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 proteins1. 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 mRNA2. 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 material3,4.

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 all5.

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 microvesicles6. 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. Exosomes isolated by ultracentrifugation appear as cup shaped structures when imaged using electron microscope.

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. 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.

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.

**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.
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 dense components\(^1\). Mostly ultracentrifugation is used along with sucrose density gradient, so the low density exosomes float\(^2\). 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\(^3\). Size based isolation employing ultrafiltration is comparatively less time consuming and requires minimal of specialized equipment, making it a cost effective exosome isolation method\(^4\).

Polymer based precipitation methods using polyethylene glycols (PEG) are frequently used for precipitation of viruses and other small particles\(^5\)–\(^7\). The same technique of precipitation followed by (10,000 to 20,000 x g) centrifugation is being used for isolation of exosomes\(^8\). 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\(^9\). 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\(^10\).

Imunoaffinity 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\(^11\),\(^12\),\(^13\). 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\(^14\).

**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\(^20\),\(^21\).

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\(^22\),\(^23\).

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**

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Exosomal Proteins</th>
<th>Level/Expression</th>
<th>Potential Use</th>
<th>Methodology</th>
<th>Source</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Melanoma and other malignant cancers</strong></td>
<td>CD63 and Caveolin 1 enriched exosomes.</td>
<td>Elevated</td>
<td>Diagnosis</td>
<td>In-house sandwich ELISA</td>
<td>Plasma</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>β1 integrin, α6 integrin, basigin, CD 73</td>
<td>Elevated expression</td>
<td>Prognosis</td>
<td>Mass spectrometry, Western blot, Flow cytometry</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td><strong>Non-small cell lung carcinoma</strong></td>
<td>CD9, CD81</td>
<td>Elevated signal</td>
<td>Diagnosis</td>
<td>Extracellular Vesicle Array (EV Array)</td>
<td>Plasma</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>CD 63</td>
<td>Co-variation in signal as</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer Type</td>
<td>Biomarker</td>
<td>Control</td>
<td>Prognosis</td>
<td>Detection Method</td>
<td>Exosomal Fraction</td>
<td></td>
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<tr>
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<tr>
<td>(NSCLC)</td>
<td>FAM3C</td>
<td>Over</td>
<td>Prognosis</td>
<td>LTQ-FT mass spectrometry</td>
<td>NSCLC cell lines</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FASN, XPO1 and PDCD6IP proteins</td>
<td>Elevated</td>
<td>Diagnosis</td>
<td>LCFTMS/ Western blot/ Immuno-histochemistry</td>
<td>Immortalized primary prostate epithelial cells</td>
<td></td>
</tr>
<tr>
<td>Prostate Cancer (PCa)</td>
<td>ENO-1</td>
<td>Decreased</td>
<td></td>
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<td></td>
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<tr>
<td>Ovarian Cancer</td>
<td>Epithelial cell adhesion molecule (EpCam) and CD24</td>
<td>Over expression</td>
<td>Diagnosis</td>
<td>Magnetic activated cell sorting procedure (MACS)</td>
<td>Ovarian tumor derived exosomes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TGF-beta1, L1CAM, ADAM10, EMMPRIN, Claudin-4</td>
<td>Over expression</td>
<td>Diagnosis/ Prognosis</td>
<td>Fluorescent microsoopy and cytofluorographic analysis.</td>
<td>Ascites/Blood</td>
<td></td>
</tr>
<tr>
<td>Hepatitis C induced HCC</td>
<td>CD81</td>
<td>Over expression</td>
<td>Diagnosis</td>
<td>Western blot/ Immunoblotting and densitometry.</td>
<td>Exosomal serum fraction</td>
<td></td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>MUC1, β1 integrin, α6 integrin, CD44, CD10, CD 73</td>
<td>Increased surface expression</td>
<td>Diagnosis/ Prognosis</td>
<td>Flow cytometry</td>
<td>HT1376 bladder cancer cells</td>
<td></td>
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<tr>
<td></td>
<td>HBA, HBB and TACSTD2</td>
<td>Upregulated expression</td>
<td>Prognosis</td>
<td>Mass spectrometry</td>
<td>Urine</td>
<td></td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Survivin (Inhibitor of apoptosis), MUC 1, β1 integrin, CD73</td>
<td>Elevated level</td>
<td>Diagnosis/ Prognosis</td>
<td>SDS PAGE, Western Blot</td>
<td>Serum exosomes/ Saliva</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Breast cancer resistance protein (BCRP)</td>
<td>Elevated level</td>
<td>Prognostic biomarker</td>
<td>Immuno fluorescence</td>
<td>Plasma</td>
<td></td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>CD44v6, Tspan 8, EpCAM, CD104</td>
<td>Elevated level</td>
<td>Diagnosis/ Prognosis</td>
<td>Flow cytometry</td>
<td>Serum exosomes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glypican 1 (Proteoglycan) circulating exosomes (GPC1-crcExos)</td>
<td>Upregulated surface expression</td>
<td>Diagnosis</td>
<td>Mass spectrometry/ Flow cytometry</td>
<td>Serum/ Mice with pancreatic tumors</td>
<td></td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>CD81, CD63, CD9</td>
<td>Elevated</td>
<td>Diagnosis/ Prognosis</td>
<td>SDS PAGE, Western Blot</td>
<td>Colorectal cancer cell lines/Ascites</td>
<td></td>
</tr>
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<td></td>
<td>Claudin 3</td>
<td>High level of detection</td>
<td>Diagnosis</td>
<td>Nano-LC-MS/MS analysis and 1-Dimensional SDS-PAGE</td>
<td>CRC-ascites</td>
<td></td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>MAGE-1 and Her-2/neu+, CRC6</td>
<td>High expression</td>
<td>Prognostic/ Predictive</td>
<td>Protein profiling</td>
<td>Platelet Depleted Plasma</td>
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<tr>
<td></td>
<td>CxCR4</td>
<td>Down regulated expression</td>
<td></td>
<td></td>
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</tbody>
</table>

**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. Much of the work conducted on evaluating RNA as biomarker started after Valadi’s discovery of exosomal mRNA and miRNA in 2007. The amount of
miRNA is higher in exosomes as compared to their parent cells. This is further confirmed by deep sequencing of exosomal RNA species by Huang et al., that concluded miRNA is the most abundant functional RNA species in exosomes. 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. 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. For example, miR-375 and miR-141 are up-regulated in serum of prostate cancer patients as compared to normal individuals. Similarly miR-373, miR-200a, miR-200b and miR-200c be can be used as diagnostic and prognostic biomarker of ovarian cancer. miR-372 is used as a biomarker of colorectal cancer. 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).

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, and may regulate gene expression. Prostate cancer antigen 3 (PCA3) was the first identified lncRNA in Prostate Cancer. Another lncRNA HOTAIR is identified as a serum based diagnostic and prognostic biomarker of LSCC. Enrich motifs identified in exosomal IncRNA align to seed regions of one or more exosomal miRNAs in Prostate cancer. Tumour derived exosomes also contains complete functional miRNAs, proteins and small RNAs that favour tumour growth by changing cell environment. In the presence of fully functional protein machinery miRNA is translated into protein. Table 2 shows a list of RNA molecules that are up or down regulated in cancers showing their potential as biomarker.

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Biomarker</th>
<th>Level</th>
<th>Source</th>
<th>Study type</th>
<th>Potential</th>
<th>Ref.</th>
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</thead>
<tbody>
<tr>
<td>Prostate Cancer (PC)</td>
<td>miR-375 and miR-141</td>
<td>Up-regulation</td>
<td>Serum</td>
<td>Cohort Study</td>
<td>Diagnosis and Stage Determination</td>
<td>54</td>
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<tr>
<td></td>
<td>miR-107 and miR-574-3p</td>
<td>Up-regulation</td>
<td>Urine</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>miR-34a</td>
<td>Down-regulation</td>
<td>Cell lines conditioned medium (CM)</td>
<td>Cell Line Models</td>
<td>Predictive Biomarker for Response to Docetaxel in PCa Progression</td>
<td>60</td>
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<tr>
<td></td>
<td>miR-1290 and miR-375</td>
<td>Higher</td>
<td>Plasma</td>
<td>Cohort Study</td>
<td>Prognostic Biomarker in Castration-Resistant PCa</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>hsa-41, miR375, hsa-miR21 and hsa-miR574</td>
<td>Higher</td>
<td>Serum</td>
<td>Non Cohort Study</td>
<td>Discriminating Biomarker</td>
<td>71</td>
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<tr>
<td></td>
<td>miR-141</td>
<td>Up-regulation</td>
<td>Serum</td>
<td>Cohort Study</td>
<td>Diagnosis</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>miR-574-3p, miR-141-5p and miR-21-5p</td>
<td>Up-regulation</td>
<td>Urine</td>
<td>Non Cohort Study and Cell Line Model</td>
<td>Diagnosis</td>
<td>74</td>
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<tr>
<td>Ovarian Cancer</td>
<td>miR-21, miR-214, miR-200a, miR-200b, miR-200c, miR-203, miR-205 and</td>
<td>Elevated</td>
<td>Serum</td>
<td>Non Cohort Study</td>
<td>Diagnosis</td>
<td>52</td>
</tr>
</tbody>
</table>
miR-141
Let-7
High expression in SKOV-3

miR-200
Expressed only in OVCAR-3

miR-21
Over Expression

miR-30a-5p
Up-regulation

miR-373, miR-200a, miR-200b, and miR-200c
Elevated Serum Cohort Study Diagnosis and Prognosis

Breast Cancer
miR-21 and miR-146
Over expression

miR-106a
Up-regulated Plasma Non Cohort Study Biomarker for metastatic Breast Cancer

Colorectal Cancer (CRC)
let-7a, miR-1246, miR-1229, miR-23a, miR-223, miR-21, and miR-150
Higher Serum Non Cohort Study Diagnosis

miR-19a
Over expression

miR-372
Up-regulation

Lung Cancer
miR-125a-5p, miR-145 and miR-146a*
Over expression

miR-29a-3p and miR-150-5p
Down-regulation

miR-21 and miR-155
Up-regulation

Gastric Cancer (GC)
miR-214, miR-221 and miR-222
Up-regulation

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

Cervical Cancer (CC)
miR-21 and miR-146a
Up-regulation

Retinoblastoma
miR-320, miR-let-7e, and miR-21
Down-regulation

Osophageal squamous cell carcinoma (ESCC)
miR-1246
Up-regulation

Hepatocellular Carcinoma (HCC)
miR-18a, miR-221, miR-222, and miR-224
Up-regulation

miR-1246
Up-regulation

Serum Non Cohort Study Diagnosis and Prognosis

miR-18a
Up-regulation

Serum Non Cohort Study Diagnosis

miR-21
and miR-146a
Up-regulation

Cervicovaginal lavage fluid Non Cohort Study Non-invasive CC screening

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

Cervical Cancer (CC)
miR-21 and miR-146a
Up-regulation

Retinoblastoma
miR-320, miR-let-7e, and miR-21
Down-regulation

Osophageal squamous cell carcinoma (ESCC)
miR-1246
Up-regulation

Hepatocellular Carcinoma (HCC)
miR-18a, miR-221, miR-222, and miR-224
Up-regulation

miR-1246
Up-regulation

Serum Non Cohort Study Diagnosis and Prognosis


### 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. 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. 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, 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. 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.

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). 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. 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.

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>
<thead>
<tr>
<th>Cancer Type</th>
<th>Exosomal Biomarkers</th>
<th>Regulation</th>
<th>Fluid</th>
<th>Study Type</th>
<th>Diagnosis</th>
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</thead>
<tbody>
<tr>
<td>Pancreatic Cancer</td>
<td>miR-17-5p and miR-21</td>
<td>Higher</td>
<td>Serum</td>
<td>Non Cohort Study</td>
<td>Diagnosis</td>
</tr>
<tr>
<td>Laryngeal Squamous Cell Carcinoma</td>
<td>miR-21 and HOTAIR (lncRNA)</td>
<td>Higher</td>
<td>Serum</td>
<td>Non Cohort Study</td>
<td>Diagnosis and Prognosis</td>
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<tr>
<td>Papillary Thyroid Cancer (PTC)</td>
<td>miR-146b and miR-222</td>
<td>Overexpression</td>
<td>-</td>
<td>Cell Line Model</td>
<td>Biomarker of PTC recurrence</td>
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<td>Melanoma</td>
<td>miR-125b</td>
<td>Down-regulation</td>
<td>Serum</td>
<td>Non Cohort Study</td>
<td>Prognosis</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>miR-320 and miR-574-3p</td>
<td>-</td>
<td>Serum</td>
<td>Cohort Study</td>
<td>Diagnosis</td>
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<td></td>
<td>RNU6-1 (snRNA)</td>
<td>Up-regulation</td>
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</table>

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**Table 3**

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Exosomal Biomarkers</th>
<th>Regulation</th>
<th>Fluid</th>
<th>Study Type</th>
<th>Diagnosis</th>
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<tbody>
<tr>
<td>Pancreatic Cancer</td>
<td>miR-101, miR-106b, miR-122, and miR-195</td>
<td>Down-Regulation</td>
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</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Bio Fluid</th>
<th>Disease</th>
<th>Biomarker (Protein/RNA)</th>
<th>Ref.</th>
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<td>Saliva</td>
<td>Breast cancer</td>
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<tr>
<td></td>
<td>Pancreatic cancer</td>
<td>mRNA</td>
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<td></td>
<td>Sjogren’s syndrome</td>
<td>miRNA</td>
<td>90</td>
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<td></td>
<td>Head and neck cancer</td>
<td>miRNA, miRNA</td>
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<td>Ascities</td>
<td>Ovarian cancer</td>
<td>Protein (CD24, EpCAM)</td>
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<td>Protein (MMP2, MMP9, uPA)</td>
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<td>Gastric cancer</td>
<td>miRNA</td>
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<td>Colorectal cancer</td>
<td>Protein (claudin-3)</td>
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<td>CSF</td>
<td>Glioblastoma</td>
<td>miRNA (miR-21)</td>
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<td>Milk /ductal fluid</td>
<td>Breast cancer</td>
<td>miRNA (miR-16, 1246, 451 and miR-720)</td>
<td>98</td>
</tr>
<tr>
<td>Bile</td>
<td>Cholangiocarcinoma</td>
<td>miRNA</td>
<td>99</td>
</tr>
</tbody>
</table>

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 tumor 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

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