer* 2012; **106**: 768-774 [PMID: 22240788 DOI: 10.1038/bjc.2011.595]
123 **Dick JE**. Stem cell concepts renew cancer research. *Blood* 2008; **112**: 4793-4807 [PMID: 19064739 DOI: 10.1182/blood-2008-08-077941]
124 **Wang H**, Rana S, Giese N, Büchler MW, Zöller M. Tspan8, CD44v6 and α6beta4 are biomarkers of migrating pancreatic cancer-initiating cells. *Int J Cancer* 2013; **133**: 416-426 [PMID: 23338841 DOI: 10.1002/ijc.28044]
125 **Ischenko I**, Seeliger H, Kleespies A, Angele MK, Eichhorn ME, Jauch KW, Bruns CJ. Pancreatic cancer stem cells: new understanding of tumorigenesis, clinical implications. *Langenbecks Arch Surg* 2010; **395**: 1-10 [PMID: 19421768 DOI: 10.1007/s00423-009-0502-z]
126 **Quante M**, Wang TC. Stem cells in gastroenterology and hepatology. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 724-737 [PMID: 19884893 DOI: 10.1038/nrgastro.2009.195]
127 **Lonardo E**, Hermann PC, Heeschen C. Pancreatic cancer stem cells - update and future perspectives. *Mol Oncol* 2010; **4**: 431-442 [PMID: 20580623 DOI: 10.1016/j.molonc.2010.06.002]
128 **Rupp AK**, Rupp C, Keller S, Brase JC, Ehehalt R, Fogel M, Moldenhauer G, Marmé F, Sültmann H, Altevogt P. Loss of EpCAM expression in breast cancer derived serum exosomes: role of proteolytic cleavage. *Gynecol Oncol* 2011; **122**: 437-446 [PMID: 21601258 DOI: 10.1016/j.ygyno.2011.04.035]
129 **Keller S**, König AK, Marmé F, Runz S, Wolterink S, Koensgen D, Mustea A, Sehouli J, Altevogt P. Systemic presence and tumor-growth promoting effect of ovarian carcinoma released exosomes. *Cancer Lett* 2009; **278**: 73-81 [PMID: 19188015 DOI: 10.1016/j.canlet.2008.12.028]
130 **Li J**, Sherman-Baust CA, Tsai-Turton M, Bristow RE, Roden RB, Morin PJ. Claudin-containing exosomes in the peripheral circulation of women with ovarian cancer. *BMC Cancer* 2009; **9**: 244 [PMID: 19619303 DOI: 10.1186/1471-2407-9-244]
131 **Khan S**, Jutzy JM, Valenzuela MM, Turay D, Aspe JR, Ashok A, Mirshahidi S, Mercola D, Lilly MB, Wall NR. Plasma-derived exosomal survivin, a plausible biomarker for early detection of prostate cancer. *PLoS One* 2012; **7**: e46737 [PMID: 23091600 DOI: 10.1371/journal.pone.0046737]
132 **Nilsson J**, Skog J, Nordstrand A, Baranov V, Mincheva-Nilsson L, Breakefield XO, Widmark A. Prostate cancer-derived urine exosomes: a novel approach to biomarkers for prostate cancer. *Br J Cancer* 2009; **100**: 1603-1607 [PMID: 19401683 DOI: 10.1038/sj.bjc.6605058]
133 **Mitchell PJ**, Welton J, Staffurth J, Court J, Mason MD, Tabi Z, Clayton A. Can urinary exosomes act as treatment response markers in prostate cancer? *J Transl Med* 2009; **7**: 4 [PMID: 19138409 DOI: 10.1186/1479-5876-7-4]
134 **Logozzi M**, De Milito A, Lugini L, Borghi M, Calabrò L, Spada M, Perdicchio M, Marino ML, Federici C, Iessi E, Brambilla D, Venturi G, Lozupone F, Santinami M, Huber V, Maio M, Rivoltini L, Fais S. High levels of exosomes expressing CD63 and caveolin-1 in plasma of melanoma patients. *PLoS One* 2009; **4**: e5219 [PMID: 19381331 DOI: 10.1371/journal.pone.0005219]
135 **Koga K**, Matsumoto K, Akiyoshi T, Kubo M, Yamanaka N, Tasaki A, Nakashima H, Nakamura M, Kuroki S, Tanaka M, Katano M. Purification, characterization and biological significance of tumor-derived exosomes. *Anticancer Res* 2005; **25**: 3703-3707 [PMID: 16302729]
136 **Bauer M**, Pelkmans L. A new paradigm for membrane-organizing and -shaping scaffolds. *FEBS Lett* 2006; **580**: 5559-5564 [PMID: 16996501]
137 **Nguyen J**, Szoka FC. Nucleic acid delivery: the missing pieces of the puzzle? *Acc Chem Res* 2012; **45**: 1153-1162 [PMID: 22428908 DOI: 10.1021/ar3000162]
138 **Vickers KC**, Remaley AT. Lipid-based carriers of microRNAs and intercellular communication. *Curr Opin Lipidol* 2012; **23**: 91-97 [PMID: 22418571 DOI: 10.1097/MOL.0b013e328350a425]
139 **Zakharova L**, Svetlova M, Fomina AF. T cell exosomes induce cholesterol accumulation in human monocytes via phosphatidylserine receptor. *J Cell Physiol* 2007; **212**: 174-181 [PMID: 17299798]
140 **Feng D**, Zhao WL, Ye YY, Bai XC, Liu RQ, Chang LF, Zhou Q, Sui SF. Cellular internalization of exosomes occurs through phagocytosis. *Traffic* 2010; **11**: 675-687 [PMID: 20136776 DOI: 10.1111/j.1600-0854.2010.01041.x]
141 **Zech D**, Rana S, Büchler MW, Zöller M. Tumor-exosomes and

leukocyte activation: an ambivalent crosstalk. *Cell Commun Signal* 2012; **10**: 37 [PMID: 23190502 DOI: 10.1186/1478-811X-10-37]
142 **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**: 563-571 [PMID: 17900673]
143 **Morelli AE**, Larregina AT, Shufesky WJ, Sullivan ML, Stolz DB, Papworth GD, Zahorchak AF, Logar AJ, Wang Z, Watkins SC, Falo LD, Thomson AW. Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. *Blood* 2004; **104**: 3257-3266 [PMID: 15284116]
144 **Atay S**, Gercel-Taylor C, Taylor DD. Human trophoblast-derived exosomal fibronectin induces pro-inflammatory IL-1β production by macrophages. *Am J Reprod Immunol* 2011; **66**: 259-269 [PMID: 21410811 DOI: 10.1111/j.1600-0897.2011.00995.x]
145 **Mathivanan S**, Lim JW, Tauro BJ, Ji H, Moritz RL, Simpson RJ. Proteomics analysis of A33 immunoaffinity-purified exosomes released from the human colon tumor cell line LIM1215 reveals a tissue-specific protein signature. *Mol Cell Proteomics* 2010; **9**: 197-208 [PMID: 19837982 DOI: 10.1074/mcp.M900152-MCP200]
146 **Perez-Hernandez D**, Gutiérrez-Vázquez C, Jorge I, López-Martín S, Ursa A, Sánchez-Madrid F, Vázquez J, Yáñez-Mó M. The intracellular interactome of tetraspanin-enriched microdomains reveals their function as sorting machineries toward exosomes. *J Biol Chem* 2013; **288**: 11649-11661 [PMID: 23463506 DOI: 10.1074/jbc.M112.445304]
147 **Lakkaraju A**, Rodriguez-Boulan E. Itinerant exosomes: emerging roles in cell and tissue polarity. *Trends Cell Biol* 2008; **18**: 199-209 [PMID: 18396047 DOI: 10.1016/j.tcb.2008.03.002]
148 **Tian T**, Wang Y, Wang H, Zhu Z, Xiao Z. Visualizing of the cellular uptake and intracellular trafficking of exosomes by live-cell microscopy. *J Cell Biochem* 2010; **111**: 488-496 [PMID: 20533300 DOI: 10.1002/jcb.22733]
149 **Skokos D**, Botros HG, Demeure C, Morin J, Peronet R, Birkenmeier G, Boudaly S, Mécheri S. Mast cell-derived exosomes induce phenotypic and functional maturation of dendritic cells and elicit specific immune responses in vivo. *J Immunol* 2003; **170**: 3037-3045 [PMID: 12626558]
150 **Parolini I**, Federici C, Raggi C, Lugini L, Palleschi S, De Milito A, Coscia C, Iessi E, Logozzi M, Molinari A, Colone M, Tatti M, Sargiacomo M, Fais S. Microenvironmental pH is a key factor for exosome traffic in tumor cells. *J Biol Chem* 2009; **284**: 34211-34222 [PMID: 19801663 DOI: 10.1074/jbc.M109.041152]
151 **Xu D**, Sharma C, Hemler ME. Tetraspanin12 regulates ADAM10-dependent cleavage of amyloid precursor protein. *FASEB J* 2009; **23**: 3674-3681 [PMID: 19587294 DOI: 10.1096/fj.09-133462]
152 **Arduise C**, Abache T, Li L, Billard M, Chabanon A, Ludwig A, Mauduit P, Boucheix C, Rubinstein E, Le Naour F. Tetraspanins regulate ADAM10-mediated cleavage of TNF-α and epidermal growth factor. *J Immunol* 2008; **181**: 7002-7013 [PMID: 18981120]
153 **Gutiérrez-López MD**, Gilsanz A, Yáñez-Mó M, Ovalle S, Lafuente EM, Domínguez C, Monk PN, González-Alvaro I, Sánchez-Madrid F, Cabañas C. The sheddase activity of ADAM17/TACE is regulated by the tetraspanin CD9. *Cell Mol Life Sci* 2011; **68**: 3275-3292 [PMID: 21365281 DOI: 10.1007/s00018-011-0639-0]
154 **Potolicchio I**, Carven GJ, Xu X, Stipp C, Riese RJ, Stern LJ, Santambrogio L. Proteomic analysis of microglia-derived exosomes: metabolic role of the aminopeptidase CD13 in neuropeptide catabolism. *J Immunol* 2005; **175**: 2237-2243 [PMID: 16081791]
155 **Le Naour F**, André M, Boucheix C, Rubinstein E. Membrane microdomains and proteomics: lessons from tetraspanin microdomains and comparison with lipid rafts. *Proteomics* 2006; **6**: 6447-6454 [PMID: 17109380]
156 **Yañez-Mó M**, Barreiro O, Gonzalo P, Batista A, Megías D, Genís L, Sachs N, Sala-Valdés M, Alonso MA, Montoya MC, Sonnenberg A, Arroyo AG, Sánchez-Madrid F. MT1-MMP collagenolytic activity is regulated through association with tetraspanin CD151 in primary endothelial cells. *Blood* 2008; **112**: 3217-3226 [PMID: 18663148 DOI: 10.1182/blood-2008-02-139394]
157 **Hendrix A**, Westbroek W, Bracke M, De Wever O. An ex(o)citing machinery for invasive tumor growth. *Cancer Res* 2010; **70**: 9533-9537 [PMID: 21098711 DOI: 10.1158/0008-5472.CAN-10-3248]
158 **Grange C**, Tapparo M, Collino F, Vitillo L, Damasco C, Deregibus MC, Tetta C, Bussolati B, Camussi G. Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. *Cancer Res* 2011; **71**: 5346-5356 [PMID: 21670082 DOI: 10.1158/0008-5472.CAN-11-0241]
159 **Lafleur MA**, Xu D, Hemler ME. Tetraspanin proteins regulate membrane type-1 matrix metalloproteinase-dependent pericellular proteolysis. *Mol Biol Cell* 2009; **20**: 2030-2040 [PMID: 19211836 DOI: 10.1091/mbc.E08-11-1149]
160 **Hakulinen J**, Sankkila L, Sugiyama N, Lehti K, Keski-Oja J. Secretion of active membrane type 1 matrix metalloproteinase (MMP-14) into extracellular space in microvesicular exosomes. *J Cell Biochem* 2008; **105**: 1211-1218 [PMID: 18802920 DOI: 10.1002/jcb.21923]
161 **Nieuwland R**, van der Post JA, Lok CA, Kenter G, Sturk A. Microparticles and exosomes in gynecologic neoplasias. *Semin Thromb Hemost* 2010; **36**: 925-929 [PMID: 21049392 DOI: 10.1055/s-0030-1267046]
162 **Park JE**, Tan HS, Datta A, Lai RC, Zhang H, Meng W, Lim SK, Sze SK. Hypoxic tumor cell modulates its microenvironment to enhance angiogenic and metastatic potential by secretion of proteins and exosomes. *Mol Cell Proteomics* 2010; **9**: 1085-1099 [PMID: 20124223 DOI: 10.1074/mcp.M900381-MCP200]
163 **Nakada M**, Miyamori H, Kita D, Takahashi T, Yamashita J, Sato H, Miura R, Yamaguchi Y, Okada Y. Human glioblastomas overexpress ADAMTS-5 that degrades brevican. *Acta Neuropathol* 2005; **110**: 239-246 [PMID: 16133547]
164 **Lo Cicero A**, Majkowska I, Nagase H, Di Liegro I, Troeberg L. Microvesicles shed by oligodendroglioma cells and rheumatoid synovial fibroblasts contain aggrecanase activity. *Matrix Biol* 2012; **31**: 229-233 [PMID: 22406378 DOI: 10.1016/j.matbio.2012.02.005]
165 **Ginestra A**, La Placa MD, Saladino F, Cassarà D, Nagase H, Vittorelli ML. The amount and proteolytic content of vesicles shed by human cancer cell lines correlates with their in vitro invasiveness. *Anticancer Res* 1998; **18**: 3433-3437 [PMID: 9858920]
166 **Ngora H**, Galli UM, Miyazaki K, Zöller M. Membrane-bound and exosomal metastasis-associated C4.4A promotes migration by associating with the α(6)β(4) integrin and MT1-MMP. *Neoplasia* 2012; **14**: 95-107 [PMID: 22431918]
167 **Sangaletti S**, Colombo MP. Matricellular proteins at the crossroad of inflammation and cancer. *Cancer Lett* 2008; **267**: 245-253 [PMID: 18471960 DOI: 10.1016/j.canlet.2008.03.027]
168 **Clayton A**, Mitchell JP, Court J, Mason MD, Tabi Z. Human tumor-derived exosomes selectively impair lymphocyte responses to interleukin-2. *Cancer Res* 2007; **67**: 7458-7466 [PMID: 17671216]
169 **Delcayre A**, Shu H, Le Pecq JB. Dendritic cell-derived exosomes in cancer immunotherapy: exploiting nature's antigen delivery pathway. *Expert Rev Anticancer Ther* 2005; **5**: 537-547 [PMID: 16001959]
170 **Artavanis-Tsakonas K**, Kasperkovitz PV, Papa E, Cardenas ML, Khan NS, Van der Veen AG, Ploegh HL, Vyas JM. The tetraspanin CD82 is specifically recruited to fungal and bacterial phagosomes prior to acidification. *Infect Immun* 2011; **79**: 1098-1106 [PMID: 21149584 DOI: 10.1128/IAI.01135-10]
171 **Tumne A**, Prasad VS, Chen Y, Stolz DB, Saha K, Ratner DM,

Ding M, Watkins SC, Gupta P. Noncytotoxic suppression of human immunodeficiency virus type 1 transcription by exosomes secreted from CD8+ T cells. *J Virol* 2009; **83**: 4354-4364 [PMID: 19193788 DOI: 10.1128/JVI.02629-08]

172 **Taylor DD**, Gercel-Taylor C. Exosomes/microvesicles: mediators of cancer-associated immunosuppressive microenvironments. *Semin Immunopathol* 2011; **33**: 441-454 [PMID: 21688197 DOI: 10.1007/s00281-010-0234-8]

173 **Zhang HG**, Kim H, Liu C, Yu S, Wang J, Grizzle WE, Kimberly RP, Barnes S. Curcumin reverses breast tumor exosomes mediated immune suppression of NK cell tumor cytotoxicity. *Biochim Biophys Acta* 2007; **1773**: 1116-1123 [PMID: 17555831]

174 **Ashiru O**, Boutet P, Fernández-Messina L, Agüera-González S, Skepper JN, Valés-Gómez M, Reyburn HT. Natural killer cell cytotoxicity is suppressed by exposure to the human NKG2D ligand MICA*008 that is shed by tumor cells in exosomes. *Cancer Res* 2010; **70**: 481-489 [PMID: 20068167 DOI: 10.1158/0008-5472.CAN-09-1688]

175 **Yuan XK**, Zhao XK, Xia YC, Zhu X, Xiao P. Increased circulating immunosuppressive CD14(+)HLA-DR(-/low) cells correlate with clinical cancer stage and pathological grade in patients with bladder carcinoma. *J Int Med Res* 2011; **39**: 1381-1391 [PMID: 21986138]

176 **Lima LG**, Chammas R, Monteiro RQ, Moreira ME, Barcinski MA. Tumor-derived microvesicles modulate the establishment of metastatic melanoma in a phosphatidylserine-dependent manner. *Cancer Lett* 2009; **283**: 168-175 [PMID: 19401262 DOI: 10.1016/j.canlet.2009.03.041]

177 **Lee HM**, Choi EJ, Kim JH, Kim TD, Kim YK, Kang C, Gho YS. A membranous form of ICAM-1 on exosomes efficiently blocks leukocyte adhesion to activated endothelial cells. *Biochem Biophys Res Commun* 2010; **397**: 251-256 [PMID: 20529672 DOI: 10.1016/j.bbrc.2010.05.094]

178 **Khalil AA**, Kabapy NF, Deraz SF, Smith C. Heat shock proteins in oncology: diagnostic biomarkers or therapeutic targets? *Biochim Biophys Acta* 2011; **1816**: 89-104 [PMID: 21605630 DOI: 10.1016/j.bbcan.2011.05.001]

179 **Elsner L**, Muppala V, Gehrmann M, Lozano J, Malzahn D, Bickeböller H, Brunner E, Zientkowska M, Herrmann T, Walter L, Alves F, Multhoff G, Dressel R. The heat shock protein HSP70 promotes mouse NK cell activity against tumors that express inducible NKG2D ligands. *J Immunol* 2007; **179**: 5523-5533 [PMID: 17911639]

180 **Dai S**, Wan T, Wang B, Zhou X, Xiu F, Chen T, Wu Y, Cao X. More efficient induction of HLA-A*0201-restricted and carcinoembryonic antigen (CEA)-specific CTL response by immunization with exosomes prepared from heat-stressed CEA-positive tumor cells. *Clin Cancer Res* 2005; **11**: 7554-7563 [PMID: 16243831]

181 **Hurwitz MD**, Kaur P, Nagaraja GM, Bausero MA, Manola J, Asea A. Radiation therapy induces circulating serum Hsp72 in patients with prostate cancer. *Radiother Oncol* 2010; **95**: 350-358 [PMID: 20430459 DOI: 10.1016/j.radonc.2010.03.024]

182 **Xiu F**, Cai Z, Yang Y, Wang X, Wang J, Cao X. Surface anchorage of superantigen SEA promotes induction of specific antitumor immune response by tumor-derived exosomes. *J Mol Med* (Berl) 2007; **85**: 511-521 [PMID: 17219095]

183 **Chen T**, Guo J, Yang M, Zhu X, Cao X. Chemokine-containing exosomes are released from heat-stressed tumor cells via lipid raft-dependent pathway and act as efficient tumor vaccine. *J Immunol* 2011; **186**: 2219-2228 [PMID: 21242526 DOI: 10.4049/jimmunol.1002991]

184 **Zeelenberg IS**, van Maren WW, Boissonnas A, Van Hout-Kuijer MA, Den Brok MH, Wagenaars JA, van der Schaaf A, Jansen EJ, Amigorena S, Théry C, Figdor CG, Adema GJ. Antigen localization controls T cell-mediated tumor immunity. *J Immunol* 2011; **187**: 1281-1288 [PMID: 21705625 DOI: 10.4049/jimmunol.1003905]

185 **Hood JL**, San RS, Wickline SA. Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis. *Cancer Res* 2011; **71**: 3792-3801 [PMID: 21478294 DOI: 10.1158/0008-5472.CAN-10-4455]

186 **Sheldon H**, Heikamp E, Turley H, Dragovic R, Thomas P, Oon CE, Leek R, Edelmann M, Kessler B, Sainson RC, Sargent I, Li JL, Harris AL. New mechanism for Notch signaling to endothelium at a distance by Delta-like 4 incorporation into exosomes. *Blood* 2010; **116**: 2385-2394 [PMID: 20558614 DOI: 10.1182/blood-2009-08-239228]

187 **Benameur T**, Tual-Chalot S, Andriantsitohaina R, Martínez MC. PPARα is essential for microparticle-induced differentiation of mouse bone marrow-derived endothelial progenitor cells and angiogenesis. *PLoS One* 2010; **5**: e12392 [PMID: 20811625 DOI: 10.1371/journal.pone.0012392]

188 **Castellana D**, Zobairi F, Martinez MC, Panaro MA, Mitolo V, Freyssinet JM, Kunzelmann C. Membrane microvesicles as actors in the establishment of a favorable prostatic tumoral niche: a role for activated fibroblasts and CX3CL1-CX3CR1 axis. *Cancer Res* 2009; **69**: 785-793 [PMID: 19155311 DOI: 10.1158/0008-5472.CAN-08-1946]

189 **Chairoungdua A**, Smith DL, Pochard P, Hull M, Caplan MJ. Exosome release of β-catenin: a novel mechanism that antagonizes Wnt signaling. *J Cell Biol* 2010; **190**: 1079-1091 [PMID: 20837771 DOI: 10.1083/jcb.201002049]

190 **Hupalowska A**, Miaczynska M. The new faces of endocytosis in signaling. *Traffic* 2012; **13**: 9-18 [PMID: 21752167 DOI: 10.1111/j.1600-0854.2011.01249.x]

191 **Bobrie A**, Krumeich S, Reyal F, Recchi C, Moita LF, Seabra MC, Ostrowski M, Théry C. Rab27a supports exosome-dependent and -independent mechanisms that modify the tumor microenvironment and can promote tumor progression. *Cancer Res* 2012; **72**: 4920-4930 [PMID: 22865453 DOI: 10.1158/0008-5472.CAN-12-0925]

192 **Aliotta JM**, Lee D, Puente N, Faradyan S, Sears EH, Amaral A, Goldberg L, Dooner MS, Pereira M, Quesenberry PJ. Progenitor/stem cell fate determination: interactive dynamics of cell cycle and microvesicles. *Stem Cells Dev* 2012; **21**: 1627-1638 [PMID: 22214238 DOI: 10.1089/scd.2011.0550]

193 **Pan Q**, Ramakrishnaiah V, Henry S, Fouraschen S, de Ruiter PE, Kwekkeboom J, Tilanus HW, Janssen HL, van der Laan LJ. Hepatic cell-to-cell transmission of small silencing RNA can extend the therapeutic reach of RNA interference (RNAi). *Gut* 2012; **61**: 1330-1339 [PMID: 22198713 DOI: 10.1136/gutjnl-2011-300449]

194 **Al-Nedawi K**, Meehan B, Micallef J, Lhotak V, May L, Guha A, Rak J. Intercellular transfer of the oncogenic receptor EGFRv Ⅲ by microvesicles derived from tumour cells. *Nat Cell Biol* 2008; **10**: 619-624 [PMID: 18425114 DOI: 10.1038/ncb1725]

195 **Balaj L**, Lessard R, Dai L, Cho YJ, Pomeroy SL, Breakefield XO, Skog J. Tumour microvesicles contain retrotransposon elements and amplified oncogene sequences. *Nat Commun* 2011; **2**: 180 [PMID: 21285958 DOI: 10.1038/ncomms1180]

196 **Demory Beckler M**, Higginbotham JN, Franklin JL, Ham AJ, Halvey PJ, Imasuen IE, Whitwell C, Li M, Liebler DC, Coffey RJ. Proteomic analysis of exosomes from mutant KRAS colon cancer cells identifies intercellular transfer of mutant KRAS. *Mol Cell Proteomics* 2013; **12**: 343-355 [PMID: 23161513 DOI: 10.1074/mcp.M112.022806]

197 **Verweij FJ**, Middeldorp JM, Pegtel DM. Intracellular signaling controlled by the endosomal-exosomal pathway. *Commun Integr Biol* 2012; **5**: 88-93 [PMID: 22482020]

198 **Higginbotham JN**, Demory Beckler M, Gephart JD, Franklin JL, Bogatcheva G, Kremers GJ, Piston DW, Ayers GD, McConnell RE, Tyska MJ, Coffey RJ. Amphiregulin exosomes increase cancer cell invasion. *Curr Biol* 2011; **21**: 779-786 [PMID: 21514161 DOI: 10.1016/j.cub.2011.03.043]

199 **Antonyak MA**, Li B, Boroughs LK, Johnson JL, Druso JE, Bryant KL, Holowka DA, Cerione RA. Cancer cell-derived microvesicles induce transformation by transferring tissue transglutaminase and fibronectin to recipient cells. *Proc Natl*

Acad Sci USA 2011; **108**: 4852-4857 [PMID: 21368175 DOI: 10.1073/pnas.1017667108]

200 **Peinado H**, Alečković M, Lavotshkin S, Matei I, Costa-Silva B, Moreno-Bueno G, Hergueta-Redondo M, Williams C, García-Santos G, Ghajar C, Nitadori-Hoshino A, Hoffman C, Badal K, Garcia BA, Callahan MK, Yuan J, Martins VR, Skog J, Kaplan RN, Brady MS, Wolchok JD, Chapman PB, Kang Y, Bromberg J, Lyden D. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat Med* 2012; **18**: 883-891 [PMID: 22635005 DOI: 10.1038/nm.2753]

201 **Khan S**, Jutzy JM, Aspe JR, McGregor DW, Neidigh JW, Wall NR. Survivin is released from cancer cells via exosomes. *Apoptosis* 2011; **16**: 1-12 [PMID: 20717727 DOI: 10.1007/s10495-010-0534-4]

202 **Webber J**, Steadman R, Mason MD, Tabi Z, Clayton A. Cancer exosomes trigger fibroblast to myofibroblast differentiation. *Cancer Res* 2010; **70**: 9621-9630 [PMID: 21098712 DOI: 10.1158/0008-5472.CAN-10-1722]

203 **Cho JA**, Park H, Lim EH, Lee KW. Exosomes from breast cancer cells can convert adipose tissue-derived mesenchymal stem cells into myofibroblast-like cells. *Int J Oncol* 2012; **40**: 130-138 [PMID: 21904773 DOI: 10.3892/ijo.2011.1193]

204 **Wysoczynski M**, Ratajczak MZ. Lung cancer secreted microvesicles: underappreciated modulators of microenvironment in expanding tumors. *Int J Cancer* 2009; **125**: 1595-1603 [PMID: 19462451 DOI: 10.1002/ijc.24479]

205 **Putz U**, Howitt J, Doan A, Goh CP, Low LH, Silke J, Tan SS. The tumor suppressor PTEN is exported in exosomes and has phosphatase activity in recipient cells. *Sci Signal* 2012; **5**: ra70 [PMID: 23012657]

206 **Janowska-Wieczorek A**, Wysoczynski M, Kijowski J, Marquez-Curtis L, Machalinski B, Ratajczak J, Ratajczak MZ. Microvesicles derived from activated platelets induce metastasis and angiogenesis in lung cancer. *Int J Cancer* 2005; **113**: 752-760 [PMID: 15499615]

207 **Jung T**, Castellana D, Klingbeil P, Cuesta Hernández I, Vitacolonna M, Orlicky DJ, Roffler SR, Brodt P, Zöller M. CD44v6 dependence of premetastatic niche preparation by exosomes. *Neoplasia* 2009; **11**: 1093-1105 [PMID: 19794968]

208 **McCready J**, Sims JD, Chan D, Jay DG. Secretion of extracellular hsp90α via exosomes increases cancer cell motility: a role for plasminogen activation. *BMC Cancer* 2010; **10**: 294 [PMID: 20553606 DOI: 10.1186/1471-2407-10-294]

209 **Luga V**, Zhang L, Viloria-Petit AM, Ogunjimi AA, Inanlou MR, Chiu E, Buchanan M, Hosein AN, Basik M, Wrana JL. Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. *Cell* 2012; **151**: 1542-1556 [PMID: 23260141 DOI: 10.1016/j.cell.2012.11.024]

210 **Hong BS**, Cho JH, Kim H, Choi EJ, Rho S, Kim J, Kim JH, Choi DS, Kim YK, Hwang D, Gho YS. Colorectal cancer cell-derived microvesicles are enriched in cell cycle-related mRNAs that promote proliferation of endothelial cells. *BMC Genomics* 2009; **10**: 556 [PMID: 19930720 DOI: 10.1186/1471-2164-10-556]

211 **Al-Nedawi K**, Meehan B, Rak J. Microvesicles: messengers and mediators of tumor progression. *Cell Cycle* 2009; **8**: 2014-2018 [PMID: 19535896]

212 **Mineo M**, Garfield SH, Taverna S, Flugy A, De Leo G, Alessandro R, Kohn EC. Exosomes released by K562 chronic myeloid leukemia cells promote angiogenesis in a Src-dependent fashion. *Angiogenesis* 2012; **15**: 33-45 [PMID: 22203239 DOI: 10.1007/s10456-011-9241-1]

213 **Huan J**, Hornick NI, Shurtleff MJ, Skinner AM, Goloviznina NA, Roberts CT, Kurre P. RNA trafficking by acute myelogenous leukemia exosomes. *Cancer Res* 2013; **73**: 918-929 [PMID: 23149911 DOI: 10.1158/0008-5472.CAN-12-2184]

214 **Meckes DG**, Shair KH, Marquitz AR, Kung CP, Edwards RH, Raab-Traub N. Human tumor virus utilizes exosomes for intercellular communication. *Proc Natl Acad Sci USA* 2010; **107**: 20370-20375 [PMID: 21059916 DOI: 10.1073/pnas.1014194107]

215 **Gourzones C**, Gelin A, Bombik I, Klibi J, Vérillaud B, Guigay J, Lang P, Témam S, Schneider V, Amiel C, Baconnais S, Jimenez AS, Busson P. Extra-cellular release and blood diffusion of BART viral micro-RNAs produced by EBV-infected nasopharyngeal carcinoma cells. *Virol J* 2010; **7**: 271 [PMID: 20950422 DOI: 10.1186/1743-422X-7-271]

216 **Roccaro AM**, Sacco A, Maiso P, Azab AK, Tai YT, Reagan M, Azab F, Flores LM, Campigotto F, Weller E, Anderson KC, Scadden DT, Ghobrial IM. BM mesenchymal stromal cell-derived exosomes facilitate multiple myeloma progression. *J Clin Invest* 2013; **123**: 1542-1555 [PMID: 23454749]

217 **Yang M**, Chen J, Su F, Yu B, Su F, Lin L, Liu Y, Huang JD, Song E. Microvesicles secreted by macrophages shuttle invasion-potentiating microRNAs into breast cancer cells. *Mol Cancer* 2011; **10**: 117 [PMID: 21939504 DOI: 10.1186/1476-4598-10-117]

218 **Zhang Y**, Liu D, Chen X, Li J, Li L, Bian Z, Sun F, Lu J, Yin Y, Cai X, Sun Q, Wang K, Ba Y, Wang Q, Wang D, Yang J, Liu P, Xu T, Yan Q, Zhang J, Zen K, Zhang CY. Secreted monocytic miR-150 enhances targeted endothelial cell migration. *Mol Cell* 2010; **39**: 133-144 [PMID: 20603081 DOI: 10.1016/j.molcel.2010.06.010]

219 **Umezu T**, Ohyashiki K, Kuroda M, Ohyashiki JH. Leukemia cell to endothelial cell communication via exosomal miRNAs. *Oncogene* 2013; **32**: 2747-2755 [PMID: 22797057 DOI: 10.1038/onc.2012.295]

220 **Fabbri M**, Paone A, Calore F, Galli R, Gaudio E, Santhanam R, Lovat F, Fadda P, Mao C, Nuovo GJ, Zanesi N, Crawford M, Ozer GH, Wernicke D, Alder H, Caligiuri MA, Nana-Sinkam P, Perrotti D, Croce CM. MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response. *Proc Natl Acad Sci USA* 2012; **109**: E2110-E2116 [PMID: 22753494 DOI: 10.1073/pnas.1209414109]

221 **Kogure T**, Lin WL, Yan IK, Braconi C, Patel T. Intercellular nanovesicle-mediated microRNA transfer: a mechanism of environmental modulation of hepatocellular cancer cell growth. *Hepatology* 2011; **54**: 1237-1248 [PMID: 21721029 DOI: 10.1002/hep.24504]

222 **Pap E**, Pállinger E, Pásztói M, Falus A. Highlights of a new type of intercellular communication: microvesicle-based information transfer. *Inflamm Res* 2009; **58**: 1-8 [PMID: 19132498 DOI: 10.1007/s00011-008-8210-7]

223 **Lespagnol A**, Duflaut D, Beekman C, Blanc L, Fiucci G, Marine JC, Vidal M, Amson R, Telerman A. Exosome secretion, including the DNA damage-induced p53-dependent secretory pathway, is severely compromised in TSAP6/Steap3-null mice. *Cell Death Differ* 2008; **15**: 1723-1733 [PMID: 18617898 DOI: 10.1038/cdd.2008]

224 **Bianco F**, Perrotta C, Novellino L, Francolini M, Riganti L, Menna E, Saglietti L, Schuchman EH, Furlan R, Clementi E, Matteoli M, Verderio C. Acid sphingomyelinase activity triggers microparticle release from glial cells. *EMBO J* 2009; **28**: 1043-1054 [PMID: 19300439 DOI: 10.1038/emboj.2009.45]

225 **Taraboletti G**, D'Ascenzo S, Borsotti P, Giavazzi R, Pavan A, Dolo V. Shedding of the matrix metalloproteinases MMP-2, MMP-9, and MT1-MMP as membrane vesicle-associated components by endothelial cells. *Am J Pathol* 2002; **160**: 673-680 [PMID: 11839588]

226 **Hao S**, Moyana T, Xiang J. Review: cancer immunotherapy by exosome-based vaccines. *Cancer Biother Radiopharm* 2007; **22**: 692-703 [PMID: 17979572]

227 **Tan A**, De La Peña H, Seifalian AM. The application of exosomes as a nanoscale cancer vaccine. *Int J Nanomedicine* 2010; **5**: 889-900 [PMID: 21116329 DOI: 10.2147/IJN.S13402]

228 **Bobrie A**, Théry C. Unraveling the physiological functions of exosome secretion by tumors. *Oncoimmunology* 2013; **2**: e22565 [PMID: 23483742]

229 **Morse MA**, Garst J, Osada T, Khan S, Hobeika A, Clay TM, Valente N, Shreeniwas R, Sutton MA, Delcayre A, Hsu DH,

Le Pecq JB, Lyerly HK. A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. *J Transl Med* 2005; **3**: 9 [PMID: 15723705]

230 **Dai S**, Wei D, Wu Z, Zhou X, Wei X, Huang H, Li G. Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer. *Mol Ther* 2008; **16**: 782-790 [PMID: 18362931 DOI: 10.1038/mt.2008.1]

231 **Viaud S**, Théry C, Ploix S, Tursz T, Lapierre V, Lantz O, Zitvogel L, Chaput N. Dendritic cell-derived exosomes for cancer immunotherapy: what's next? *Cancer Res* 2010; **70**: 1281-1285 [PMID: 20145139 DOI: 10.1158/0008-5472.CAN-09-3276]

232 **Shen C**, Hao SG, Zhao CX, Zhu J, Wang C. Antileukaemia immunity: effect of exosomes against NB4 acute promyelocytic leukaemia cells. *J Int Med Res* 2011; **39**: 740-747 [PMID: 21819704]

233 **Hartman ZC**, Wei J, Glass OK, Guo H, Lei G, Yang XY, Osada T, Hobeika A, Delcayre A, Le Pecq JB, Morse MA, Clay TM, Lyerly HK. Increasing vaccine potency through exosome antigen targeting. *Vaccine* 2011; **29**: 9361-9367 [PMID: 22001882 DOI: 10.1016/j.vaccine.2011.09.133]

234 **Rountree RB**, Mandl SJ, Nachtwey JM, Dalpozzo K, Do L, Lombardo JR, Schoonmaker PL, Brinkmann K, Dirmeier U, Laus R, Delcayre A. Exosome targeting of tumor antigens expressed by cancer vaccines can improve antigen immunogenicity and therapeutic efficacy. *Cancer Res* 2011; **71**: 5235-5244 [PMID: 21670078 DOI: 10.1158/0008-5472.CAN-10-4076]

235 **Lv LH**, Wan YL, Lin Y, Zhang W, Yang M, Li GL, Lin HM, Shang CZ, Chen YJ, Min J. Anticancer drugs cause release of exosomes with heat shock proteins from human hepatocellular carcinoma cells that elicit effective natural killer cell antitumor responses in vitro. *J Biol Chem* 2012; **287**: 15874-15885 [PMID: 22396543 DOI: 10.1074/jbc.M112.340588]

236 **Claas C**, Seiter S, Claas A, Savelyeva L, Schwab M, Zöller M. Association between the rat homologue of CO-029, a metastasis-associated tetraspanin molecule and consumption coagulopathy. *J Cell Biol* 1998; **141**: 267-280 [PMID: 9531564]

237 **Gesierich S**, Berezovskiy I, Ryschich E, Zöller M. Systemic induction of the angiogenesis switch by the tetraspanin D6.1A/CO-029. *Cancer Res* 2006; **66**: 7083-7094 [PMID: 16849554]

238 **Marleau AM**, Chen CS, Joyce JA, Tullis RH. Exosome removal as a therapeutic adjuvant in cancer. *J Transl Med* 2012; **10**: 134 [PMID: 22738135 DOI: 10.1186/1479-5876-10-134]

239 **Safaei R**, Larson BJ, Cheng TC, Gibson MA, Otani S, Naerdemann W, Howell SB. Abnormal lysosomal trafficking and enhanced exosomal export of cisplatin in drug-resistant human ovarian carcinoma cells. *Mol Cancer Ther* 2005; **4**: 1595-1604 [PMID: 16227410]

240 **Yin J**, Yan X, Yao X, Zhang Y, Shan Y, Mao N, Yang Y, Pan L. Secretion of annexin A3 from ovarian cancer cells and its association with platinum resistance in ovarian cancer patients. *J Cell Mol Med* 2012; **16**: 337-348 [PMID: 21435174 DOI: 10.1111/j.1582-4934.2011.01316.x]

241 **Chen X**, Liang H, Zhang J, Zen K, Zhang CY. microRNAs are ligands of Toll-like receptors. *RNA* 2013; **19**: 737-739 [PMID: 23554231 DOI: 10.1261/rna.036319.112]

242 **Lotvall J**, Valadi H. Cell to cell signalling via exosomes through esRNA. *Cell Adh Migr* 2007; **1**: 156-158 [PMID: 19262134]

243 **Tan A**, Rajadas J, Seifalian AM. Exosomes as nano-theranostic delivery platforms for gene therapy. *Adv Drug Deliv Rev* 2013; **65**: 357-367 [PMID: 22820532 DOI: 10.1016/j.addr.2012.06.014]

244 **Seow Y**, Wood MJ. Biological gene delivery vehicles: beyond viral vectors. *Mol Ther* 2009; **17**: 767-777 [PMID: 19277019 DOI: 10.1038/mt.2009.41]

245 **Alvarez-Erviti L**, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol* 2011; **29**: 341-345 [PMID: 21423189 DOI: 10.1038/nbt.1807]

246 **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**: 185-191 [PMID: 23032975 DOI: 10.1038/mt.2012.180]

247 **Sun D**, Zhuang X, Xiang X, Liu Y, Zhang S, Liu C, Barnes S, Grizzle W, Miller D, Zhang HG. A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. *Mol Ther* 2010; **18**: 1606-1614 [PMID: 20571541 DOI: 10.1038/mt.2010.105]

248 **Zhuang X**, Xiang X, Grizzle W, Sun D, Zhang S, Axtell RC, Ju S, Mu J, Zhang L, Steinman L, Miller D, Zhang HG. Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. *Mol Ther* 2011; **19**: 1769-1779 [PMID: 21915101 DOI: 10.1038/mt.2011.164]

249 **Chalmin F**, Ladoire S, Mignot G, Vincent J, Bruchard M, Remy-Martin JP, Boireau W, Rouleau A, Simon B, Lanneau D, De Thonel A, Multhoff G, Hamman A, Martin F, Chauffert B, Solary E, Zitvogel L, Garrido C, Ryffel B, Borg C, Apetoh L, Rébé C, Ghiringhelli F. Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells. *J Clin Invest* 2010; **120**: 457-471 [PMID: 20093776 DOI: 10.1172/JCI40483]

250 **Maguire CA**, Balaj L, Sivaraman S, Crommentuijn MH, Ericsson M, Mincheva-Nilsson L, Baranov V, Gianni D, Tannous BA, Sena-Esteves M, Breakefield XO, Skog J. Microvesicle-associated AAV vector as a novel gene delivery system. *Mol Ther* 2012; **20**: 960-971 [PMID: 22314290 DOI: 10.1038/mt.2011.303]

251 **Kota J**, Chivukula RR, O'Donnell KA, Wentzel EA, Montgomery CL, Hwang HW, Chang TC, Vivekanandan P, Torbenson M, Clark KR, Mendell JR, Mendell JT. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell* 2009; **137**: 1005-1017 [PMID: 19524505 DOI: 10.1016/j.cell.2009.04.021]

252 **Kosaka N**, Takeshita F, Yoshioka Y, Hagiwara K, Katsuda T, Ono M, Ochiya T. Exosomal tumor-suppressive microRNAs as novel cancer therapy: "exocure" is another choice for cancer treatment. *Adv Drug Deliv Rev* 2013; **65**: 376-382 [PMID: 22841506 DOI: 10.1016/j.addr.2012.07.011]

P- Reviewer: Guo CY **S- Editor:** Wen LL
L- Editor: A **E- Editor:** Liu XM

百世登

Baishideng®

Published by **Baishideng Publishing Group Co., Limited**
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road, Wan Chai, Hong Kong, China
Telephone: +852-6555-7188
Fax: +852-3177-9906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com