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A N I N T E R N A T I O N A L J O U R N A L

Exosomes as Biomarker of Cancer

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ABSTRACT

Rapid advances in medicine and biotechnology resulted in the development of non-invasive diagnostic and prognostic biomarkers enabling convenient and accurate detection. Exosomes has recently emerged as non-invasive biomarker for a number of diseases including cancer. Exosomes are the small endosome originated membranous vesicles secreted in a number of biological fluids such as serum, saliva, urine, ascites, cerebrospinal fluid, etc. Exosomes contain microRNA proteins and mRNA which can be used as disease specific biomarkers. Here we reviewed recent advancement in the field of exosomes as diagnostic biomarker for cancer along with a brief overview of their biogenesis, function and isolation.

Key words: Exosome, Biomarker, Cancer biomarker

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INTRODUCTION

Exosomes are membrane bound extra cellular vesicles that originate from late endosome, ranging in size from 30 to 150 nano meter. These are released from several types of the cells and can be found circulating in almost all biological fluids. Exosomes were first described with reference to mammalian reticulocytes as circulating vesicles derived from multi vesicular bodies, containing membrane associated proteins[1]. During the last decade a number of studies shaped our understanding regarding composition and function of exosomes. It is known that exosomes carry different molecular components of the cells from which they originate. These include proteins, lipids, microRNA and mRNA[2]. Exosomes were once considered as a mechanism to secrete unwanted substances, but the detection of functional mRNA and microRNA in exosomes has generated enormous interest in studying their role in a variety of human pathologies and development. Exosomes act as a medium of communication between mammalian cells by mediating exchange of genetic material[3,4].

The lumen of exosomes is filled with cytoplasm, of the cell of their origin; they are a valuable sample of cell's interior showing enormous diagnostic potential. The main advantages that make exosomes, a promising tool in cancer diagnosis and prognosis include their ability to represent a global landscape of tumour heterogeneity that cannot be appreciated using traditional methods of mutation analysis.

Secondly analysis of circulating exosomes is much safer alternate to currently used invasive biopsies that are very difficult to perform repeatedly. Moreover the personalized nature of exosome based diagnosis like microRNA profiling is highly specific as compared to low specificity of conventional serum biomarkers that imparts marginal advantage in terms of personalized diagnosis if any at all[5].

BIOGENESIS OF EXOSOMES

Biogenesis of exosomes starts with the invagination of late endosomal membrane resulting in the formation of smaller vesicles in the lumen of late endosomes /multi vesicular bodies (MVBs). Membrane proteins that are selected for degradation are sorted into intra luminal vesicles of MVBs before fusion with lysosome. Alternatively MVBs fuse with cell membrane and release their luminal vesicles as exosomes (Figure 1). Large vesicles 100 to 1000 nm released directly from cell membrane are called microvesicles[6]. The very similar and somewhat overlapping size range of exosomes and microvesicles makes their separation difficult.

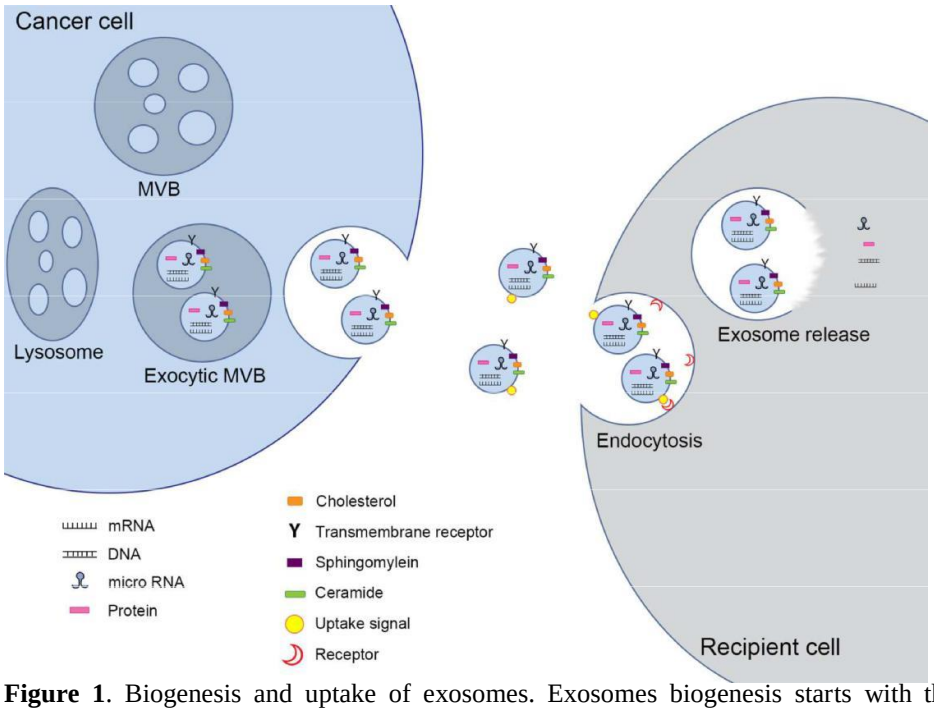

Figure 1. Biogenesis and uptake of exosomes. Exosomes biogenesis starts with the formation of intraluminal vesicles in late endosomes following cargo sorting. Both ESCRT dependent and ESCRT independent lipid driven pathways are involved in formation of multi vesicular bodies, MVBs. Exocytic MVB fuse with plasma membrane in a Rab GTPases regulated fashion. Exosome membrane is enriched in sphingomyelin, cholesterol, and ceramide whereas lumen of vesicle is filled with miRNA, mRNA, DNA and proteins. Exosomes released from cancer cell are taken up through endocytosis by neighbouring cells. Once endocytosed by recipient cell exosomes release their cargo, resulting in altered regulation of a variety of biological functions of recipient cell.

Endosomal sorting complex required for transport (ESCRT) is the multi protein complex that regulates formation of MVBs and its components for example Tsg101 is often found associated with exosomes.

Other protein markers found attached with exosomal membrane are also reminiscent of its origin including Rab GTPase, Annexins , SNAREs, Alix and flotillin[7].Exosomes isolated by ultracentrifugation appear as cup shaped structures when imaged using electron microscope[8].

Exosome content database, ExoCarta shows 9,769 proteins, 1,116 lipids, 3,408 mRNAs, and 2,838 miRNAs that were identified in exosomes from multiple organisms[9]. Proteins like Tsg101, tetraspanins,

CD63 and CD81 are commonly found with exosomes and can be used as exosome markers. The lipid content of exosomes includes cholesterol, sphingolipids, phospholipids, and bisphosphates[10].

Biological function of exosomes depends on their ability to recognise recipient cells. Specificity in target cell recognition is known from studies where B cell exosomes selectively recognize follicular dendritic cells and exosomes from human intestinal epithelial cells targeted dendritic cells[11,12].

ISOLATION OF EXOSOMES

Different groups investigating exosomal vesicles lack agreement on a universal method for exosome isolation from different body fluids. This is because of exosome size variation, variations in protein/lipid composition or varying percentages of non-specific component aggregation on exosome surface. All these factors affect sedimentation properties of exosomes and can interfere with purification. With the advancement of molecular detection techniques, even minute exosomal components can be quantified. Furthermore co-isolation of contamination other than exosomes creates another level of complexity in the interpretation of exosomal analysis data. The methods used for exosome isolation include ultracentrifugation, ultrafiltration, polymer – based precipitation and immunoaffinity, purification[13].

Ultracentrifugation, a "gold standard" method for isolation of exosomes, traditionally employs a centrifugal force in excess of 100,000 x g to a solution of various macromolecules, resulting in sedimentation of high density molecules from the centrifuge axis to less denser components[13]. Mostly ultracentrifugation is used along with sucrose density gradient, so the low density exosomes float[14]. The method is not fit for high throughput clinical applications due to its labour intensive nature. Ultracentrifugation consumes more time requires expensive laboratory equipments and highly trained personnel[15].

Size based isolation employing ultrafiltration is comparatively less time consuming and requires minimal of specialized equipment, making it a cost effective exosome isolation method[16].

Polymer based precipitation methods using polyethylene glycols (PEG) are frequently used for precipitation of viruses and other small particles[17-19]. The same technique of precipitation followed by (10,000 to 20,000 x g) centrifugation is being used for isolation of exosomes[20]. Commercial products such as Total Exosome Isolation by Life Technologies, ExoSpin by Cell Guidance Systems and ExoQuick by System Biosciences enables fast exosome precipitation from various biological fluids such as milk, blood, urine, amniotic fluid, serum, etc[15]. Various groups have compared commercially available exosome precipitation reagents reporting variation in yield and level of purity that can be achieved for subsequent downstream analysis[21].

Immunoaffinity capture is another promising new approach for isolating specific exosomes by affinity purification using lectins and antibodies against CD9, CD81, CD63, CD82, EpCAM, Alix and Rab5. For this approach to work, antibodies are immobilized on media like magnetic beads, chromatographic plates, matrices, and filters[14,15,22]. Use of specific antibodies gives this method selectivity in isolating subpopulations from circulating exosomes while making it somewhat less desirable method in terms of capturing the true exosome and tumour heterogeneity in clinical samples[13].

EXOSOMAL PROTEINS AS DIAGNOSTIC BIOMARKERS

Proteomics is a rapidly emerging field due to advancement in biotechniques and instrumentation. The research and development in proteomics has led to improvements in disease prognosis and diagnosis especially with reference to use of proteins as biomarker. Exosomes also have various proteins either enclosed within the vesicles or present on surface membrane. Latest techniques enabled researchers to detect, quantitate and characterize the proteins of exosomes. Peptide libraries can be prepared from isolated exosomes for comparison of protein profiles. The exosomal proteins have emerged as non-invasive diagnostic and prognostic biomarkers for many types of cancers[23,24].

Research conducted on exosomes shed in urine during various diseases has led to the development of an entire database of urinary exosome proteins, isolated from healthy human donors. Based on protein mass spectrometric analysis data obtained by NHLBI Epithelial Systems Biology Laboratory, their components, synthesis and functions have been catalogued as well[25, 26].

Table 1 lists exosomal proteins that can be used as potential biomarkers for various cancers. Some exosomes were derived from body fluids of patients including urine, serum, saliva, plasma, ascites, CSF, etc. while others were isolated from experimental cell lines.

Table 1. Exosomal Proteins in Different Cancers

Diseases	Exosomal Proteins	Level/Expression	Potencial Use	Methodology	Source	Ref.
Melanoma and other malignant cancers	CD63 and Caveolin 1 enriched exosomes.	Elevated	Diagnosis	In-house sandwich ELISA	Plasma	27
	β1 integrin, α6 integrin, basigin, CD 73.	Elevated surface expression	Prognosis	Mass spectrometry, Western blot, Flow cytometry		28
Non-small cell lung carcinoma	CD9, CD81	Elevated signal	Diagnosis	Extracellular Vesicle Array (EV Array)	Plasma	29
	CD 63	Co-variation in signal as				

(NSCLC)		compared to control				
	FAM3C	Over expression	Prognosis	LTQ-FT mass spectrometry	NSCLC cell lines	[30]
Prostate Cancer (PCa)	FASN, XPO1 and PDCD6IP proteins	Elevated expression	Diagnosis	LCFTMS/ Western blot/ Immuno-histochemistry	Immortalized primary prostate epithelial cells	[31]
	ENO-1	Decreased expression				
Ovarian Cancer	Epithelial cell adhesion molecule (EpCAM) and CD24	Over expression	Diagnosis	Magnetic activated cell sorting procedure (MACS)	Plasma/ Ovarian tumor derived exosomes	[32] [33]
	TGF-beta1, MAGE3/6, L1CAM, ADAM10, EMMPRIN, Claudin-4	Over expression	Diagnosis/ Prognosis	Fluorescent microscopy and cytofluorographic analysis.	Ascites/Blood	[34]
	TSG101 and Alix	Over expression	Prognosis	Protein profiling	Ovarian cancer cell lines	
Hepatitus C induced HCC	CD81	Over expression	Diagnosis	Immunoblotting and densitometry.	Exosomal serum fraction	[20]
Bladder cancer	MUC1, β1 integrin, α6 integrin, CD44, CD10, CD 73	Increased surface expression	Diagnosis/ Prognosis	Mass spectrometry/ Western blot/ Flow cytometry	HT1376 bladder cancer cells	[28]
	HBA, HBB and TACSTD2	Upregulated expression	Prognosis	Mass spectrometry	Urine	
Breast cancer	Survivin (Inhibitor of apoptosis), MUC 1, β1 integrin, CD73	Elevated level	Diagnosis/ Prognosis	SDS PAGE, Protein staining, Western Blot	Serum exosomes/ Saliva	[35]
	Breast cancer resistance protein (BCRP)	Elevated level	Prognostic biomarker	Immuno fluorescence	Plasma	[36]
Pancreatic cancer	CD44v6, Tspan 8, EpCAM, CD104	Elevated level	Diagnosis/ Prognosis	Flow cytometry	Serum exosomes	[37]
	Glypician 1 (Proteoglycan) circulating exosomes (GPC1+crExos)	Upregulated surface expression	Diagnosis	Mass spectrometry/ Flow cytometry	Serum/ Mice with pancreatic tumors	[38]
Colorectal cancer	CD81, CD63, CD9	Elevated	Diagnosis/ Prognosis	SDS PAGE, Protein staining, Western Blot	Colorectal cancer cell lines./Ascites	[39]
	Claudin 3	High level of detection	Diagnosis	Nano-LC-MS/MS analysis and 1-Dimensional SDS-PAGE	CRC-ascites	[33]
Gastric cancer	MAGE-1 and Her-2/neu+, CRC6	High expression	Prediction/ Prognosis Little diagnostic Value	Protein profiling	Platelet Depleted Plasma	[40]
	CxCR4	Down regulated expression				

EXOSOMAL NUCLEIC ACIDS AS BIOMARKER

Exosomes found in body fluids contain significant amount of different RNA species such as mRNA, miRNA, (micro RNA), snRNA (small nuclear RNA) and lncRNA (long non coding RNA) as well as DNA. Recently fragmented ribosomal RNA (rRNA) is discovered as major specie of exosomal RNA[41-45]. Much of the work conducted on evaluating RNA as biomarker started after Valadi's discovery of exosomal mRNA and miRNA in 2007[3]. The amount of

miRNA is higher in exosomes as compared to their parent cells 46. This is further confirmed by deep sequencing of exosomal RNA species by Huang et al. that concluded miRNA is the most abundant functional RNA specie in exosomes[47]. These discoveries stirred up interest in using miRNA as biomarker of different diseases.

miRNA are short, non-coding single stranded RNA molecules, having length up to 19-23 nucleotides. They regulate gene expression mostly by targeting 3´untranslated regions of mRNAs at post transcriptional level. miRNA plays a vital role in different biological processes that includes apoptosis, cell cycle control and are also associated with disease such as cancers and neurodegenerative disorders[48,49]. The composition and concentration of exosomal miRNAs varies among diseased and healthy individuals. This variation shows the potential of using exosome derived miRNAs as non-invasive biomarker. Several studies conducted on different types of cancer have reported cancer specific exosomal miRNAs as biomarker[50-53]. For example, miR-375 and miR-141 are up-regulated in serum of prostate cancer patients as compared to normal individuals[54]. Similarly miR-373, miR-200a, miR-200b and miR-200c can be used as diagnostic and prognostic biomarker of ovarian cancer. miR-372 is used as a biomarker of colorectal cancer[56]. Some exosomal miRNAs can be diagnostic or prognostic biomarker of more than one cancer while others are specific for particular cancer. For example, miR-21 is diagnostic biomarker of ovarian, breast, cervical, retinoblastoma, gastric, pancreatic, cervical cancer and laryngeal squamous cell carcinoma (LSCC)[57-63].

Besides miRNA exosomes also contains long non-coding RNA (lncRNA) that range in size from several 100-1000 bases. Transcribed in diseased and normal cells, the exact function of lncRNA is not clear, while there are some indications that lncRNA acts as a sponge for miRNA[64, 65, 66]. Prostate cancer antigen 3 (PCA3) was the first identified lncRNA in Prostrate Cancer [67]. Another lncRNA HOTAIR is identified as a serum based diagnostic and prognostic biomarker of LSCC[63]. Enrich motifs identified in exosomal lncRNA align to seed regions of one or more exosomal miRNAs in Prostate cancer. Tumour derived exosomes also contains complete functional mRNAs, proteins and small RNAs that favour tumour growth by changing cell environment. In the presence of fully functional protein machinery mRNA is translated into protein[3, 50, 68]. Table 2 shows a list of RNA molecules that are up or down regulated in cancers showing their potential as biomarker.

Table 2. Types of RNA as Biomarker in Different Cancers

Pathology	Biomarker	Level	Source	Study type	Potential	Ref.
Prostate Cancer (PC)	miR-375 and miR-141	Up-regulation	Serum	Cohort Study	Diagnosis and Stage Determination	54
	miR-107 and miR-574-3p	Up-regulation	Urine			
	miR-34a	Down-regulation	Cell line conditioned medium (CM)	Cell Line Models	Predictive Biomarker for Response to Docetaxel in PCa Progression	69
	miR-1290 and miR-375	Higher	Plasma	Cohort Study	Prognostic Biomarker in Castration-Resistant PCa	70
	hsa-41 miR375, hsa-miR21 and hsa-miR574	Higher	Serum	Non Cohort Study	Discriminating Biomarker	71
	miR-141	Up-regulation	Serum	Cohort Study	Diagnosis	72
	miR-574-3p, miR-141-5p and miR-21-5p	Up-regulation	Urine	Non Cohort Study and Cell Line Model	Diagnosis	73
Ovarian Cancer	miR-21, miR-214, miR-200a, miR-200b, miR-200c, miR-203, miR-205 and	Elevated	Serum	Non Cohort Study	Diagnosis	52

	miR-141					
	Let-7	High expression in SKOV-3	Cell Culture Media	Cell Line Models (SKOV-3 and OVCAR-3)	Diagnosis	[74]
	miR-200	Expressed only in OVCAR-3				
	miR-21	Over Expression	-	Non Study Cohort	Diagnosis	[75]
	miR-30a-5p	Up-regulation	Urine	Non Study Cohort and Cell Line Model	Diagnostic and Therapeutic target of Ovarian Serous Carcinoma	[76]
	miR-373, miR-200a, miR-200b and miR-200c	Elevated	Serum	Cohort Study	Diagnosis and Prognosis	[55]
Breast Cancer	miR-21 and miR-146[a]	Over expression	Plasma	Non Cohort Study	Diagnosis	[62]
	miR-106a	Up-regulated	Plasma	Non Cohort Study	Biomarker for metastatic Breast Cancer	[77]
Colorectal Cancer (CRC)	let-7a, miR-1246, miR-1229, miR-23a, miR-223, miR-21, and miR-150	Higher	Serum	Non Cohort Study	Diagnosis	[62]
	miR-19a	Over expression	Serum	Non Cohort Study	Prognostic Biomarker	[78]
	miR-372	Up-regulation	Serum	Non Cohort Study	Diagnosis and Prognosis	[56]
Lung Cancer	miR-125a-5p, miR-145 and miR-146a*	Over expression	Serum	Non Cohort Study	Diagnosis	[79]
	miR-29a-3p and miR-150-5p	Down-regulation	Plasma	Cohort Study	Predict response To Radiation Therapy	[80]
	miR-21 and miR-155	Up-regulation	Serum and Conditioned media	Non Study Cohort (Cell lines and recurrent nude mouse xenograft)	Diagnosis	[81]
Gastric Cancer (GC)	miR-214, miR-221 and miR-222	Up-regulation	Serum	Non Cohort Study	Diagnosis and Prognosis	[82]
	miR-21 and miR-1225-5p	Higher	Malignant ascites and Peritoneal lavage fluid	Non Cohort Study	Diagnosis and Prognosis of Peritoneum Dissection of Gastric Cancer	[60]
Cervical Cancer (CC)	miR-21 and miR-146a	Up-regulation	Cervicovaginal lavage Fluid	Non Cohort Study	Non-invasive CC screening	[58]
Retinoblastoma	miR-320, miR-let-7e, and miR-21	Down-regulation	Plasma	Non Cohort Study	Diagnosis	[59]
Osophageal squamous cell carcinoma (ESCC)	miR-1246	Up-regulation	Serum	Non Cohort Study	Diagnosis and Prognosis	[83]
Hepatocellular Carcinoma (HCC)	miR-18a, miR-221, miR-222, and miR-224	Up-regulation	Serum	Non Cohort Study	Diagnosis	[84]

	miR-101, miR-106b, miR-122, and miR-195	Down-Regulation				
Pancreatic Cancer	miR-17-5p and miR-21	Higher	Serum	Non Cohort Study	Diagnosis	61
Laryngeal Squamous Cell Carcinoma	miR-21and HOTAIR (lncRNA)	Higher	Serum	Non Cohort Study	Diagnosis and Prognosis	63
Papillary Thyroid Cancer (PTC)	miR-146b and miR-222	Over expression	-	Cell Line Model	Biomarker of PTC recurrence	85
Melanoma	miR-125b	Down-regulation	Serum	Non Cohort Study	Prognosis	86
Glioblastoma	miR-320 and miR-574-3p	-	Serum	Cohort Study	Diagnosis	42
	RNU6-1 (snRNA)	Up-regulation				

EXOSOMES FROM OTHER BIOFLUIDS AS BIOMARKER

Exosomes biomarkers have extensively been reported in biological fluid such as blood, plasma and urine. But recently several exosomes biomarkers have been identified in saliva, bronchoalveolar lavage fluid, cerebrospinal fluid, amniotic fluid, breast milk, semen, synovial fluid, bile and malignant ascites[87-89]. Several studies demonstrated that exosomal micro RNA from human saliva can be used as diagnostic biomarker. For example, in 2009 Micheal and his co-workers isolated and characterized the miRNA carrying exosomes from saliva. They reported that miRNA in exosomes of Sjogran's syndrome patients vary from that of healthy persons[90]. These miRNA (hsa-miR-150, hsa-miR-29b, miRPlus-17829, miRPlus-17841, miRPlus-17848, miRPlus-17858) can be used as a diagnostic biomarkers in future. A year later, Palanisamy et al. found that salivary exosomes also contain several protein and mRNA[87], which have a potential to be used as biomarkers. Breast cancer exosomes interacts with cells of salivary gland, which in turn change the composition of salivary gland cell derived exosomes both proteomically and transcriptomically[91]. These promising discoveries might lead to the development of saliva based biomarkers for breast cancer.Recently it has been establish that salivary exosomes may be used to early detection of pancreatic cancer. Seven genes (Apbb1ip, Aspn, BCO31781, Daf2, FOXP1, Gng2 and Incenp) in saliva derived exosomes after the development of pancreatic cancer. Principe and co-workers highlighted the importance of saliva for early diagnosis of head and neck cancer[93].

A number of exosomal cancer biomarkers were isolated from ascetic fluid. Examples include exosomes of ovarian carcinoma patients that derives from ascities were over-expressing CD24 protein and epithelial cell adhesion molecules (EpCAM)[94]. Tokuhisa and his co-workers reported that high expression of exosomal miR-21 and miR1225-5p may serve as a promising prognostic biomarker of gastric cancer in malignant ascites samples[60]. Recently in 2015 it has been reported that miRNA contents of CSF derived exosomes can be used as a potential biomarker for therapeutic observation of glioblastoma patients[95].

Table 3 shows different exosomal cancer biomarkers identified in body fluids other than peripheral blood.

Further research in this domain will definitely help in the development of new exosomal biomarkers.

Table 3. Different Types of Exosomal Cancer Biomarker in Body-Fluids.

Bio Fluid	Disease	Biomarker (Protein/RNA)	Ref.
Saliva	Breast cancer		91
	Pancreatic cancer	mRNA	87,92
	Sjogren's syndrome	miRNA	90
	Head and neck cancer	mRNA, miRNA	93
Ascities	Ovarian cancer	Protein (CD24, EpCAM)	94
		Protein (MMP2, MMP9, uPA)	96
	Gastric cancer	miRNA	60
	Colorectal cancer	Protein (claudin-3)	97
CSF	Glioblastoma	miRNA (miR-21)	95
Milk /ductal fluid	Breast cancer	miRNA (miR-16, 1246, 451 and miR-720)	98
Bile	Cholangiocarcinoma	miRNA	99

CONCLUSION AND FUTURE PROSPECTS

As compared to other biomarkers which are detected in body fluids, exosomal biomarkers give high sensitivity and specificity. Given the name of liquid biopsy, exosomes contain the valuable samples derived from within the cancer cells and stably packaged to survive in blood circulation and other body fluids. Exosomes are secreted by cancer cells during tumour progression and have a great potential to become a routine laboratory practices in future. However their diversity needs to be fully explored before standardised diagnostic procedures can be developed.

REFERENCES

1. Pan B-T, Johnstone RM. Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor. Cell. 1983;33(3):967-78.
2. Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nature Reviews Immunology. 2002;2(8):569-79.
3. 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. Nature cell biology. 2007;9(6):654-9.
4. Bang C, Thum T. Exosomes: New players in cell–cell communication. The international journal of biochemistry & cell biology. 2012;44(11):2060-4.
5. An T, Qin S, Xu Y, Tang Y, Huang Y, Situ B, et al. Exosomes serve as tumour markers for personalized diagnostics owing to their important role in cancer metastasis. Journal of extracellular vesicles. 2015;4.
6. Booth AM, Fang Y, Fallon JK, Yang J-M, Hildreth JE, Gould SJ. Exosomes and HIV Gag bud from endosome-like domains of the T cell plasma membrane. The Journal of cell biology. 2006;172(6):923-35.
7. van Niel G, Porto-Carreiro I, Simoes S, Raposo G. Exosomes: a common pathway for a specialized function. Journal of biochemistry. 2006;140(1):13-21.
8. Conde-Vancells J, Rodriguez-Suarez E, Embade N, Gil D, Matthiesen R, Valle M, et al. Characterization and comprehensive proteome profiling of exosomes secreted by hepatocytes. Journal of proteome research.2008;7(12):5157-66.
9. Keerthikumar S, Chisanga D, Ariyaratne D, Al Saffar H, Anand S, Zhao K, et al. ExoCarta: a web-based compendium of exosomal cargo. Journal of molecular biology. 2015.
10. Laulagnier K, Motta C, Hamdi S, Sébastien R, Fauvelle F, Pageaux J-F, et al. Mast cell-and dendritic cellderived exosomes display a specific lipid composition and an unusual membrane organization. Biochemical Journal.2004;380(1):161-71.
11. Mallegol J, Van Niel G, Lebreton C, Lepelletier Y, Candalh C, Dugave C, et al. T84-intestinal epithelial exosomes bear MHC class II/peptide complexes potentiating antigen presentation by dendritic cells. Gastroenterology. 2007;132(5):1866-76.
12. Denzer K, van Eijk M, Kleijmeer MJ, Jakobson E, de Groot C, Geuze HJ. Follicular dendritic cells carry MHC class II-expressing microvesicles at their surface. The Journal of Immunology. 2000;165(3):1259-65.

13. Taylor DD, Shah S. Methods of isolating extracellular vesicles impact down-stream analyses of their cargoes. Methods. 2015;87:3-10.
14. Théry C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Current protocols in cell biology. 2006:3.22. 1-3.. 9.
15. Zeringer E, Barta T, Li M, Vlassov AV. Strategies for isolation of exosomes. Cold Spring Harbor protocols. 2015;2015(4):pdb. top074476.
16. Cheruvanky A, Zhou H, Pisitkun T, Kopp JB, Knepper MA, Yuen PS, et al. Rapid isolation of urinary exosomal biomarkers using a nanomembrane ultrafiltration concentrator. American Journal of Physiology-Renal Physiology. 2007;292(5):F1657-F61.
17. Yamamoto KR, Alberts BM, Benzinger R, Lawhorne L, Treiber G. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus purification. Virology. 1970;40(3):734-44.
18. Adams A. Concentration of Epstein-Barr virus from cell culture fluids with polyethylene glycol. Journal of General Virology. 1973;20(3):391-4.
19. Lewis GD, Metcalf TG. Polyethylene glycol precipitation for recovery of pathogenic viruses, including hepatitis A virus and human rotavirus, from oyster, water, and sediment samples. Applied and environmental microbiology. 1988;54(8):1983-8.
20. Lin J, Li J, Huang B, Liu J, Chen X, Chen X-M, et al. Exosomes: novel biomarkers for clinical diagnosis. The Scientific World Journal. 2015;2015.
21. Rekker K, Saare M, Roost AM, Kubo A-L, Zarovni N, Chiesi A, et al. Comparison of serum exosome isolation methods for microRNA profiling. Clinical biochemistry. 2014;47(1):135-8.
22. Chen C, Skog J, Hsu C-H, Lessard RT, Balaj L, Wurdinger T, et al. Microfluidic isolation and transcriptome analysis of serum microvesicles. Lab on a chip. 2010;10(4):505-11.
23. Simpson RJ, Jensen SS, Lim JW. Proteomic profiling of exosomes: current perspectives. Proteomics. 2008 Oct;8(19):4083-99. PubMed PMID: 18780348.
24. Choi DS, Kim DK, Kim YK, Gho YS. Proteomics of extracellular vesicles: Exosomes and ectosomes. Mass spectrometry reviews. 2015 Jul-Aug;34(4):474-90. PubMed PMID: 24421117.
25. Pisitkun T, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. Proc Natl Acad Sci U S A. 2004;101(36):13368-73.
26. Gonzales PA, Pisitkun T, Hoffert JD, Tchapyjnikov D, Star RA, Kleta R, et al. Large-scale proteomics and phosphoproteomics of urinary exosomes. J Am Soc Nephrol. 2009;20(2):363-79.
27. Logozzi M, De Milito A, Lugini L, Borghi M, Calabro L, Spada M, et al. High levels of exosomes expressing CD63 and caveolin-1 in plasma of melanoma patients. PLoS One. 2009;4(4):17.
28. Blackwell RH, Franzen CA, Flanigan RC, Kuo PC, Gupta GN. The untapped potential of urine shed bladder cancer exosomes: biomarkers, signaling, and therapeutics. Bladder. 2014;1(1):e7.
29. Jakobsen KR, Paulsen BS, Baek R, Varming K, Sorensen BS, Jorgensen MM. Exosomal proteins as potential diagnostic markers in advanced non-small cell lung carcinoma. J Extracell Vesicles. 2015;4(26659).
30. Wang L-Z, Soo RA, Thuya WL, Wang TT, Guo T, Lau JA, et al., editors. Exosomal protein FAM3C as a potential novel biomarker for non-small cell lung cancer. ASCO Annual Meeting Proceedings; 2014.
31. Duijvesz D, Burnum-Johnson KE, Gritsenko MA, Hoogland AM, Vredenbregt-van den Berg MS, Willemsen R, et al. Proteomic profiling of exosomes leads to the identification of novel biomarkers for prostate cancer. PLoS One. 2013;8(12).
32. Beach A, Zhang HG, Ratajczak MZ, Kakar SS. Exosomes: an overview of biogenesis, composition and role in ovarian cancer. J Ovarian Res. 2014;7(14):1757-2215.

33. An T, Qin S, Xu Y, Tang Y, Huang Y, Situ B, et al. Exosomes serve as tumour markers for personalized diagnostics owing to their important role in cancer metastasis. J Extracell Vesicles. 2015;4(27522).
34. Liang B, Peng P, Chen S, Li L, Zhang M, Cao D, et al. Characterization and proteomic analysis of ovarian cancer-derived exosomes. J Proteomics. 2013;80:171-82.
35. Yu Dd, Wu Y, Shen Hy, Lv Mm, Chen Wx, Zhang Xh, et al. Exosomes in development, metastasis and drug resistance of breast cancer. Cancer science. 2015;106(8):959-64.
36. Chen Y, Wang L, Zhu Y, Chen Z, Qi X, Jin L, et al. Breast cancer resistance protein (BCRP)-containing circulating microvesicles contribute to chemoresistance in breast cancer. Oncology letters. 2015 Dec;10(6):3742-8. PubMed PMID: 26788201. Pubmed Central PMCID: 4665209.
37. Madhavan B, Yue S, Galli U, Rana S, Gross W, Muller M, et al. Combined evaluation of a panel of protein and miRNA serum-exosome biomarkers for pancreatic cancer diagnosis increases sensitivity and specificity. International journal of cancer. 2015 Jun 1;136(11):2616-27. PubMed PMID: 25388097. Epub 2014/11/13. eng.
38. Melo SA, Luecke LB, Kahlert C, Fernandez AF, Gammon ST, Kaye J, et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. Nature. 2015 Jul 9;523(7559):177-82. PubMed PMID: 26106858. Pubmed Central PMCID: 4825698. Epub 2015/06/25. eng.
39. Chiba M, Kimura M, Asari S. Exosomes secreted from human colorectal cancer cell lines contain mRNAs, microRNAs and natural antisense RNAs, that can transfer into the human hepatoma HepG2 and lung cancer A549 cell lines. Oncology reports. 2012;28(5):1551-8.
40. Kalniņa Z, Meistere I, Kikuste I, Tolmanis I, Zayakin P, Linē A. Emerging blood-based biomarkers for detection of gastric cancer. World journal of gastroenterology. 2015;21(41):11636.
41. Simpson RJ, Kalra H, Mathivanan S. ExoCarta as a resource for exosomal research. Journal of extracellular vesicles. 2012;1.
42. Manterola L, Guruceaga E, Pérez-Larraya JG, González-Huarriz M, Jauregui P, Tejada S, et al. A small noncoding RNA signature found in exosomes of GBM patient serum as a diagnostic tool. Neuro-oncology.2014:not218.
43. Ahadi A, Brennan S, Kennedy PJ, Hutvagner G, Tran N. Long non-coding RNAs harboring miRNA seed regions are enriched in prostate cancer exosomes. Scientific reports. 2016;6.
44. Schageman J, Zeringer E, Li M, Barta T, Lea K, Gu J, et al. The complete exosome workflow solution: from isolation to characterization of RNA cargo. BioMed research international. 2013;2013.
45. Jenjaroenpun P, Kremenska Y, Nair VM, Kremenskoy M, Joseph B, Kurochkin IV. Characterization of RNA in exosomes secreted by human breast cancer cell lines using next-generation sequencing. PeerJ. 2013;1:e201.
46. Goldie BJ, Dun MD, Lin M, Smith ND, Verrills NM, Dayas CV, et al. Activity-associated miRNA are packaged in Map1b-enriched exosomes released from depolarized neurons. Nucleic acids research. 2014:gku594.
47. Huang X, Yuan T, Tschannen M, Sun Z, Jacob H, Du M, et al. Characterization of human plasma-derived exosomal RNAs by deep sequencing. BMC genomics. 2013;14(1):1.
48. Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, et al. The microRNA spectrum in 12 body fluids. Clinical chemistry. 2010;56(11):1733-41.
49. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nature Reviews Cancer. 2006;6(11):857-66.
50. Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, Curry WT, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nature cell biology.2008;10(12):1470-6.
51. Silva J, Garcia V, Zaballos A, Provencio M, Lombardia L, Almonacid L, et al. Vesicle-related microRNAs in plasma of nonsmall cell lung cancer patients and correlation with survival. European Respiratory Journal. 2011;37(3):617-23.

52. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecologic oncology. 2008;110(1):13-21.
53. Rabinowits G, Gercel-Taylor C, Day JM, Taylor DD, Kloecker GH. Exosomal microRNA: a diagnostic marker for lung cancer. Clinical lung cancer. 2009;10(1):42-6.
54. Bryant R, Pawlowski T, Catto J, Marsden G, Vessella R, Rhees B, et al. Changes in circulating microRNA levels associated with prostate cancer. British journal of cancer. 2012;106(4):768-74.
55. Meng X, Muller V, Milde-Langosch K, Trillsch F, Pantel K, Schwarzenbach H. Diagnostic and prognostic relevance of circulating exosomal miR-373, miR-200a, miR-200b and miR-200c in patients with epithelial ovarian cancer. Oncotarget. 2016.
56. Yu J, Jin L, Li W, Jiang L, Hu Y, Zhi Q, et al. Serum miR-372 is a diagnostic and prognostic biomarker in patients with early colorectal cancer. Anti-cancer agents in medicinal chemistry. 2015.
57. Kumar S, Keerthana R, Pazhanimuthu A, Perumal P. Overexpression of circulating miRNA-21 and miRNA-146a in plasma samples of breast cancer patients. Indian journal of biochemistry & biophysics. 2013;50(3):210-4.
58. Liu J, Sun H, Wang X, Yu Q, Li S, Yu X, et al. Increased exosomal microRNA-21 and microRNA-146a levels in the cervicovaginal lavage specimens of patients with cervical cancer. International journal of molecular sciences. 2014;15(1):758-73.
59. Liu SS, Wang YS, Sun YF, Miao LX, Wang J, Li YS, et al. Plasma microRNA-320, microRNA-let-7e and microRNA-21 as novel potential biomarkers for the detection of retinoblastoma. Biomedical reports. 2014;2(3):424-8.
60. Tokuhisa M, Ichikawa Y, Kosaka N, Ochiya T, Yashiro M, Hirakawa K, et al. Exosomal miRNAs from peritoneum lavage fluid as potential prognostic biomarkers of peritoneal metastasis in gastric cancer. PloS one. 2015;10(7):e0130472.
61. Que R, Ding G, Chen J, Cao L. Analysis of serum exosomal microRNAs and clinicopathologic features of patients with pancreatic adenocarcinoma. World journal of surgical oncology. 2013;11(1):1.
62. Ogata-Kawata H, Izumiya M, Kurioka D, Honma Y, Yamada Y, Furuta K, et al. Circulating exosomal microRNAs as biomarkers of colon cancer. PloS one. 2014;9(4):e92921.
63. Wang J, Zhou Y, Lu J, Sun Y, Xiao H, Liu M, et al. Combined detection of serum exosomal miR-21 and HOTAIR as diagnostic and prognostic biomarkers for laryngeal squamous cell carcinoma. Medical Oncology. 2014;31(9):1-8.
64. Jacquier A. The complex eukaryotic transcriptome: unexpected pervasive transcription and novel small RNAs. Nature Reviews Genetics. 2009;10(12):833-44.
65. Johnson JM, Edwards S, Shoemaker D, Schadt EE. Dark matter in the genome: evidence of widespread transcription detected by microarray tiling experiments. TRENDS in Genetics. 2005;21(2):93-102.
66. Wang Y, Xu Z, Jiang J, Xu C, Kang J, Xiao L, et al. Endogenous miRNA sponge lincRNA-RoR regulates Oct4, Nanog, and Sox2 in human embryonic stem cell self-renewal. Developmental cell. 2013;25(1):69-80.
67. Lu W, Zhou D, Glusman G, Utleg AG, White JT, Nelson PS, et al. KLK31P is a novel androgen regulated and transcribed pseudogene of kallikreins that is expressed at lower levels in prostate cancer cells than in normal prostate cells. The Prostate. 2006;66(9):936-44.
68. Sandhu S, Garzon R, editors. Potential applications of microRNAs in cancer diagnosis, prognosis, and treatment. Seminars in oncology; 2011: Elsevier.
69. Corcoran C, Rani S, O'Driscoll L. miR-34a is an intracellular and exosomal predictive biomarker for response to docetaxel with clinical relevance to prostate cancer progression. The Prostate. 2014;74(13):1320-34.
70. Huang X, Yuan T, Liang M, Du M, Xia S, Dittmar R, et al. Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer. European urology. 2015;67(1):33-41.
71. Li M, Rai AJ, DeCastro GJ, Zeringer E, Barta T, Magdaleno S, et al. An optimized procedure for exosome isolation and analysis using serum samples: Application to cancer biomarker discovery. Methods. 2015;87:26-30.

72. Li Z, Ma Y-Y, Wang J, Zeng X-F, Li R, Kang W, et al. Exosomal microRNA-141 is upregulated in the serum of prostate cancer patients. OncoTargets and therapy. 2016;9:139.
73. Samsonov R, Shtam T, Burdakov V, Glotov A, Tsyrlina E, Berstein L, et al. Lectin-induced agglutination method of urinary exosomes isolation followed by mi-RNA analysis: Application for prostate cancer diagnostic. The Prostate. 2016;76(1):68-79.
74. Kobayashi M, Salomon C, Tapia J, Illanes SE, Mitchell MD, Rice GE. Ovarian cancer cell invasiveness is associated with discordant exosomal sequestration of Let-7 miRNA and miR-200. Journal of translational medicine. 2014;12:4. PubMed PMID: 24393345. Pubmed Central PMCID: 3896684. Epub 2014/01/08. eng.
75. Cappellesso R, Tinazzi A, Giurici T, Simonato F, Guzzardo V, Ventura L, et al. Programmed cell death 4 and microRNA 21 inverse expression is maintained in cells and exosomes from ovarian serous carcinoma effusions. Cancer cytopathology. 2014;122(9):685-93.
76. Zhou J, Gong G, Tan H, Dai F, Zhu X, Chen Y, et al. Urinary microRNA-30a-5p is a potential biomarker for ovarian serous adenocarcinoma. Oncology reports. 2015;33(6):2915-23.
77. Zhao Q, Deng S, Wang G, Liu C, Meng L, Qiao S, et al. A direct quantification method for measuring plasma MicroRNAs identified potential biomarkers for detecting metastatic breast cancer. Oncotarget. 2016.
78. Matsumura T, Sugimachi K, Iinuma H, Takahashi Y, Kurashige J, Sawada G, et al. Exosomal microRNA in serum is a novel biomarker of recurrence in human colorectal cancer. British journal of cancer. 2015;113(2):275-81.
79. Wang R-J, Zheng Y-H, Wang P, Zhang J-Z. Serum miR-125a-5p, miR-145 and miR-146a as diagnostic biomarkers in non-small cell lung cancer. International journal of clinical and experimental pathology. 2015;8(1):765.
80. Dinh T-KT, Fendler W, Chałubińska-Fendler J, Acharya SS, O'Leary C, Deraska PV, et al. Circulating miR-29a and miR-150 correlate with delivered dose during thoracic radiation therapy for non-small cell lung cancer. Radiation Oncology. 2016;11(1):1.
81. Munagala R, Aqil F, Gupta RC. Exosomal miRNAs as biomarkers of recurrent lung cancer. Tumor Biology. 2016:1-12.
82. Wang M, Zhao C, Shi H, Zhang B, Zhang L, Zhang X, et al. Deregulated microRNAs in gastric cancer tissue-derived mesenchymal stem cells: novel biomarkers and a mechanism for gastric cancer. British journal of cancer. 2014;110(5):1199-210.
83. Takeshita N, Hoshino I, Mori M, Akutsu Y, Hanari N, Yoneyama Y, et al. Serum microRNA expression profile: miR-1246 as a novel diagnostic and prognostic biomarker for oesophageal squamous cell carcinoma. British journal of cancer. 2013;108(3):644-52.
84. Sohn W, Kim J, Kang SH, Yang SR, Cho J-Y, Cho HC, et al. Serum exosomal microRNAs as novel biomarkers for hepatocellular carcinoma. Experimental & molecular medicine. 2015;47(9):e184.
85. Lee JC, Zhao J-T, Gundara J, Serpell J, Bach LA, Sidhu S. Papillary thyroid cancer–derived exosomes contain miRNA-146b and miRNA-222. Journal of Surgical Research. 2015;196(1):39-48.
86. Alegre E, Sanmamed MF, Rodriguez C, Carranza O, Martin-Algarra S, Gonzalez A. Study of circulating microRNA-125b levels in serum exosomes in advanced melanoma. Archives of Pathology and Laboratory Medicine. 2014;138(6):828-32.
87. Palanisamy V, Sharma S, Deshpande A, Zhou H, Gimzewski J, Wong DT. Nanostructural and transcriptomic analyses of human saliva derived exosomes. PLoS ONE. 2010;5(1):e8577.
88. Keller S, Rupp C, Stoeck A, Runz S, Fogel M, Lugert S, et al. CD24 is a marker of exosomes secreted into urine and amniotic fluid. Kidney international. 2007;72(9):1095-102.
89. Aalberts M, van Dissel-Emiliani FM, van Adrichem NP, van Wijnen M, Wauben MH, Stout TA, et al. Identification of distinct populations of prostasomes that differentially express prostate stem cell antigen, annexin A1, and GLIPR2 in humans. Biology of reproduction. 2012;86(3):82.
90. Michael A, Bajracharya SD, Yuen PS, Zhou H, Star RA, Illei GG, et al. Exosomes from human saliva as a source of microRNA biomarkers. Oral diseases. 2010;16(1):34-8.

91. Lau CS, Wong DT. Breast cancer exosome-like microvesicles and salivary gland cells interplay alters salivary gland cell-derived exosome-like microvesicles in vitro. PLoS ONE. 2012;7(3):e33037.
92. Lau C, Kim Y, Chia D, Spielmann N, Eibl G, Elashoff D, et al. Role of pancreatic cancer-derived exosomes in salivary biomarker development. Journal of Biological Chemistry. 2013;288(37):26888-97.
93. Principe S, Hui ABY, Bruce J, Sinha A, Liu FF, Kislinger T. Tumor-derived exosomes and microvesicles in head and neck cancer: Implications for tumor biology and biomarker discovery. Proteomics. 2013;13(10-11):1608-23.
94. Runz S, Keller S, Rupp C, Stoeck A, Issa Y, Koensgen D, et al. Malignant ascites-derived exosomes of ovarian carcinoma patients contain CD24 and EpCAM. Gynecologic oncology. 2007;107(3):563-71.
95. Akers JC, Ramakrishnan V, Kim R, Phillips S, Kaimal V, Mao Y, et al. miRNA contents of cerebrospinal fluid extracellular vesicles in glioblastoma patients. Journal of Neuro-oncology. 2015;123(2):205-16.
96. Graves LE, Ariztia EV, Navari JR, Matzel HJ, Stack MS, Fishman DA. Proinvasive properties of ovarian cancer ascites-derived membrane vesicles. Cancer Research. 2004;64(19):7045-9.
97. Choi DS, Park JO, Jang SC, Yoon YJ, Jung JW, Choi DY, et al. Proteomic analysis of microvesicles derived from human colorectal cancer ascites. Proteomics. 2011;11(13):2745-51.
98. Pigati L, Yaddanapudi SC, Iyengar R, Kim D-J, Hearn SA, Danforth D, et al. Selective release of microRNA species from normal and malignant mammary epithelial cells. PLoS ONE. 2010;5(10):e13515.
99. Li L, Masica D, Ishida M, Tomuleasa C, Umegaki S, Kalloo AN, et al. Human bile contains MicroRNAladen extracellular vesicles that can be used for cholangiocarcinoma diagnosis. Hepatology. 2014;60(3):896-907.

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