A Review on the role of Exosomes & Micro vesicles in Cancer Formation with Therapeutic Application

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Approval

This project, submitted to the Dept. of Pharmacy, Faculty of Health & Life Sciences at Daffodil International University, has been deemed satisfactory for the partial fulfilment of the requirements for the Bachelor of Pharmacy (B.Pharm) degree. It is also approved for its style and content.

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Md. Jubayer Hossain
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Dedication

God is the primary recipient of my project dedication, followed by my parents, professors, & friends.

Abstract

Cancer is a complicated group of disorders characterized by the uncontrolled proliferation and spread of abnormal cells. These abnormal cells can infiltrate and destroy healthy tissues and organs, potentially leading to death. Cancer can be caused by genetic mutations, exposure to chemicals, radiation, tobacco smoke, and lifestyle choices. Over 100 distinct forms exist, each with unique traits, available therapies, and prognosis. This review project explores the relationship between cancer and exosomes and micro vesicles, two extracellular vesicles of different sizes, and their roles in carcinogenesis. Exosomes and micro vesicles are linked to cancer advancement through immune surveillance evasion, angiogenesis promotion, tumor microenvironment modification, and metastasis facilitation. They also carry oncogenic cargo such as proteins, nucleic acids, & lipids that can alter recipient cell function and contribute to the malignant phenotype. Understanding the unique functions of exosomes and micro vesicles in cancer etiology is crucial to understand the intricate interactions between tumor cells and their surrounding milieu. Additionally, since exosomes and micro vesicles reflect the molecular profile of the tumor, they show potential as diagnostic biomarkers for cancer identification and surveillance. The purpose of this project is to understand the causes of cancer and how to treat it. The information was gathered from several sources, including PubMed, Google, Google Scholar, CrossRef, and others. The publications from which the data were gathered were published between 2000 and 2024. Furthermore, focusing on the synthesis or function of exosomes and micro vesicles offers a potential therapeutic strategy for the treatment of cancer. Determining the precise roles that exosomes and micro vesicles play in the various phases of cancer development, as well as creating efficient treatment plans that specifically target these vesicles, are still difficult tasks. Further investigation into the relationship between micro vesicles, exosomes, and cancer may provide new understandings of tumor biology and eventually better ways to diagnose and treat cancer patients.

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CHAPTER ONE
INTRODUCTION

1. Introduction

Small vesicles generated by cells called exosomes and micro vesicles are essential for intercellular communication and the movement of different chemicals between cells. Extracellular vesicles play a crucial role in mediating intercellular communication by enabling the transfer of many substances, including proteins, lipids, and nucleic acids like DNA and RNA. Exosomes originate from the endosomal pathway and are usually smaller, with a diameter ranging from 30 to 150 nanometers. When multivesicular bodies (MVBs) fuse with the cell membrane, they are liberated into the extracellular milieu. They develop within MVBs. A wide range of substances, including proteins, lipids, and nucleic acids, are carried by exosomes and may provide insight into the health or illness of the parent cell. With a diameter of 100–1,000 nanometers, micro vesicles also referred to as ectosomes or shedding vesicles are bigger than exosomes. Micro vesicles, as opposed to exosomes, are created by the plasma membrane fissioning and budding directly outward. In addition, they may act as channels for intercellular communication and transport a range of biomolecules including proteins, lipids, and nucleic acids that are comparable to those found in exosomes. Exosomes and micro vesicles have been linked to a number of physiological functions, including neuronal signaling, tissue healing, and immune response modulation. They also have a significant impact on pathological situations including inflammation, cancer, and neurological illnesses. Exosomes and micro vesicles have attracted a lot of attention in biomedical research and clinical applications because of their capacity to carry bioactive compounds and their potential as biomarkers for disease diagnosis and therapeutic targets. Gaining knowledge about the biology and roles of these extracellular vesicles might be beneficial for the creation of innovative medical diagnostic methods and treatment strategies for a range of illnesses.

1.1 Exosomes:

Nearly all cell types, including fibroblasts, endothelial, epithelial, neuronal, immunological, and cancer cells, actively produce exosomes, which are nanosized vesicles [1]. Rich in a variety of bioactive substances, including lipids, proteins, nucleic acids, and metabolites, exosomes have the capacity to transmit signals across different types of cells. [2] Exosomes had been examined in a broad spectrum of body fluids, including saliva, cerebrospinal fluid, bile, blood, breast milk, and urine. This suggests that exosomes are involved in a variety of physiological reactions. [3] Exosomes' pathophysiological impacts on illnesses, particularly malignancies, have come to light recently. According to reports, exosomes generated from tumors contribute to the development of cancer malignancy by fostering cancer growth, creating a premetastatic niche, and controlling treatment resistance. Clinically, exosomes with distinct biological and pathophysiological properties have been highlighted as therapeutic targets, diagnostic indicators, or even delivery methods for anticancer drugs. Here, we provide a thorough review of exosomes in cancer cell biology and go over how intercellular interactions mediated by exosomes control the development and spread of cancer. We also provide an overview of the function of exosomes in clinical settings with respect to their molecular and biological properties.

1.2 Classifications: - Exosomes may be classified as either natural or engineered depending on whether they have undergone artificial modification. Afterwards, exosomes originating from plants and animals make up the two categories of natural exosomes. Animal-derived exosomes are further separated into normal exosomes and tumor exosomes since exosomes are generated in both normal and malignant situations. Nearly every kind of normal cell, including mesenchymal stem cells (MSC), natural killer (NK) cells, T cells, B cells, macrophages, dendritic cells (DC), and human umbilical vein endothelial cells, may create exosomes.[5][6][7][8] Mesenchymal stem cells (MSCS), for instance, are pluripotent stem cells with the ability to differentiate in several directions and self-renew. MSCs have strong paracrine effect and may produce a significant amount of exosome in addition to their ability to adapt to the tumor microenvironment. Studies have shown that exosomes containing paclitaxel (PTX) considerably reduce the development of the individual's pancreatic tumor cell line CFPAC-1 in vitro, suggesting that they may find use as drug carriers.[9] Furthermore, it has been shown that MSC exosomes contribute to the onset of several illnesses. In addition to playing a role in tissue repair and injury [10], they may also be utilized to treat liver disorders and lessen liver damage. They also have specific therapeutic impacts on neurological and heart diseases, including heart attack

[11],[12], & [13].[14] Mononuclear phagocytes, or macrophages, are involved in wound healing, inflammation, immunological control, and angiogenesis.[15] In general, macrophages may be polarized into M1 or M2 macrophages and are found throughout the body. Studies have shown that exosomes generated from macrophages may influence the microenvironment of lung tissue by modulating immune system and inflammatory signals.[16] Moreover, Exo-PTX has remarkable anti-cancer efficacy within the Lewis model of metastasis from lung cancer. The spread of cancer cells is almost exclusively associated with the transport of exosomes produced by macrophages via the respiratory route. The study suggests that macrophage-produced exosomes may have specific surface proteins and are more concentrated in cancer cells than other cell types; however, the precise method behind this phenomenon needs to be further explored.[17]One of the reasons exosomes are heterogeneous is because they may inherit a wide variety of unique biomolecules from their parent cells.[18] Furthermore, there are variations in the yield, composition, function, and drug loading of exosomes derived from various sources. Different treatment outcomes might thus arise. Co-incubation was used by Kanchanapally et al. [19]to effectively load doxorubicin (DOX) onto exosomes produced from macrophages, pancreatic cancer cells (PCCs), and pancreatic stellate cells (PSCs). As an illustration of the specificity of exosomes from various origins, exosomes produced via pancreatic stellate cells had maximum yield and high rate of medication loading, whereas exosomes originating from macrophages have the most anticancer effect. Additionally, ascites, bile, also milk, urine, plasma, & saliva are examples of biofluids in which normal exosomes may be widely distributed. Paclitaxel is a chemotherapeutic agent that has been effectively delivered using milk-derived exosomes; the exosomes' bioavailability, stability, safety, and toxicity have all been studied and shown to be within acceptable limits.[20] Exosomes found in biofluids may also exhibit specific therapeutic and diagnostic qualities. For instance, a thorough examination of circular RNA in exosome found in the blood or CSF fluids may help identify the fundamental processes behind the development of neuropsychiatric disorders. Moreover, spinal cord injury (SCI) diagnosis and prognosis may be made with the use of miRNAs transported by serum exosomes. Similarly, through analysis of the expression profile of microRNA in EC and urine-derived exosomes from suspicious patients, it was shown that differently expressed microRNA may be employed as indicators for the identification of endometrial cancer (EC).[21] Numerous exosomes may be secreted by cancerous cells, and the distinct antigens on their outside may provide details about the origin of the donor cell. Tumor exosomes are crucial for tumor growth, dissemination, immune regulation, disease diagnosis, and disease progression. Research has shown that overexpressed exosomes from colorectal cancer cells may aid normal colonic epithelial FHC cell movement. Reducing tumor exosome production is a potential therapeutic strategy for metastatic colorectal cancer. Tyrosine kinase inhibitors (TKIs) targeting the BCR-ABL1 p210 oncoprotein may be effective in treating chronic myeloid leukemia (Ph+ CML) with Philadelphia chromosomes. Tumor exosomes from TKI-treated patients can measure the persistence of active leukemia cells, providing a unique monitoring strategy to prevent CML recurrence. Further research is underway for food-derived exosomes, as mammalian and plant exosomes share structural similarities. Particles made of ginger may stop the onset of disorders associated to the liver, while ELN made of grapes, carrots, grapefruit, and ginger can keep the digestive tract in a healthy state by reducing inflammation.[25][26][27][28] Exosomes are currently categorized mostly according to their origins. The traits and uses of the many kinds of exosomes are not thoroughly examined in this categorization. Subdivisions based on immunogenicity, biological dispersion, and organophilicity may be explored in the future.[14]

1.3 Micro vesicles: - Tumor formation involves a series of cellular reprogramming processes that lead to aberrant growth and invasiveness [29]. malignant growth and dissemination need rewiring of stromal cells, in addition to malignant cells. Intrinsic events of cells, such as either epigenetic or genetic changes, or outside variables, such as either directly or indirectly cell communication, may cause this. Soluble substances including cytokines and chemokines, & expansion factors are renowned for their purpose in tumors formation. EVs are categorized into four types based on size, composition, shape, cell origin, biogenesis, and morphology: tiny exosomes from endosomes, medium-sized microvesicles from plasma membranes, giant oncosomes, and apoptotic bodies released by dying cells [31][32]. Recent research suggests that the generally used categorization of EVs, such as Exo, may not accurately reflect their biological and biochemical features [33]. To minimize ambiguity, the phrase "small EV" refers to EV extracted at or above 100,000 grams. We shall use the word Exo throughout the review, since it is still often used by writers. In cancer, EVs have been demonstrated to be required for a variety of stages during tumor formation and development. EV disrupts gene expression and signaling in cancer cells by horizontally transferring bioactive chemicals to the surrounding stroma. Malignant cells may transform benign cells into tumor-supporting ones, promoting cancer growth and dissemination. Although Exo has been extensively studied in cancer, the function of the bigger MV remains unclear. MV, unlike Exo, is freely accessible in patients' blood and can be identified using standard techniques, making it a good option for "liquid biopsies". Furthermore, MV has long been associated with metastatic development. Recent research suggests that microvascular endothelial cells (MV) may play a role in cancer genesis and detection. Initially, this was due to their ability to form blood clots, allowing tumor cells to escape. However, recent studies suggest that MV may be involved in multiple stages of tumor progression. MV and Exo are different cancer treatments, and MV may either support or contradict cancer therapy. Studies focusing on EV extraction at 10,000-20,000 g are discussed.

1.4 Classification of micro vesicles: -

Small vesicles known as micro vesicles are secreted from cells and are crucial for the transfer of proteins, lipids, and nucleic acids as well as other materials during intercellular communication. They are categorized according to their size, cargo content, and biogenesis. A general categorization of micro vesicles is as follows:

1. **Exosomes: -**

Exosomes are tiny vesicles, ranging in diameter from 30 to 150 nm, that develop within multivesicular bodies (MVBs) and are discharged into the extracellular milieu when MVBs fuse with the plasma membrane. Bioactive substances found in exosomes include proteins, lipids, and nucleic acids (including DNA fragments, mRNA, and miRNA). They play a role in immune system control, disease development, and cell-cell communication.[35]

2. **Micro vesicles: -**

Micro vesicles which have a diameter of 100 nm to 1 μm, are bigger than exosomes. The plasma membrane fissions and direct outward budding causes them to develop. Similar to exosomes, micro vesicles transport a variety of substances, including as lipids, proteins, and nucleic acids. They play a role in inflammation, coagulation, and intercellular communication.[36]

3. **Apoptotic bodies:**

During planned cell death, known as apoptosis, bigger vesicles with a diameter of 1-5 μm are released from apoptotic cells. These are known as apoptotic bodies. These vesicles are made up of cellular debris from the dying cell, organelles, and broken DNA. To aid in the removal of apoptotic cells, phagocytic cells identify and engulf apoptotic bodies.[37]

2.0 Importance of exosomes and Micro vesicles in cellular communication: -

Exosomes are essential for intercellular communication because they help transport biomolecules and have an impact on a variety of physiological and pathological functions.

1. **Transfer of Biomolecules: -**

Exosomes facilitate the transfer of genetic and signaling information between cells by delivering a cargo of proteins, lipids, and nucleic acids, including DNA fragments, mRNA, and miRNA. In order to modify cellular responses and functions, this transfer is essential.[31]

2. **Cell-Cell Signaling: -**

By transferring signaling molecules to recipient cells, exosomes facilitate intercellular communication and control a range of biological functions, including as immunological responses, differentiation, and proliferation.[30]

3. **Immune Regulation: -**

Exosomes produced by immune cells help to regulate and tolerate the immune system by delivering regulatory factors, cytokines, and antigen-presenting molecules to target cells.[38]

4. **Tumor Microenvironment**: -

Exosomes originating from cancer affect the development and spread of tumors by stimulating angiogenesis, immunological evasion, and microenvironmental remodeling. Additionally, they promote the spread of treatment resistance factors and oncogenic molecules, which heightens the aggressiveness of cancer.[18]

5. **Neuronal Communication**: -

Exosomes modulate neuronal function and homeostasis by carrying neurotransmitters, synaptic proteins, and regulatory factors between neurons and glial cells. This contributes to neuronal transmission and synaptic plasticity.[39]

6. **Regeneration and Repair**: -

Exosomes produced from stem cells or regenerative tissues have the potential to be therapeutic agents in regenerative medicine because they influence angiogenesis, differentiation, and proliferation of cells, which in turn promotes tissue repair and regeneration [40]

3.0 Biogenesis and characterization of exosomes and micro vesicles: -

3.1 Exosome biogenesis: - Published in 1946[41], the earliest and most significant investigation proving the presence of EVs was conducted.
Until the 1980s, these "fragments" were thought to be platelet "dust" or cellular debris that directly budded from the plasma membrane [42]. De Broe [43] first reported the release of these "membrane fragments" as a universal characteristic of live cells in 1977. Two trailblazers, Johnstone as well as Harding, made the important revelation in 1983 that TfR (Transferrin receptor), linked to tiny 50 Nanometer. vesicle, were emitted into the extracellular space by developing blood reticulocytes by a mechanism of receptor-mediated recycling and endocytosis. Rose Johnstone came up with the word "exosome" to describe these EVs. But this phrase was also used to describe other membrane fragments, called "exosome complexes," that were separated from biological fluids and had a size range of 40 to 1,000 nm [44]. Exosomes were first proposed in 1980 as a new means of intercellular communication and as cellular waste disposal systems. This term was later used to describe 40–100 nm vesicles that are produced during reticulocyte development as a result of the plasma membrane-fusion of multivesicular endosomes (MVEs) [45][46]. A similar process was eventually discovered a decade later to release exosomes from B lymphocytes and dendritic cells [47][48].
Based on their size and release method, EVs are now divided into three categories: Figure 1 shows the diameters of exosomes (less than 150 nm), MVs/shedding particles (100~1000 nm), and apoptotic bodies (>1000 nm). Exosomes are a subclass of extracellular nanosized membrane vesicles, distinguished by their diameter, which falls between 30 and 150 nm. When multivesicular bodies fuse with the plasma membrane, they are discharged into the extracellular environment, where their biogenesis takes place via the exocytosis of multivesicular endosomes [49].
When intraluminal vesicles in multivesicular bodies merge with the parent cell's plasma membrane, exosomes are released into the extracellular environment [2],[30]. Exosomes are multimolecular carriers that include proteins, lipids, DNA, and RNA. The molecules inside exosomes are a direct reflection of the metabolic status of the cells that produced them. Three stages comprise exome biogenesis: first, endocytic vesicles are formed by the invasion of the plasma membrane; second, MVBs are formed by the inward budding of the endosomal membrane; and third, MVBs fuse with the plasma membrane to release the contents of the vesicles, known as exosomes (Figure 2) [50]. Exosomes are made up of a lipid bilayer and a tiny, organelle-free cytoplasm. When the MVB, an endocytic pathway organelle, fuses with the plasma membrane, exosomes are released. The process of exosome biogenesis is strictly controlled. It starts with endosomes in-budding, which creates MVBs containing intraluminal vesicles (ILVs) [51], [52]. ILVs are then released extracellularly as exosomes by the MVBs merging with various cell types' cell membranes. Next, the exosomes act as messengers, interacting with other cells through vesicular docking and fusion facilitated by endosomal sorting complex required for transport (ESCRT), a crucial piece of machinery for exosome synthesis, and SNAREs complexes. ESCRT is made up of the related AAA ATPase Vps4 complex and four distinct protein complexes, ESCRT-0, -I, -II, and -III [53]. Exosome secretion is decreased by depletion of the ESCRT-0 proteins, Hrs. and TSG101, and the ESCRT-I protein, STAM1. These results are supported by the fact that exosome secretion is increased upon knockdown of ESCRT-III and related proteins, including CHMP4C, VPS4B, VTA1, and ALIX [53]. When the integral membrane protein SIMPLE is transfected, COS cells secrete more exosomes; however, the production of exosomes is inhibited by a mutant version of SIMPLE [54]. On the other hand, MVB biogenesis may happen without ESCRTs, as shown by Stuffers et al. [55]. For instance, ILVs arise when one or more of the four ESCRT complexes' essential subunits are missing, suggesting that exosomes may form via ESCRT- independent processes. Lipids are essential for the synthesis of vesicles and for transport mechanisms such membrane fission, fusion, and deformation [56]. Ceramide, for instance, is essential for the creation of exosomes. The synthesis of ceramide is carried out by sphingomyelinase 2 (nSMase2), and in Oli-neu cells, the exosomal release of the proteolipid protein is decreased when this enzyme is inhibited [57]. Exosome

biogenesis involves not just ESCRT proteins but also lipids and a number of other proteins, including heat shock proteins, tetraspanins, annexins, flotillin, lactadherin, and platelet-derived growth factor receptors [58][59][60]. Scientists led by Gerst have discovered that v-SNAREs and t-SNAREs, which are homologues of the synaptobrevin/VAMP family, help sort proteins going from the Golgi apparatus to the plasma membrane [61], [62]. They are both forward and backward processes. Functional proteins, microRNA, and mRNA make up exosomes. These include components from the nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, and endosomes, as well as proteins from the plasma membrane, cytoplasm, and endosomes. Exosomes have different protein contents depending on the kind of cell they are discharged from. Not only do they contain proteins and lipid-related proteins like TSG101, CHMP2A, CD63, and CD81, but they also contain a wide range of biomarkers such as lipids, cholesterol, sphingomyelin, ceramide, and phosphatidylserine [2, 30, 63, 64, 65, and 66]. The large molecules that make up exosomes have a big impact on their physiological and pathological states, such as Angiogenesis, inflammation, immunological reactions, cell death, neurological conditions, and cancer [67].

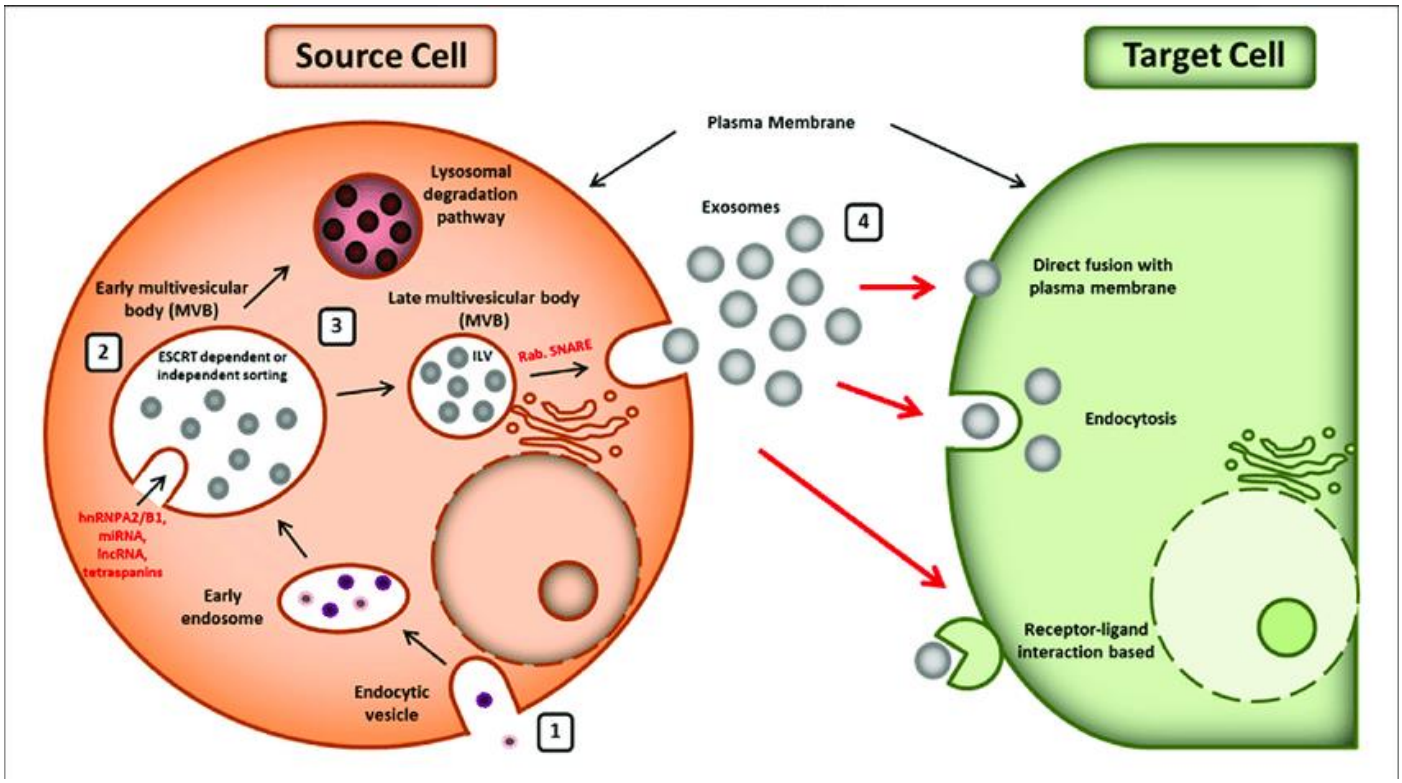

FIGURE 1: Biogenesis of exosomes

3.2 Function of exosome: -

Exosomes have the ability to combine with recipient cells and discharge their contents (Figure 1). Exosomes are crucial for cell-to-cell communication because they carry biological components like lipids, RNAs, and proteins from one cell to another [68][69][70]. Exosomes have been shown in a large body of research to perform a variety of roles, dependent upon the cell or tissue from whence they originate. Exosomes from certain immune cell subtypes, such B cells and DCs, may in particular mediate adaptive immune responses against infections and malignancies [71]. Through the transmission of oncogenes and onco-miRNAs between cancer cells as well as between cancer cells and the tumor stroma, exosomes produced from tumor cells actively participate in carcinogenesis, metastasis, and response to treatment [72]. Multiple sclerosis, transient ischemic episodes, and antiphospholipid syndrome are among the neurological conditions that are impacted by exosomes released from activated blood cells and the vascular endothelium [73]. Remarkably, recent research has shown that viruses and prions, among other pathogens, employ exosomes to transmit molecules of harmful origin between host cells. As a result, exosomes are involved in immune evasion and viral dissemination [74], [75].
Furthermore, exosomes have a great deal of promise as biomarkers for disease detection since the molecular makeup of these particles is indicative of physiological or pathophysiological alterations in the cell or tissue from which they originated.

3.3 Isolation of Exosomes: -

Differential ultracentrifugation is often used to separate exosomes from body fluids and cell culture medium due to their tiny size and low density [47], [76]. To extract dead cells and cell debris, the collected biofluid is briefly centrifuged at 300 ×g and then again at 10,000 ×g. After collecting the supernatant, it is centrifuged at 100,000 ×g for at least one hour. After that, phosphate buffer solution is used to wash the pellet containing crude exosomes in order to get rid of any leftover proteins and other impurities. Purified exosomes are obtained by subjecting the material to a second ultracentrifugation at 100,000 ×g. Additionally, a number of studies have shown that exosomes may be extracted with greater purity by using ultracentrifugation in a continuous sucrose density gradient [77],[78]. Ultracentrifugation is not ideal for clinical applications since it is labor-intensive, time-consuming, and needs costly laboratory equipment. Nonetheless, a number of recent technological developments have simplified and expedited exosome separation, making it more economical. Urinary exosomes have been quickly isolated by Cheruvanky et al. [79] and Merchant et al. [80] using ultrafiltration and microfiltration, respectively. Using lectins or antibodies against exosomal markers as CD63, CD81, EpCAM, or Rab5, immunoaffinity capture techniques have also been used to isolate exosomes [76], [81], [82]. Another technique that has been investigated for quick exosome separation is precipitation followed by centrifugation. These days, exosomes may be separated using commercial reagents like Exo Quick (System Biosciences, Mountain View, CA, USA) in a single precipitation step. Exo Quick may be more effective than ultracentrifugation when it comes to exosomal RNA analysis, since the Rekker group has shown that it is as effective as ultracentrifugation in separating serum exosomes for exosomal miRNA profiling [83].

3.4 characterization of exosomes: -

Small extracellular vesicles called exosomes are discharged into the extracellular milieu by cells. By transporting different biomolecules between cells, including as proteins, lipids, metabolites, and nucleic acids (such as DNA, mRNA, and miRNA), they perform vital roles in intercellular communication. Exosomes are characterized by examining their size, content, structure, and functional characteristics using a variety of methods.

1. Transmission Electron Microscopy (TEM): -

Exosomes may be seen at great resolution using TEM, revealing details about their size, shape, and lipid bilayer structure.[84]

2. Dynamic Light Scattering (DLS): -

DLS calculates the hydrodynamic diameter of exosomes by measuring their size distribution in solution based on their Brownian motion.[85]

3. Nanoparticle Tracking Analysis (NTA): -

Exosome size distribution and concentration are measured using NTA by following their Brownian motion in solution.[86]

4. Western Blotting: -

Specific protein markers (such as CD63, CD9, Alix, and TSG101) that are present in exosomes may be identified and confirmed using Western blot analysis.[87]

5. RNA Analysis: -

RNA sequencing, microarray analysis, and RT-qPCR are a few methods that may be used to analyze the RNA cargo (mRNA, miRNA, etc.) of exosomes.[88]

3.5 micro vesicles biogenesis: -

The direct emergence of MV occurs through the cell's surface. Molecules in the plasma membrane are rearranged in relation to Ca2+ concentrations during the peeling process., the makeup of lipids and proteins, and other factors. The translocation of phosphatidylserine from the inner to the outer membrane leaflet, which is aided by Ca2+-dependent amino phospholipid translocases such as calpain, flippasses, floppases, and scramblases, is one feature of MV that is thought to be prevalent. This procedure is described in full in [89]. Furthermore, phospholipidserine is externalized by bigger apoptotic complexes [90], [91]. Only healthy, live cells should be used for MV separation since contaminated MV may make it difficult to distinguish apoptotic bodies from MV. Ca2+ levels control the stiffness, bending, and curvature of the membrane. This results in the actin cytoskeleton underneath contracting and reorganizing, which allows MVs to pinch and grow (reviewed in [35]). Ceramide and cholesterol are two other lipids that affect the production and release of MV [92]. There is a correlation between neutral sphingomyelinase activity and exo and MV release. Lipid sphingomyelin is hydrolyzed by this enzyme to produce phosphorylcholine and ceramide. Exo release decreased when the enzyme was inhibited, while MV budding increased concurrently [93], providing evidence that the two EV subpopulations' releases are related, although via different biogenetic pathways. Apart from fats, the enzymatic apparatus responsible for regulating the cytoskeleton significantly influences the development and budding of MVs. ARF6, a small GTPase ADP-ribosylation factor, is one example of such a protein. Phospholipase D (PLD) binds extracellular signal-regulated kinase at the plasma membrane as a consequence. MV release is facilitated by the actinomyosin contraction caused by the ERK-activated signaling cascade, which is started downstream of myosin light chain kinase (MLCK) [94]. Similar to MV, LO is thought to have its origins in the plasma membrane. LO shedding has only been connected to cancerous cells that have developed amoeboid characteristics in order to become more motile and invasive, in contrast to MV ([32]). Actin dynamics regulators essential for the development of MV include RhoA and other small GTPases of the Rho family, such as RHO-associated protein kinases [95]. The last step in MV release and synthesis is controlled by the endosomal sorting complex required for transport (ESCRT), which is known to play a role in exobiogenesis [96]. Tumor susceptibility gene 101 and arrestin domain-containing protein 1 are two late endosomal proteins that cooperate to transfer TSG101 from the endosome to the plasma membrane, where it releases MV [97].

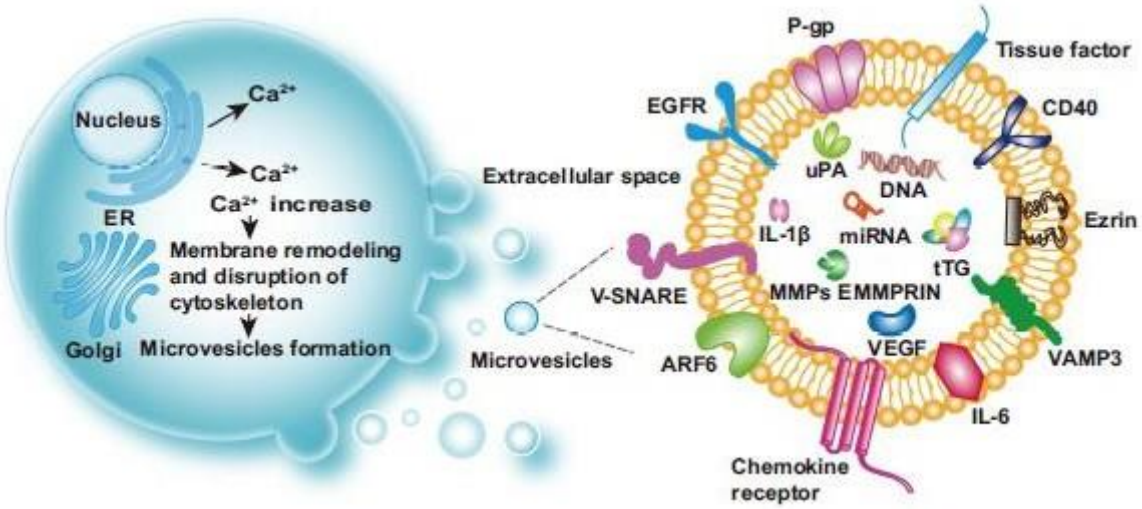

FIGURE 2: -micro vesicles biogenesis

3.6 Characterization and Cargo of MVs: -

various proteins, some of which are found on their surfaces, may be transported by MVs.Consequently, some markers such as the extracellular matrix metalloproteinase inducer (EMMPRIN), CD40, MMPs, ARF6, and others may be used to detect MVs. [98]. 32, 99, 100, and 101 Membrane protein enzymes, growth factor receptors, cytokines, chemokines, lipids, and nucleic acids, including oncogenic genes, genomic DNA, and miRNAs, are all transported by MVs [102].[103]. [104]. According to Lo Cicero et al. [105], constitutive partners were present in low quantities, and Hsc70 was mostly detected on MVs. Bioactive materials, for example, RNAs, proteins, and miRNAs, affect the phenotypic and function of receptor cells and contribute to the invasion of tumors. [106] Tumor cells' surface transmembrane glycoprotein, EMMPRIN (also called CD147) [101], stimulates the production of MMPs by surrounding fibroblasts or tumor cells. [107] These MMPs may break down the basement membrane, which makes it easier for cancerous tumor cells to proliferate, invade, and spread. [108] Menck et al.'s research [109] shows that MVs may be identified using EMMPRIN. The P38/MAPK signaling pathway in tumor cells may be activated by highly glycosylated EMMPRIN, promoting invasion and metastasis.

4.0 Distinctions Among Micro vesicles, Exosomes: -

Table:1

Vesicle Type	**micro vesicles**	**Exosomes**
source	membrane of plasma	MVBs, or multivesicular bodies
Biogenesis	separating from the membrane of plasma	Plasma membrane fusion with endosomes
Size (nm)	100–1000	40–160
Markers	integrin, selectin, ARF6, EMMPRIN, and CD40	Tetraspanins, flotillin, ESCRT, TSG101, ALIX, CD63, CD81, HSP, and
topic	membrane and cytoplasmic mRNA, non-coding RNAs, and proteins, also receptors	Major Histocompatibility Complex molecules, cytoplasmic and membrane proteins, non-coding RNAs, and mRNA
Major pathway	Ca2+-dependent	ESCRT– dependent
identification technique	EM, FACS	Western blotting
Isolation methods	Centrifugation (10,000–20,000 g) magnetophoretic sorting or immunoaffinity chromatography	Ultracentrifugation (≥100,000 g), immunoprecipitation (ExoQuick), SEC

5.0 Exosomes as Biomarker of Cancer: -

1. Exosomal Proteins as Biomarkers: -

Exosomes include a large number of proteins linked to cancer, and since exosome membranes shield luminal proteins from external protease degradation,[110],[111] proteins from TEEXs are emerging as prognostic and diagnostic biomarkers for cancer. Table 1a provides an overview of exosomal proteins for the diagnosis of cancer. Globally, pancreatic cancer is thought to be the deadliest disease due to its high metastasis and invasiveness.[112] The best course of action is still surgical resection. Nevertheless, the majority of patients had incurable malignancies due to locally progressed or variable degrees of metastasis at the time of diagnosis.[113] Pancreatic cancer cell secretory exosomes were shown to be heavily loaded with Glypican-1 (GPC-1), an overexpressed proteoglycan that is membrane-anchored. Serum from patients with pancreatic cancer was precisely and sensitively shown to contain GPC-1+ exosomes. Moreover, it was shown that the tumor burden and the patients' preoperative and postoperative survival were correlated with the amounts of GPC-1+ exosomes. Melo et al. established a crucial foundation and demonstrated the efficacy of using exosomal proteins in the detection of pancreatic cancer.[114] Furthermore, pancreatic ductal adenocarcinoma (PDAC) patients have elevated blood levels of cytoskeleton-associated protein 4 (CKAP 4) compared to healthy persons using ELISA utilizing recently developed anti-CKAP 4 monoclonal antibodies (mAbs). Exosomes in serum that contain CKAP 4 might potentially be a different biomarker for PDAC.[115] Of all lung cancers, non-small cell lung cancer (NSCLC) is the most prevalent kind and is regarded as the most aggressive tumor with the greatest global morbidity and death rate. Forty According to a research, individuals with non-small cell lung cancer (NSCLC) had substantially higher blood levels of exosomal expression of extracellular matrix protein 1 (ECM1) and alpha-2-HS-glycoprotein (AHSG). Furthermore, it was discovered that carcinoembryonic antigen (CEA) with AHSG or ECM1 may provide a more accurate diagnostic result than CEA by itself. The diagnostic potential of NSCLC was increased by the trinity of AHSG, ECM1, and CEA, which had a greater diagnostic power than any of them alone.[116] Gastric cancer (GC) is a major cause of tumor death and a worldwide public health problem because to its high incidence and mortality rates in various communities, particularly Asian and Hispanic Americans.[117]Fu et al. recently discovered, using mass spectrometry (MS), that the tripartite motif-containing 3 (TRIM3) protein levels were reduced in exosomes from GC patients' blood and GC tissues.[118] Additional research revealed that there could be a negative correlation between the expression levels of certain miRNAs and TRIM in GC; that is, an increase in miRNA expression is associated with a decrease in TRIM expression. The most basic and universal mechanism that regulates many physiological processes in cells and is essential to cellular signal transmission is protein phosphorylation.[119] The precise function of phosphorylated proteins in bodily fluids for disease detection is still unclear, and only a small number of phosphoproteins have been found to far as viable biomarkers to differentiate between illness and health. Thankfully, a recent research showed that phosphoproteins in plasma exosomes are a very viable option for disease biomarkers and might eventually replace other technologies used in cancer screening and monitoring.[120] Furthermore, exosomes produced from lung cancer (LC) exhibit important lung cancer (LC) phosphoproteins, such as extracellular regulated protein kinases (ERK) and epidermal growth factor receptor (EGFR), and they retain their original phosphorylation state.[121]Cancer cells treated with tyrosine kinase inhibitors (TKIs) showed changes in the phosphorylation of proteins in their secretory exosomes. As a result, variations in exosome protein phosphorylation may be used to track how well TKIs work as a treatment in LC.[122] According to studies on exosome proteomics, TEXs are essential for the development of tumors as well as for their proliferation, metastasis, and maintenance of survival.[123] Nevertheless, challenges remain in the proteomics research of exosomes, particularly with regard to exosome quality criteria. Proteomics is a crucial research technique in the post gene age that should be used in conjunction with transcriptomics and genomics to thoroughly study exosomes for their applications.

2. NUCLEIC ACIDS AS BIOMARKER: -

Exosomes, which are present in bodily fluids, are rich in DNA and a variety of RNA species, including mRNA, miRNA (micro-RNA), snRNA (small nuclear RNA), and lncRNA (long non-coding RNA). As a primary specie of exosomal RNA, recently fragmented ribosomal RNA (rRNA) has been identified [124][125][126][127][128]. With Valadi's 20073 discovery of exosomal mRNA and miRNA, a significant amount of research was done on RNA as a biomarker. Exosomes have a greater concentration of miRNA than their parent cells [129]. Deep sequencing of exosomal RNA species by Huang et al. further supports this, finding that miRNA is the most prevalent functional RNA species in exosomes [130]. The use of miRNA as a biomarker for many illnesses has gained attention as a result of these findings. miRNAs are single-stranded RNA molecules that are small and non-coding, with a maximum length of nucleotides. They primarily target 3\untranslated regions of mRNAs at the post transcriptional stage to control gene expression. miRNA is essential for many biological processes, such as apoptosis and cell cycle regulation. It is also linked to diseases including cancer and neurological disorders [131]. Both healthy and sick people have different exosomal miRNA concentrations and compositions. This variant demonstrates the possibility for non-invasive biomarker applications using exosome-derived miRNAs. Exosomal miRNAs unique to cancer have been identified as biomarkers in a number of investigations on various cancer types [132][133][134][135]. For instance, compared to healthy people, prostate cancer patients' blood levels of miR-375 and miR-141 are elevated.[228]. MiR-373, miR-200a, miR-200b, and miR-200c are also useful as ovarian cancer biomarkers for diagnosis and prognosis. One colorectal cancer biomarker that is utilized is miR-37256. While certain exosomal miRNAs are exclusive to a single malignancy, others may be used as prognostic or diagnostic biomarkers for several cancers. MiR-21, for instance, is a diagnostic biomarker for pancreatic, ovarian, breast, cervical, retinoblastoma, gastric, and laryngeal squamous cell carcinoma (LSCC)

In addition to miRNA, long non-coding RNA (lncRNA) with a length of several hundred to thousand bases is also present in exosomes. The precise role of lncRNA, which is expressed in both healthy and sick cells, is unknown, but there are hints that it may serve as a sponge for miRNA [136], [137], and [138]. The first lncRNA in Prostate Cancer [139] was discovered as PCA3, or prostate cancer antigen 3. As a diagnostic and prognostic biomarker of LSCC [140] based on serum, another lncRNA known as HOTAIR has been discovered. In prostate cancer, enrich motifs found in exosomal lncRNA correspond to seed areas of one or more exosomal miRNAs. Complete functioning mRNAs, proteins, and short RNAs that modify the cell environment to promote tumor development are also present in exosomes originating from tumors. When the necessary protein machinery is present, mRNA is translated into protein [64], [102], [141].

6.0 Micro vesicles as Biomarker of Cancer: -

Extracellular vesicles (EVs) have attracted interest as possible indicators in the medical context due to a growing body of in vitro research showing cancer cells secrete huge amounts of EVs carrying stuff particular to tumors. Up until now, the majority of studies have concentrated on finding cancer-related vesicles in the intricate combination of plasma EV. Particularly for solid tumors (like glioblastoma and pancreatic cancer) that are not readily accessible without intrusive procedures, this seems to be a viable way for determining the genetic composition of the tumor and identifying traits that may be addressed. Unlike typical soluble "tumor markers" in blood, MV may detect non-secreted, membrane-bound, or even intracellular compounds. Consequently, the range of identifiable tumor-associated variables is expanded by MV-based liquid biopsy, which may aid in accurate patient risk assessment, treatment monitoring, and diagnosis. Tumor micro velocities (MV) in blood are thought to be of potential importance as early diagnostic biomarkers since malignancies seem to enter the circulation relatively early [142]. The sensitivity of detection may be enhanced by their much larger abundance as compared to the very sparse CTCs. Recently, it was shown that tumor EV, which is made up of both big and microscopic vesicles, may pass across the BBB in both glioma patients and an orthotopic glioblastoma xenotransplant model employing human cancer stem cells. [143]. According to this discovery, tumor-specific modifications in EV were found in the blood of patients with inferior gliomas [144], the majority of whom still had an intact BBB. This study lends more credence to the idea of employing EV as early cancer biomarkers. Tumors are known to be quite diverse, which is another crucial element to remember. This situation is presently receiving more and more attention due to innovative single cell analysis tools. Although EV are thought to provide a more complete picture, a biopsy from a small portion of the tumor only partially reflects the molecular makeup of the whole tumor. This may also apply to individuals with metastatic cancer that has progressed, since metastases often exhibit differences from the original tumor and from one another, perhaps indicating the expression of distinct markers or therapeutic targets [145],[146]. Curiously, a preliminary glioblastoma investigation reported finding oncogenic EGFRvIII transcripts in plasma-derived EV in two patients who had received a tumor tissue biopsy diagnosis of EGFRvIII-negative [147]. It may thus be hypothesized that EV may more precisely reflect tumor heterogeneity than restricted samples. The initial step in attempting to specifically detect tumor-derived MV in blood is to discover cancer-specific components that are not expressed on MV released by other cell groups. Tumor must thus be well characterized; this has often been done on vesicles generated from cancer cell lines and then translated to patient samples. In light of the cellular transcriptome landscape, MV may be a more useful indicator of mutation status or gene amplifications for cancer diagnosis than Exo, according to a recent publication [148]. MV of leukemia cell lines was revealed to include particular tumorigenic genes (e.g., HOXA9, MEIS1) as well as characteristic oncogenic fusion transcripts [149],[150]. Similar findings were made in a glioblastoma model where tumor-associated gene genomic DNA (gDNA) sequences were found to have a distinct distribution in either apoptotic bodies, MV or Exo isolated from human cancer stem cells, or in the blood of mice that were transplanted into other bodies. Notable sequences found in MV included PIK3CA, EGFR, AKT1, and MDM2[151]. Still up for debate, however, is whether mutation detection using cell-free DNA is preferable to vesicle-associated transcript analysis. The findings of the Krug et al. group are encouraging in this regard. They showed that a combination analysis of circulating tumor and EV DNA, as opposed to circulating tumor DNA alone, enhanced the sensitivity of identifying EGFR mutations in the plasma of lung cancer patients [152]. It has been shown that MV and Exo differ in their RNA composition [153]. Although a number of population-specific miRNAs have been found for both MV and Exo, there seems to be a tendency suggesting that microRNA in MV is comparatively less enriched than miRNAs in Exo [154],[155],[156]. Furthermore, a comparative analysis of the Micro vesicle and Exo from the cells revealed an enrichment relative to the whole cell, with some microRNA that were nearly unnoticeable colon cancer cell lysates identified at high levels (>1000 TPM) [157]. Many important investigations have shown that the majority of RNA sequences present at rates of considerably fewer than one copy of each

EV, as a result, it is uncertain whether sufficient quantities of RNA molecules travel to EV to functionally influence recipient cells. [158],[159],[160]. Consequently, the remaining portion of this study focuses on MV- associated proteins, which have the added advantage of potentially serving as targets for targeted medication delivery in addition to indicators. Western blotting, flow cytometry, and several proteomic techniques have all been used extensively to assess the protein composition of EV. With the use of cutting-edge targeted proteomic collection techniques like selected (or multiple) reaction monitoring (SRM/MRM), it is possible to identify a pre-selected subset of certain target peptides with excellent quantitative accuracy and sensitivity. Initially, the complete EV proteome was extensively characterized using shot-gun proteomics.

Thus, using these techniques makes it easier to find and validate biomarker pipelines on a wide scale in biological materials that are complicated (like blood) [161]. SRM/MRM has the potential to be used in future EV-related biomarker research, as shown by a recent work [162] that effectively used it to find new possibility biomarkers for prostate cancer on Exo in urine. It has been suggested that compared to Exo, the MV proteome is more comparable to the cellular proteome [163]. Certain proteins from the cellular plasma membrane, however, are not present on MV, and few of them are significantly concentrated, indicating the possibility of distinct sorting processes [164]. Table 1 lists the majority of MV-associated tumor antigens that have been identified and used as biomarkers in cancer patients' blood. When it comes to hematologic malignancies, MV bearing the corresponding antigen linked to the malignancy (for example, CD30 for Hodgkin lymphoma and CD38 for multiple myeloma) were discovered in cancer patients but were virtually absent in healthy controls. For solid tumors, similar findings have been reported. Human epidermal growth factor receptor 2 (Her2/Neu), an oncogenic receptor, was discovered and shown to be considerably enhanced on blood-derived MV from patients with stomach cancer in one of the early investigations [166]. For colorectal cancer, this conclusion has been validated in the interim [167].

Advanced tumor stage was observed to be associated with a higher number of MV bearing the tumor antigen EMMPRIN in the biggest research to date, which included 330 cancer patients with advanced solid tumors and 103 healthy, non-cancer controls [168]. Examining MV's co-expression of EMMPRIN with other tumor-associated molecules, like EGFR1, MUC1/CA 15-3, or epithelial cell adhesion molecule, revealed that these numbers were not only significantly higher in cancer patients than in controls, but also linked to a poor overall survival. These findings suggest that MV may be a useful prognostic biomarker for cancer. Since EpCAM is overexpressed in tumors originating from epithelial cells, making it a legitimate tumor marker, it is one of the most researched antigens. Patients with liver disorders could be classified as either having or not having liver tumors (including hepatocellular or cholangiocellular carcinoma) thanks to the use of EpCAM in conjunction with other tissue-specific markers like the asialoglycoprotein receptor 1 (ASGPR1), which is only shown in liver cells [169]. In keeping with this, after the surgical removal of the tumor, patients' levels of EpCAM+ MV decreased and were linked to the size of the tumor in patients with colorectal cancer [169], [170]. Caveolin-1 (CAV1) was not detectable in healthy persons but was detected on 5–10% of huge EV (1–10 m) isolated by filtering with a size profile similar to the 10,000 g pellet, according to another study utilizing samples plasma from individuals whose prostate cancer has spread [171]. Moreover, highly concentrated tumor MV could be concealed in the varied blood MV combination, necessitating the development of novel methods for the targeted enrichment of tumor MV populations. One suggestion [172] is to remove platelet-derived MV before analysis, since this kind of MV makes up the bulk of MV in blood. Recently, a methodology for separating distinct subgroups of large and small EV from tumor tissue in this case, Malignant Lentigo was reported [173],[174]. This is a critical step that will enable the separation of MV from original tumor tissues and metastases, enabling the identification of biomarkers that are distinct to patients as well as tumors. The MV profiles from the main tumor and its metastases, as well as the MV found in bodily fluids, may then be compared to learn more about the development of clones and to advise the choice and monitoring of therapies.

Table 2. Common tumor antigens found on MV collected from patients

Marker	Subtypes of Cancer	Technique
CD13	AML/CML leukemia Myelodysplastic syndrome	Flow cytometry
CD138	several Malignant Lentigo	fluorescence-activated cell sorting
CD19	Leukemia (CLL) in non-Hodgkin lymphoma Morbus Walden Strom	Flow cytometry
CD30	Lymphoma Hodgkin	fluorescence-activated cell sorting
CD38	Several Malignant Lentigo	(FC)
c-Met	stomach cancer	Western blot
Caveolin-1	Prostate cancer	(FC)
EGFR	Colorectal	(FACS)
EGFRvIII	GBM	Immunofluorescence
Extracellular Matrix Metalloproteinase Inducer(EMMPRIN)	Colorectal cancer	Immunofluorescence
Epithelial cell adhesion molecule	Cancers of the breast, lungs, head, and neck Pancreatic cancer and colorectal cancer	Western blot
EpCAM/EMMPRIN	Lung cancer, and colorectal cancer	Flow cytometry
EpCAM/ASGPR1	Cholangiocarcinoma and Hepatocellular Carcinoma	Western blot
FAK	Breast cancer	(FACS)
HepPar1	Hepatocellular carcinoma	(FC)
Her2/Neu	Gastric cancer	(FACS)
MUC1	Breast cancer	Western blot

CHAPTER TWO
Literature review

7.

1. Weihua Li, Chuanyun Li, Tong Zhou, Xiuhong Liu, Xiaoni Liu, Xiuhui Li, Dexi Chen. https://doi.org/10.1186/s12943-017-0706-8 : -

A brand-new class of cancer biomarkers is being identified using exosomes. A nanoscale, bilayered vesicle, exosomes are released into all bodily fluids by different types of living cells. Exosomes are seen as encouraging, clinically-relevant indicators of cancer development and response to treatment, based on our growing, if imperfect, understanding of their synthesis, cell secretion, and the molecular and genetic makeup unique to cancer cells. Cancer cell-derived or cancer plasma-derived exosomes have distinct properties, which are confirmed by preliminary proteomic, genomic, and functional profiling. A number of clinical conditions, including cancer, are correlated with changes in the protein or nucleic acid profiles of exosomes in plasma. Prior research on the use of exosomes in cancer detection and therapy, however, mostly examined miRNAs. Applications of exosomal proteins for early-stage cancer detection, monitoring, and prognostic assessment may be investigated with the advent of quick large-scale exosomal content generation, purification, extraction, and screening. The use of exosomal proteins in cancer diagnostics has advanced recently, and we have outlined those achievements below.

2. Yang Liu1, Ke Shi1, Yong Chen2, Xianrui Wu1, Zheng Chen1, Ke Cao3, Yongguang Tao4, Xiang Chen5, Junlin Liao6* and Jianda Zhou1*. https://doi.org/10.3389/fonc.2021.639159: -

Extracellular vesicle-derived exosomes have the ability to transfer Nucleic acids, fats, and proteins, and other materials to target cells, therefore stimulating or suppressing different signaling pathways. Furthermore, it is thought that exosomes have a variety of roles in the initiation and advancement of cancers originating from various tissue origins. These roles include modifying the tumor microenvironment, stimulating angiogenesis, invasion, and metastasis, as well as controlling the immune system's ability to eliminate tumor cells. It is still unknown, nevertheless, exactly what chemical pathways exosomes use to engage in these many operations. We outline the advancements in the study of exosomes generated from tumor cells in the development of cancer in this review.
We also talk about the potential uses of exosomes in conjunction with specially designed chemotherapy medications to treat cancer.

3. Yuju Zhou, Ying Zhang, Huan Gong, Siqi Luo and Yan Cui. https://doi.org/10.3390/ijms222212204: -

Exosomes are little extracellular vesicles that are released by several cell types and may be found widely in a range of bodily fluids. Exosomes may have a role in controlling the tumor microenvironment and influencing the growth and spread of tumors, according to recent studies. Owing to their wide involvement in cancer research, exosomes are now at the center of efforts to find a novel cancer treatment. Exosomes have the potential to be very effective therapeutic delivery vehicles for tiny chemicals, proteins, and RNAs intended for cancer cells. Proteins, lipids, and nucleic acids transported by exosomes are being investigated as possible targets for cancer therapy as well as as promising biomarkers for cancer diagnosis and prognosis. Furthermore, exosomes from various origins act differently in cancer applications. In this review, we describe the precise process by which exosomes influence the tumor-microenvironment communication and list the therapeutic and diagnostic uses of exosomes in cancer.

4. Shuangli Zhu, Shiyu Li, Ming Yi, Ning Li, Kongming Wu. https://doi.org/10.2147/IJN.S325448 : -

Tumor cells and other cells in the tumor microenvironment release micro vesicles, which are extracellular vesicles with a diameter of 100–1000 nm. Tumor-derived micro vesicles have been linked to medication resistance and tumor start and development, according to an increasing number of studies. Tumor-derived micro vesicles may also be used to create tumor vaccines as they include a range of immunogenic chemicals and reduce the tumor's response to immunotherapy. Furthermore, tumor-derived micro vesicles isolated from bodily fluids may be used as biomarkers for prognostication or cancer detection due to their great stability. Tumor-derived micro vesicles may also be used to treat cancer patients by delivering oncolytic adenovirus and chemotherapy medications, or by helping tumor regenerating cells overcome their drug resistance. The main features of tumor-derived micro vesicles are outlined in this review, with an emphasis on their biological traits, role in tumor growth, and therapeutic uses.

5. Roberta Valenti, Veronica Huber, Manuela Iero, Paola Filipazzi, Giorgio Parmiani, and Licia Rivoltini. https://doi.org/10.1158/0008-5472.can-07-0520:-

Exosomes, or tumor-released micro vesicles, are often seen in the bodily fluids of cancer patients and are thought to have a role in the development of tumors. Recent research has shown that the production of micro vesicles by human melanoma and colorectal carcinoma cells may stimulate monocyte differentiation into myeloid-derived suppressor cells, which aid in tumor development and immune evasion. These results highlight the critical function that these extracellular organelles play in altering tumor-stromal interactions and hence fostering cancer.

CHAPTER THREE
Purpose of the study

8.0. Purpose of the Study

The study of the correlation between exosomes and micro vesicles in cancer serves several important purposes:

1. **Understanding cancer progression:** Investigating the correlation between exosomes and micro vesicles in cancer provides insights into the mechanisms underlying tumor growth, invasion, and metastasis. By elucidating how these extracellular vesicles contribute to cancer progression, researchers can identify potential therapeutic targets to impede tumor development and spread.

2. **Identify the role of exosomes for Cancer formation:** Exosomes are involved in the development, spread, and metastasis of cancer in many ways. Exosomes are tiny membrane vesicles that are released into the extracellular milieu by the majority of cell types, including cancer cells. Proteins, lipids, and nucleic acids including DNA, mRNA, and microRNAs are among the many biomolecules they include. Exosomes have the ability to alter the microenvironment of tumors and accelerate the development of cancer.

3. **Identify the role of Micro vesicles for cancer formation:** Similar to exosomes, Micro vesicles are tiny membrane-bound vesicles that are secreted into the extracellular milieu by cells, including cancer cells. Micro vesicles have unique biogenesis routes and features, despite some similarities with exosomes. Through its facilitating of cell-to-cell communication, modulation of the tumor microenvironment, promotion of metastasis, induction of EMT, modulation of the immune response, and contribution to treatment resistance, micro vesicles play a variety of important functions in the genesis and progression of cancer. Novel therapeutic and diagnostic techniques for the treatment of cancer may result from better understanding of the roles played by micro vesicles in cancer biology.

CHAPTER FOUR

METHODOLOGY

9.0. Methodology

Literature Search: A thorough search of the literature was done using a variety of platforms, such as Google, Google Scholar, PubMed, CrossRef, and others. After a comprehensive investigation, around 20 documents—a combination of research and review papers—were found. These records served as the basis for composing a written report, which required a careful examination of the chosen articles. A particular focus was placed on locating relevant sources that support the goals of the research. It is important to highlight that every reference included in the article came from publications that were released between 2000 and 2024.

Data Analysis: The collected data underwent thorough data analysis once relevant literature was gathered. The technique of analysis used made it easier to draw significant conclusions from the collected study data. The material was systematically arranged thematically such that each portion of the study focused on a particular aspect of the subject being studied. Through careful balancing of the theme organization with the goals of the research, the article successfully provided a thorough review of the topic.

CHAPTER FIVE
RESULTS AND DISCUSSION

10. Results and Discussion

10.1 Results

Role of exosomes in cancer progression: -

1. Exosomes in Tumor Progression: -

Exosomes are involved in regular cellular function, but when they aren't working properly, they may cause cancer. Exosomes alter the flow and cell division of tumor microenvironments, thereby stimulating the immune system. In a number of human malignancies, they cause the epithelial-mesenchymal transition and interconversion to the mesenchymal-epithelial transition [175]. Exosomes play a part in chemotherapy resistance, metastasis, and cancer. But dendritic cell-produced exosomes may be altered to stimulate immunological responses against tumors (also known as "dexosomes") [18], [176]. Exosome have a variety of functions in tumor development. They could alter the expression of some genes. EVs often experience after translation modifications, such as ubiquitination. Cancer and other diseases may be exacerbated by dysregulation of ubiquitination and deubiquitylation, which also control the metabolic reprogramming of cancer cells [177]. Numerous malignancies, including invasive breast cancer, lung and pancreatic cancer, cell carcinomas of the neck also head, have been related to exosomes carrying Wnt5b [178]. Apart from their impact on expression of genes, Additionally, they could participate in post-translation modifications, as will be discussed later. Mannose and sialic acid residues have been shown to be abundant in ovarian cancer EVs [179], suggesting altered glycosylation. In myeloid leukemia, exosomes promote Src phosphorylation, which aids in angiogenesis. Therapeutic interventions targeting this kind of phosphorylation are possible [180].

2. Exosomes in Cancer Immunology: -

Exosomes may be able to evade immune surveillance because they include ligand 1 for the organized cell death receptor (PD-L1), That directly inhibit CD8+ T lymphocytes' ability to fight cancer in vivo [181],[182]. Regarding the prospects of anti-PD-L1 treatment for cancer, some writers are optimistic [183],[184]. Extracellular vesicle-surface PD-L1 is linked to immunosuppression, tumor patients' disease progression, and changed immunotherapy response [185],[186].

3. The role of exosomes in angiogenesis and Lymph angiogenesis: -

Microenvironments inside cells exert a significant influence on the progression of cancer. The release of exosomes by cancer cells serves to precondition the surrounding tissue environments, facilitating both local spreading and distant metastasis through the delivery of inflammatory and other factors [187],[188],[189]. Notably, exosomes derived from breast cancer have been found to enhance the adhesion of cells to extracellular matrix proteins, thereby promoting metastasis [190]. Numerous studies have reported on the role of exosomes in inducing metastases. These tumor-derived exosomes have the potential to impact various factors related to epithelial-mesenchymal transition (EMT) and evasion of immune surveillance, such as - catenin, caveolin-1, and transforming growth factor beta (TGF) [191]. Furthermore, the development of new blood and lymph vessels is intimately linked to the growth and dissemination of malignancies. Growth factors that support lymph angiogenesis, as well as angiogenic factors that promote tumor angiogenesis, are secreted by cells inside the tumor and the surrounding tissue. It's interesting to note that non-coding RNA-loaded exosomes generated from tumor cells also take part in these activities. [192],[193]. Recent studies have demonstrated that exosomal miRNAs derived from cancer cells play a role in promoting angiogenesis and/or lymph angiogenesis through different signaling pathways that affect both endothelial and non-endothelial cells [194][195][196][197], [198]. Additionally, exosomes produced from tumors that carry certain microRNA, such as microRNA-21 or let-7a under hypoxic stress, have been shown to induce M2 polarization of macrophages, thereby stimulating tumor- associated angiogenesis and lymph angiogenesis [199],[200]. Additionally, long non-coding RNAs (lncRNAs) present within exosomes have been implicated in these processes [201][202][203]. For example, lnc RNAs H19 and HOTAIR are known to stimulate angiogenesis through the production and release of growth factors for vascular endothelial cells [204],[205], while other lnc RNAs function by sequestering microRNAs [206],[207]. Furthermore, it has been shown that exosomal Circular RNA -100338 may control angiogenesis to encourage HCC (Hepatocellular carcinoma) metastases [208]. Exosomes may also transport other components that are essential for fostering angiogenesis. Research

has shown that Annexin II, a factor linked to carcinogenesis, is transferred by breast cancer cell exosomes both in vivo and in vitro [209]. Interestingly, hypoxic areas of tumors with poor chemo responsiveness are where new blood vessel creation is most often seen [210]

4.Tumor-derived exosomes in cancer metastasis: -

The most frequent reason for mortality from cancer is metastasis. Cancer metastasis requires multistep mechanisms. Stephen Paget proposed the "seed and soil" concept in 1889, which claims that the connection between tumor cells, often called the "seed," and certain organ microenvironments, also called the "soil," determines metastasis. [211] Metastatic tumor cells release bioactive molecules that reconstruct the extracellular matrix and modify leading cells in organ locations, including bone marrow progenitor cells, CAF, and tumor-associated neutrophils, to create premetastatic niches before cancer metastasis. Paget's "seed and soil" theory was expanded upon in 1980 [212] by Hart and Fidler, who noted that contact with the tumor microenvironment in particular specific organs had a significant impact on metastatic colonization, hence contributing to the nonrandom pattern of cancer spread. However, the regulatory pathways controlling organ-specific metastasis are poorly understood. Lyden's group's research shows that tumor-derived exosome aid in premetastatic niches by triggering progenitor cells of hematopoietic stem cells from bone marrow that display VEGFR1 through the exchange of exosome-mediated MET oncoprotein..[213] The exact same group has recently shown that the integrin expression patterns of exosomes originating from tumors serve as "ZIP codes" that guide exosomes to certain tissues and organs, resulting in organotropism during metastatic spread.[214]Exosomal integrins α6β4 and α6β1 showed a good correlation with lung metastasis based on proteomics and clinical relevance studies, whereas exosome integrin αvβ5 showed a strong correlation with liver metastasis.[215] Additionally, tumor-derived exosomes activated Src and increased pro-inflammatory S100 gene expression in target organ recipient cells, resulting in the formation of premetastatic niches.[216] As evidence for the aforementioned research, other investigations have shown that exosomes originating from tumors transport enzymes known to modify extracellular matrix, including MMP2 or MMP9. This results in the breakdown of extracellular matrix and facilitates the invasion and spread of cancer. [217] Similar to this, MIF-1 found in pancreatic cancer-derived exosomes increases the TGF expression of Kupffer cells in the liver, which in turn causes rising in fibronectin levels synthesis by HSCs.[218] The redesigned milieu subsequently amplifies the recruitment of macrophages generated from bone marrow, which helps the liver build a premetastatic niche and creates the right circumstances for metastasis.[219] The functional influence of tumor-derived exosomes on the invasiveness of malignancies highlights their pathological importance on cancer spread.[220] Exosome-mediated cancer invasion clearly requires both exosome secretion and invadopodium formation simultaneously.[221] Ab27a knockdown-induced inhibition of exosome biogenesis or secretion dramatically decreased extracellular matrix digestion and the development of mature invadopods.[222] Interestingly, invadopodia are important locations for exosome secretion, indicating a synergistic relationship between exosomes and the regulation of invasive activity.[223] Additionally, tumor-derived exosomes have the potential to transport miRNAs from cancer cells that have metastasized to less metastatic cells. This might lead to changes in gene expression inside less metastatic cells, thereby facilitating metastasis.[224] One of the main causes of illness and death for cancer patients is brain metastases. The blood-brain barrier (BBB) is very selectively permeable, yet metastatic cancer cells may nevertheless enter the central nervous system (CNS). Exosomes produced by metastatic cancer cells have been shown in recent research to be capable of damaging the BBB's structure and functionality.[225],[226]miRNAs, including miR-105 and miR-181c, endothelium cells in the circulatory system absorb exosomes that are present in cancer, which then cause cytoskeleton to be abnormally localized or target tight junction proteins, so causing the degradation of vascular endothelial barriers.[227], [228]The compromised capillary endothelial barriers therefore allow cancer to spread to the brain.

TABLE 3 | Exosomes' roles in the development of cancer.

Origin of exosomes	Chemical	Sort	Stage of cancer development	Sort of action	References
breast cancer cells that have spread.	MicroRNA 105	MicroRNA	Remodeling of the tumor microenvironment	A movement regulator that works by focusing on the tight junction protein ZO-1	229
Exosomes generated from fibroblasts and linked to cancer	nourishment	Lipids, TCA-cycle intermediates, & amino acids	Remodeling of the tumor's microenvironment	Stop the oxidative phosphorylation of cells.	230
cells associated with bladder cancer	Annexin A2 and casein kinase IIa	Protein	epithelial-to-mesenchymal transition	Promote epithelial-to-mesenchymal transition	231
Cells of lung adenocarcinoma	MicroRNA -499a-5p	MicroRNA	epithelial-to-mesenchymal transition	The M-TOR signaling pathway facilitates lung cancer cell proliferation, EMT, and migration.	232
stem cells for tumors	MicroRNA -19b-3p	MicroRNA	epithelial-to-mesenchymal transition	EMT is brought on by tumor stem cells displaying the PTEN gene.	233
Sub durative prolonged hematoma	MicroRNA -144-5p	MicroRNA	Angiogenesis	encourages angiogenesis that is very porous and prevents hematoma absorption	234
stomach cancerous cells	MicroRNA -155	MicroRNA	Angiogenesis	Box-o3, which targets endothelial	235

stem cells from bone marrow	unidentified	unidentified	Angiogenesis	Encourage flap survival and lessen the likelihood of necrosis	236
cells of nasopharyngeal cancer	MicroRNA - 17-5p	MicroRNA	Angiogenesis	focusing on BAMBI by controlling the AKT/VEGF-A signaling pathway encourages angiogenesis, nasopharyngeal cancer cell development, and migration.	237
cells associated with ovarian cancer	MicroRNA - 205	MicroRNA	Angiogenesis	It induces vascularization, which facilitates the spread of tumor cells.	238
Pancreatic cancerous cell	MicroRNA - 27a	MicroRNA	Angiogenesis	BTG2 stimulated human vascular capillary cells' angiogenesis.	239
cell for breast cancer	S100	nourishment	Angiogenesis	The Src kinase signaling pathway is activated, which increases pulmonary vascular leakage.	240
Macrophages of the M2-type	MicroRNA - 21-5p & MicroRNA - 155-5p	MicroRNA	Movement and invasion	It has the ability to interact with the BRG1 encoding sequence, suppress BRG1 expression, and encourage colorectal cancer invasion and migration.	241

A cell from colorectal cancer	First secretion protein that is activated by calcium (CAPS1)	Protein's	Migration	Normal migration of FHC epithelial cells was encouraged.	242
cells associated with liver cancer	CLEC3B gene	DNA	Angiogenesis & tumor growth	AMPK and VEGF signaling pathways drive HCC metastasis & angiogenesis.	243
cells associated with breast cancer	Cluster of Differentiation 47	Protein's	Tumor metastasis and immunological regulation	This substance may facilitate immune evasion by macrophages and T cells, promote tumor metastasis, migration, and invasion, and enable tumor cells to bypass detection and elimination by T lymphocytes and NK nuclear macrophages.	244
cells associated with liver cancer	CD81		Tumor metastasis and immunological escape	encourages the spread of liver cancer cells in HCC brought on by viral hepatitis C	245
Endothelium lymphaticus cells	MicroRNA -503-3p MicroRNA - 4269& MicroRNA - 30e-3p	MicroRNA	Spreading	The goal is to regulate the tumor's microenvironment and the communication. prevent the spread of breast cancer	246

10.2 Role of MVs in cancer progression: -

MVs may have a role in the formation of metastatic ecosystems via interactions between tumor cells and cells produced from bone marrow [247]. The original tumor secretes MVs, which draw hematopoietic cells in. The development of MVs released by platelets inside the main tumor may then foster an environment that encourages the tumor cells' propensity to become malignant [248]. MVs derived from hematopoietic stem cells have the potential to draw cancer cells to sites in the bone or other metastatic locales, so starting the process of secondary metastatic site formation [249].

1. The involvement of MVs in escape from apoptosis: -

MV release functions as a defense mechanism for cell survival by removing intracellular stress [250]. The strictly controlled process of apoptosis, also known as programmed cell death, is essential for tissue homoeostasis and development [251]. One of the primary apoptotic executioner enzymes, caspase 3, is present in MVs separated from live cells' conditioned media but not in the releasing cells [252]. This was further supported by different research that found that transfecting cells with caspase 3 restored the inhibition of MV release in caspase-3-deficient MCF-7 cells. Numerous investigations have shown that cancer cells release MVs containing caspase-3 to stop the buildup of intracellular caspase 3 [253]. Evidence that inhibiting MV release causes caspase 3 to accumulate within cells, causing apoptosis, provided support for this theory. Thus, it is thought that the release of MVs carrying caspase-3 contributes to cell survival [254]. Numerous studies have shown a connection between MDR and MV release. Research by Shedden et al. [255] revealed that chemo insensitive cancer cell lines express more genes linked to membrane shedding than chemo sensitive cells, providing more evidence of the association between MV shedding and cancer cell survival. Furthermore, doxorubicin, a chemotherapeutic drug, was found in greater amounts in MVs that were produced from cancer cells that were resistant to chemotherapy [256]. Additionally, Safeaei et al. [257] showed that compared to micro vesicles (MVs) generated by cisplatin-sensitive cells, exosomes from cancer cells that were cisplatin-insensitive contained two to six times more of the chemotherapy medication cisplatin.

2. MVs and escape from the immune system: -

MVs are released in response to complement activation [258]. Sims et al. [259] showed that the release of C5b-9-bearing MVs prevented apoptosis in human platelets co-cultured with a sub lytic concentration of the MAC (membrane attack complex), C5b-9. Most cells are protected by MV release in response to external stimuli via a mechanism known as "complement resistance" [260]. Cancer cells use the same way to evade complement lysis. Additionally, MVs containing the complement membrane cofactor protein CD46 are excreted by cancer cells. This protein inactivates C4b and C3b, which lowers inflammation de microtumors [288]. Cancer cells increase their ability to survive by expressing FasL (Fas ligand) (CD95L), a ligand of the death receptor Fas (CD95), which induces T-cell apoptosis and weakens the adaptive immune system [261]. Moreover, MVs generated from cancer cells fuse with monocyte plasma membranes, hindering the cells' ability to differentiate into dendritic cells [262]. It has been shown that released MVs express LMP-1 (latent membrane protein-1), an immune suppressor transmembrane protein that inhibits T-cell proliferation and thereby compromises immune system function, in tumors developed in patients with Epstein-Barr virus infection [75]. Additionally, it was shown that tumor cell-derived MVs inhibited T-lymphocyte function via transforming growth factor receptor β1 (TGFβ1)-mediated mechanisms [263].

3. MVs contribute to metastasis and angiogenesis: -

Proteases like MMP (matrix metalloproteinase)-2 and MMP-9, which break down collagen and promote tumor development, are found in MVs. The potential of MVs to enhance tumor invasiveness is eliminated when MMP-2 and MMP-9 are inhibited [264]. Moreover, uPA (urokinase-type plasminogen activator), which catalyzes the transformation of plasminogen into plasmin, is present in tumor-derived MVs. Because plasmin breaks down fibrin, the extracellular matrix is also broken down, which promotes the formation of tumors [265]. Additionally, fibrin coats cancer cells to prevent them from being recognized and attacked by the immune system, and the fibrin matrix also promotes the formation of new blood vessels [266].TF, a factor that promotes thrombosis, is present in MVs from cancer patients.

The latter MVs transport and accumulate their pro-coagulant TF at the site of injury after being captured by activated platelets [267]. Moreover, these MVs have the potential to combine with platelets, resulting in coagulation and the release of growth factors [268]. Additionally, VEGF (vascular endothelial growth factor) and other growth factors are encoded by mRNA found on cancer cell-derived MVs. These vesicles fuse with monocytes to transmit their nucleic acids and stimulate the creation of growth factors [269]. MVs produced from cancer cells have been shown to modify T cell functions and exhibit immunosuppressive properties [270]. It is possible that this immune system suppression is a factor in the metastasis and dissemination of tumor cells. Because cancer cells encased in TF-bearing MVs are shielded from immune system examination, this dissemination may be hematological, resulting from activated platelets that express and carry PSGL (Pselectin glycoprotein) [271]. Additionally, TF-bearing MVs' procoagulant qualities cause fibrin to develop intravascularly, which facilitates cancer cells' adhesion to the vessel wall [272]. On the other hand, MVs generated from cancer cells help the immune system attack by transferring tumor antigens to antigen-presenting cells. MVs are also released by antigen-presenting cells, and these vesicles inhibit the development of mouse tumors [273]. Anti-cancer treatment may target MV release by obstructing the positive effects of MV release on tumor development. Serine/threonine kinases called ROCK (Rho-associated kinase) I and II influence cell migration, morphology, adhesion, and release of microvesicles [274]. It has been investigated whether dendritic cell-derived MVs may be a potential cancer treatment for advanced non-small-cell lung cancer, colorectal cancer, and metastatic melanoma [275]. Anti-cancer treatment may consider targeting inhibition of MV release as a possible target due to its association with many processes linked to tumour progression. According to a different research, B-lymphoblastoid cells treated with heat (42◦C) release exosomes with different protein compositions to regulate micro vesicles (MVs), which include large amounts of heat-shock protein [276]. Proteomic investigations of cancer-cell-derived MVs may be used to measure the effectiveness of anti-cancer medications. These MVs may also show the effects of chemotherapy and serve as an early biomarker for medication evaluation. Proteomics may also be used to bladder cancer, as shown by the finding that MVs had higher amounts of eight proteins than the control group [277]. Other indicators to take into consideration for early cancer diagnosis include cancer-specific mRNA or miRNA [278]. Moreover, MV measurement before to chemotherapeutic drug delivery may serve as a predictive indication for cancer patients' survival rate. Measuring the protein content of MVs may be a useful early indicator to evaluate the efficiency of anti-cancer treatment in examining the effectiveness of anti-cancer medications [279]. Moreover, circulating MVs reveal tumor-specific markers that may be helpful in the early diagnosis of cancer, and individuals at high cancer risk may benefit from the identification of cancer-specific mRNA and miRNA [280].

4. Drug resistance: -

In cancer treatment, drug resistance in solid tumors has been an unresolved pharmacological issue up till now. Pharmacological resistance comes in two flavors: acquired resistance, which develops during treatment, and intrinsic (inherent) resistance, which occurs when resistance to chemotherapy already exists before to beginning a pharmacological treatment program [281]. Novel medications that might target cancer cells selectively were developed to address this issue. Nevertheless, as of yet, these medications have not reduced the prevalence of drug resistance [282]. Pharmacokinetic (marked intratumorally changes in drug exposure) or pharmacodynamic (failure to evoke cytotoxicity) classifications are two alternate ways to classify drug resistance [283]. The interaction of many impacted pathways results in the development of resistance to anti-cancer medications. One of the main treatment options in oncology is chemotherapy, particularly when radiation is not an effective way to treat cancer that has spread [284]. Chemotherapy-responsive cancer cells have a variety of mechanisms that work together to confuse the cytotoxic effects of chemotherapy drugs [285]. Anti-cancer medications work by inhibiting certain phases in the DNA replication process, such as nucleoside production, DNA interaction, and cell mitosis prevention, in order to target cell proliferation [286]. Chemoresistance is the outcome of alterations in the biology of cancer cells brought about by chemotherapy. Since p53 is a short-lived protein, an endogenous inhibitor called HDM2 (human double minute 2) promotes p53 degradation. The transcription factor p53 is an essential regulator of the cell stress response, which is activated by a broad range of stimuli such as DNA damage, hypoxia, loss of cell–cell contact, and inappropriate oncogene activation [287]. When a cell is under stress, p53 stabilizes, which triggers p53 post-translational modification and its interaction with cooperating factors and DNA [288].

5. Association of p53 in cancer drug resistance: -

On chromosome 17, the p53 gene is a tumor-suppressor gene [289]. The protein p53 attaches to DNA inside the cell, causing another gene to become active and generate the protein p21, which then interacts with CDK2 (cyclin-dependent kinase 2), a protein that accelerates cell division. A cell that has complexed with CDK2 is unable to proceed to the subsequent phase of cell division. As a consequence, a series of actions are set in motion that lessen the impact of harm by up-regulating a number of genes, including p21, which controls the course of the cell cycle during the G1 phase and causes the cell cycle to be suspended. Cell viability and survival are facilitated by cell-cycle suspension, which permits DNA and cellular damage repair [290], [291]. In the event that DNA damage cannot be repaired, p53 triggers the production of apoptosis inducers, such as Fas and Bax, to prevent damaged DNA from being replicated in daughter cells. The crucial function of p53 as a tumor-suppressor gene in cell biology is shown by its functional duality [292]. This important role is demonstrated by the fact that about 53% of cancers have p53 mutations. These high percentages of p53 mutations impair p53's ability to control the cell cycle and induce apoptosis by upregulating pro-apoptotic genes like FAS (CD95/Apo-1) [197], NOXA (NADPH oxidase activator), BAX, and TRAIL-R2 (tumor necrosis-factor-related apoptosis-inducing ligand receptor 2) (DR5). As a result, it is anticipated that p53 will play a significant role in determining how well genotoxic chemotherapy drugs work to treat cancer [293]. Loss of p53 activity has been shown to give resistance to a variety of anti-cancer medications, including 5-FU (5-fluorouracil), in colorectal cancer by preventing the start of apoptosis after chemotherapeutic treatment [294]. Consequently, the possibility that gene therapy might boost the effectiveness of chemotherapy is being researched. Still, a number of studies have been unable to establish a connection between p53 mutations and resistance to anti-cancer drugs [295]. Furthermore, the manner that various tissues employ their repair pathways varies, making it extremely harder to anticipate how well chemotherapeutic drugs would work [296]. A number of processes contribute to the development of drug resistance, such as drug inactivation, processing of drug-induced damage, evasion of apoptosis, increased drug efflux and reduced drug inflow [297]. Several studies have shown that in cells treated with 5-FU, defective p53 leads to treatment resistance (due to an incapacity to undergo apoptosis, the primary target of chemotherapy medicines).

10.3 Table 4: -

Components		Types of cancer	Original Sources	Role	Processes
miRNA	microRNA -9	Gliomas	Glioma's cell	Metastasis	Angiogenesis
	microRNA -26a		stem cells from gliomas		
	microRNA -29a		stem cells from gliomas	suppression of the immune system	stimulate the myeloid-derived suppressor cells to differentiate
	microRNA -92a				
	microRNA -21	Oral cancer of squamous cells	carcinoma cell with oral squamous cells		inhibit T cells
	microRNA -128-3p	colon cancer	A cell from colorectal cancer	Resistance to drugs	preventing the transition between epithelium and mesenchymal cells
	microRNA -25-3p			Metastasis	Angiogenesis
	microRNA -92a-3p		Fibroblasts linked to cancer	resistance to chemotherapy	Mesenchymal-epithelial transition and the Wnt/beta-catenin signalling pathway
	microRNA -151a	Glioblastoma versatile	The multiforme glioblastoma cell		The route reliant on loss of miR-151a
	microRNA -223	carcinoma of the ovary epithelium	cancer-related macrophage		PI3K/AKT pathway-MiR-223/PTEN
	microRNA -155	Apical ductal carcinoma in humans	Macrophages linked to tumors	Resistance to drugs	inhibiting apoptosis
	microRNA -196a	Cancer of the head and neck	Fibroblasts linked to cancer		Connecting CDKN1B and ING5
	microRNA -501	stomach cancer	stomach cancerous cell		inhibition of caspase-9/-3, activation of Akt, and suppression of BLID
	microRNA -221		generated mesenchymal stem cells from bone marrow		Encourage matrix adherence and the spread of metastatic lesions
	microRNA -21	Cancer of the ovaries	Fibroblasts linked to cancer		binding the target APAF1 and inhibiting ovarian cancer apoptosis while fostering medication tolerance

	microRNA -142-3p	Cancer of the colon	Bone marrow-derived mesenchymal stem cell	Metastasis	Increasing the stemness of stromal cells
	microRNA -301a-3p	tumor of the pancreas	Pancreatic tumor cell	Metastasis	Set off the M2 polarization of EMT and macrophages.
	microRNA -193a-3p	Lung cancer	Bone marrow-derived mesenchymal stem cell	Metastasis	Signaling via STAT3 and the epithelial-mesenchymal transition
	microRNA -210-3p				
	microRNA -5100				
	microRNA -23a		Lung cancer cell		Angiogenesis
	microRNA -27b-3p	Multiple myeloma	Cancer-associated fibroblast		modifying the microenvironment of the bone marrow
	microRNA -105	Breast cancer	Breast cancer cell		Reprogramming of metabolism and modification of molecular properties
	microRNA -126				
	microRNA -122	Gastric cancer	Gastric cancer cell		reducing the absorption of glucose by niche cells
	microRNA -130a				Angiogenesis
	microRNA -103	Hepatocellular carcinoma	Hepatocellular carcinoma cell		Reduce the integrity of the endothelium junction
	microRNA -21				Activate fibroblasts linked to malignancy to produce angiogenic cytokines
	microRNA -23a			suppression of the immune system	Suppress T function
	microRNA -23a	Nasopharyngeal carcinoma	Nasopharyngeal carcinoma cell	Metastasis	Angiogenesis
	microRNA -19b-3p	Colorectal cancer			Mesenchymal-epithelial transition
Long noncoding RNA	SBF2 AS1	The glioblastoma	Cells of glioblastoma	Resistance to drugs	restoring the tumor microenvironment via reconstruction

	LNCARSR gene	Cancer of the renal cells	Cells of renal cell carcinoma		raising c-MET and AXL expression
	Long non-coding RNAs -UCA1	Cancer of the bladder	Cells associated with bladder cancer	Metastasis	altering the tumor microenvironment
	Long non-coding RNAs APC1	colon cancer	A cell from colorectal cancer	Antitumor	Prevent angiogenesis
	Long non-coding RNAs 19		linked to cancer	Resistance to Chemoresistance	
			fibroblast		
circRNA	circular PTGR1	Cancer of the liver	cell of hepatocellular carcinoma	Metastasis	
	circular NRIP1	stomach cancer	stomach cancerous cell		AKT/mTOR axis-related energy metabolism is activated when miR-149-5p expression is blocked.
Additional non-coding RNA	Y RNA hY4	Chronic leukemia of lymphocytes	Cells with persistent lymphocytic leukemia	suppression of the immune system	Cytokines should be released, and PDL-1 should be activated.
	PSMA3	stem cells found in mesenchymal tissue	several myelomas	medicine resistance	Transform multiple myeloma cells' resistance to proteasome inhibitors
Protein	ANXA6	cancer of the breast	breast cancerous cell		Increase the number of Ly6C (+) CCR2(+) monocytes and induce Ccl2 in the lung premetastatic niche.
	Death-programmed ligand-1	GBM.	GBM. cell	suppression of the immune system	impede the activation of T cells
	70 heat shock protein	Maternal cancer	cancerous cell		Make the myeloid-derived suppressor cell active.

10.4 CLINICAL APPLICATIONS OF EXOSOMES: -

Exosomes may be used as therapeutic targets and diagnostic indicators because they carry certain bioactive chemicals that are important at different stages of cancer development. This helps explain how exosomes contribute to the pathophysiological development of malignancies. A condition known as medication resistance linked to exosomes is starting to show up in different kinds of cancer. Thus, understanding the processes behind exosome-mediated therapeutic resistance to cancer might provide important insights for precision cancer treatments. Furthermore, since the makeup of their membranes is comparable to that of the majority of body cells, since they do not trigger an immune response, exosomes have been used in anticancer medication delivery systems.

1. Tumor exosomes as potential biomarkers: -

Over time, cancer is always changing due to mutations and selection processes that intensify the disease's propensity to spread. Exosomal payloads have a striking similarity within the cell state the initial released cell after being separated from their parent cells. Therefore, in terms of diagnosis, prognosis, and illness monitoring, real-time detection of alterations within exosomal payloads might provide insightful information for the essential requirement of precision medicine. For instance, early cancer identification raises the likelihood of a good treatment result and increases the survival rate; for this reason, the necessity for sensitive and accurate biomarkers cannot be overstated. Finding tumor markers in liquid biopsies has many advantages over tissue biopsies, including being less invasive, simple to collect, quick, and affordable. Furthermore, the abundance of dynamic data that can be easily obtained from a patient is helpful in identifying biomarkers for early cancer diagnosis in such individuals. Specifically, exosomes based on lipids provide a more resilient and concentrated transport system for delicate biological components found in bloodstream fluids including serum, plasma, urine, and saliva. Under standard storage settings, the biological components in exosomes produced from blood plasma are very stable (lasting more than 90 days).[298] Furthermore, it is often shown that people with illness have noticeably increased levels of exosomes in their bodily fluids. It should be mentioned that while circulating tumor cells (CTC) and cell-free DNAs may be quickly and easily evaluated in liquid biopsies, their qualities in relation to the initiation and spread of cancer are debatable and restricted when compared to tumor exosomes. It has been found that the homologous EGFR, or epidermal growth factor receptor, has distinctive mutations carried by cfDNAs.primary tumors, but increased circulating tumor DNAs clearing mechanisms are often seen in the kidney or liver, suggesting a lack of knowledge about the toxicity and stability of cfDNAs.[299] It is quite uncommon to find circulating tumor cells in peripheral blood. Therefore, it is rather challenging to separate and identify these uncommon circulating tumor cells from all other hematological and immunological cells, and there is an urgent need for an efficient separation and detection strategy.[300] It has been discovered that certain integrin expression patterns of exosomes produced from tumors may serve as "ZIP codes" to guide organotrophic spread. This is the first bioactive chemical that can forecast the metastases of cancer to particular organs, and it links exosomal integrins to the possibility for organotrophic metastatic spread. Similar to this, a particular exosome signature that includes MET, HSP90 isoform, heat shock protein 70 (HSP70), very late antigen 4 (VLA-4), tyrosinase-related protein 2 (TYRP2), and HSP70 was found being highly stated when melanoma is in its advanced stages. and can be used as a substitute for liquid biopsies in the diagnosis of aggressive melanoma.[301] Exosomes generated from tumor-derived exosomes are a source of biomarkers indicating the condition of parental cancer cells, as clinically shown by the presence of particular EGFR VIII in exosomes taken from serum samples of Glioblastoma patients. Furthermore, exosome gene expressions in urine have been shown to be associated with patients who have high-grade prostate cancer, providing further evidence for the benefits of exosomes in cancer detection and prognosis However, in exosomes taken from patients who have prostate cancer that has spread, the production of androgen receptor splice type 7 RNA is a reliable marker of the start of resistance to endocrine therapy [302].

2. Tumor exosomes as therapeutic targets: -

The development of cancer is linked to the cargo of exosomes generated from tumors. Therefore, different therapeutic approaches have been suggested as potential cancer therapies. These include blocking exosome formation, secretion, and exosome-mediated cell-cell communication, as well as ablation of certain active exosomal cargos. In fact, blocking ESCRT-dependent mechanisms that drive exosomes biogeny, like sphingomyelinases11 or ALIX signaling60 or syndexcan, has been demonstrated to have a negative impact on the generation of exosomes and the advancement of cancer. The size and manner in which multivesicular endosomes attach to the plasma membrane are regulated by Rab27, a small GTPase, another crucial protein involved in exosome secretion. The growth and metastasis of cancer were decreased after inhibiting Rab27a. It has been shown that by concentrating on the process of exosome uptake via endocytosis or micropinocytosis, chlorpromazine, a blocker of clathrin-mediated endocytosis, decreases cancer malignancy in vitro. 309 Additionally, the regulation of exosome absorption by recipient cells is dependent on specific glycosylation sequences on the surface peptides of exosomes generated from tumors. Based on this finding, it seems that modifications to the glycosylation of exosomal peptides might play a major role in driving cancerous growth. Lately, For the extra-medullary hemofiltration of exosomes from circulation as a possible cancer therapeutic method, a specific plasmapheresis platform has been proposed. [310] The implication is that therapeutic reagents have an additional way to prevent oncogenic signals in malignancies by eliminating exosomes through the circulatory system. All together, these findings suggest that one possible route to the development of treatments for cancer may be to obstruct the production and discharge of exosomes derived from malignancies.

3. The drug delivery functions of exosomes: -

Natural exosome vesicles, like liposomes, have drawn a lot of interest as drug delivery systems. To start with, exosomes with a nanometric size have an elevated level of transferability across cells. Moreover, bioactive compounds are protected from degradation by outside factors by the protective bilayer of lipid architecture in exosomes. In addition, exosomes have reduced immunogenicity and noxious, in contrast to other drug delivery methods. [311] Lastly, exosomes that have certain proteins on the outer layer, like integrins, have the capacity to target certain organs with precision. These characteristics of exosomes suggest that they might be useful drug-delivery vehicles for proteins, siRNAs, or chemotherapy medications. Interestingly, exosomes have fatal impacts in Danio-Rerio brain cancers by transferring anticancer medications across the blood-brain barrier. Parental cancer cells may endocytose exosomes containing anticancer drugs produced by autologous cancers, which causes the parental cancer cells to become more cytotoxic. Targeting specificity was achieved by fusing αv integrin-specific RGD (Arg-Gly-Asp) peptide on exosomes packed with anticancer medication (doxorubicin), which greatly enhanced exosome absorption by αv integrin-positive cancer cells and suppressed the growth of malignancy. Exosomes are novel cell-free vaccinations that are intrinsic to immunotherapy. [312] In patients with advanced non-small-cell lung cancer, cancer-related antigens integrated inside exosomes originating from the dendritic cells of autologous stimulate immunological responses against cancer. Exosomes derived from interferon-γ-mature dendritic cells were used in subsequent research to hasten NK and T cell antitumor immune responses. A higher percentage of survival without progression and a growth in the activity of NK cells were seen in patients with advanced non-small-cell lung cancer.
[313] All of these studies demonstrated that exosomes function as possible drug delivery systems or cell-free vaccinations in anticancer therapies.

4. Exosomes in cancer therapy: -

Because exosomes are non-toxic and non-immunogenic, they may be a useful medication delivery method in cancer treatment. Adriamycin and paclitaxel have been delivered by exosomes for targeted cancer treatment, with low immunogenicity and toxicity seen [314, 315]. Furthermore, a wide variety of cell types are capable of producing exosomes. Furthermore, exosomes have a greater incidence of tumor cell penetration than liposomes. Exosomes also have the ability to target certain cells and tissues by means of particular proteins; as a result, they may be used to deliver medications intended for cancer cells. Exosomes may also readily cross a variety of barriers, including the blood-brain barrier, because of their tiny size [316]. Furthermore, exosomes are readily manipulable to improve their ability to target cancer cells [317]. Tumor-derived exosomal RNA from blood and other body fluids may be employed as biomarkers in cancer screening and diagnosis, according to mounting evidence [318]. Bioengineered exosomes have been utilized to specifically target cancer cells, such as CSCs, with anti-cancer medications and functional RNAs. Exosome-based CSC targeting is one of the most promising methods for creating cancer treatments. The donor cells are genetically altered to manufacture certain proteins on the exosome membrane in order to make modified exosomes. Exosomes have the ability to target certain CSC producers, including as CD44, CD24, CD133, and CD200 [319]. Since dendritic cells (DCs) are important for the main and secondary immune responses, exosomes generated from DCs may be used as a targeted cancer treatment. Exosomes generated from astrocytes deliver miRNAs that target PTEN to tumor cells that have spread elsewhere, suppressing PTEN expression. CCL2 release increases the recruitment of IBA1-expressing myeloid cells and the seeding of metastases when PTEN is lost in invasive tumor cells. Furthermore, GE11 peptide-expressing exosomes have been engineered into human embryonic kidney cells. The Epidermal Growth Factor Receptor (EGFR), which is expressed by a large number of tumor cells of epithelial origin, may be bound by these exosomes. Furthermore, these exosomes have the ability to transfer certain non-coding RNAs, such miRNA let7a, to the intended cells. Exosomes loaded with chemotherapeutic drugs have recently been shown to be a revolutionary technique in cancer treatment. The anti-tumor effects of these medications have been enhanced by the exosomes. Exosomes laden with paclitaxel are utilized to treat a variety of malignancies, such as pancreatic, lung, and prostate cancer. Furthermore, in a mouse model of colon cancer, exosomes laden with doxorubicin caused the tumor's size to decrease. Furthermore, doxorubicin-containing exosomes demonstrated effective anti-tumor effects in the animal model of inflammatory cancers. Exosomes laden with doxorubicin also shown a high degree of efficacy in destroying breast cancer cells. To get the naïve T cells to fight the tumor cells, exosomes produced by DCs might be

employed to deliver tumor antigens. To produce the tumor antigen human mucin1 (hMUC1), exosomes generated from two mouse cell lines that differ in terms of MHC types were used. It has been shown that these exosomes efficiently stimulate the immune system [320] (Fig. 3). Exosomes may be useful as delivery systems for precise and targeted delivery of functional proteins to cancer cells. One protein that causes cancer cells to undergo apoptosis is called Tumor Necrosis Factor (TNF)-related Apoptosis-inducing Ligand (TRAIL). The TRAIL-secreting exosomes shown remarkable effectiveness in causing tumor cells to undergo apoptosis, which in turn led to a decrease in the size of the tumor [321]. Many different kinds of malignancies have high expression levels of the anti-apoptotic protein surviving. Surviving's normal function is compromised by an inactivating mutation (T34A). Pancreatic cancer cells undergo apoptosis when exosomes carrying this particular mutant Surviving are transferred [322] (Fig. 3). Additionally, therapeutic RNAs like miRNAs and siRNAs have been delivered to cancer cells via exosomes. In hepatocellular carcinoma, miRNA-122 increases chemosensitivity. The delivery of miR-122 enhanced chemosensitivity of the HCC cells occurs via exosomes produced from adipose mesenchymal stem cells. Exosomes were used to transfer siRNA against RAD51 to Hela and HT1080 cells, which caused the receiving cancer cells to undergo a significant amount of cell death [323]. Exosomes generated from HEK293 and MSC were used to deliver siRNA targeting PKL-1 in bladder cancer cells, resulting in decreased expression of polo-like kinase 1 (PLK-1) (Fig. 3).

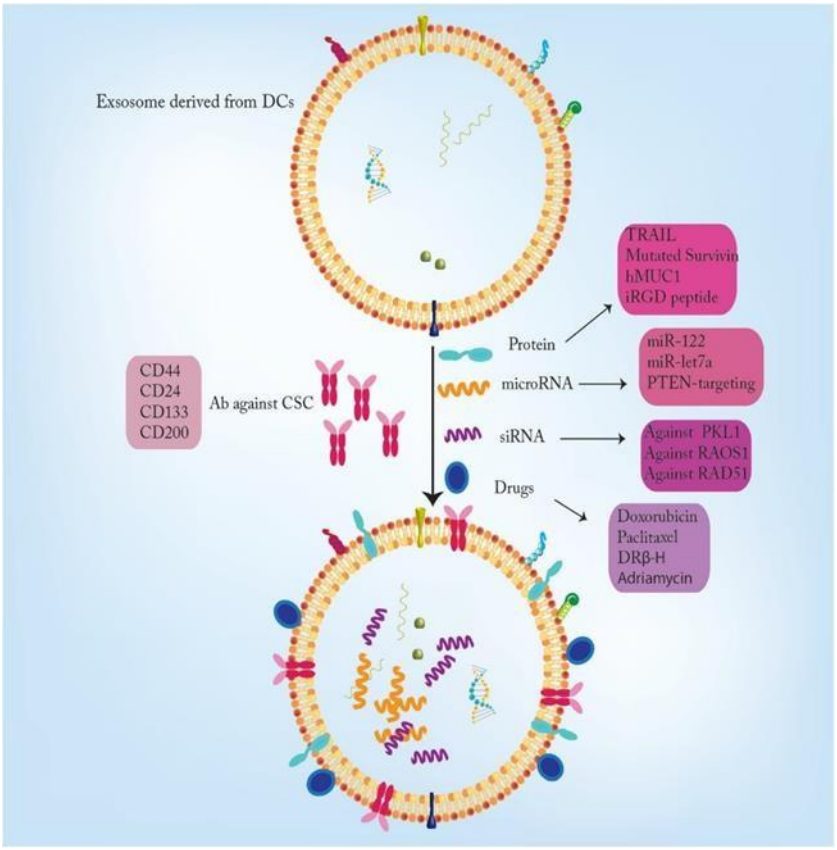

Fig. 3 Application of exosomes in cancer treatment. DC-derived exosomes may be used to transfer functional RNAs like siRNA and microRNA as well as anti-cancer medications like chemotherapeutic medicines. Peptides may also be administered to the intended cancer cells, particularly CSCs.

10.5. CLINICAL APPLICATIONS OF MICROVESICLES: -

1. **Cancer Vaccines: -** The principle behind tumor vaccines is to boost T cells' antitumor immune system by exposing them to tumor antigens from MVs. Antigens may be delivered by TMVs since they are capable of carrying a range of immunogenic compounds, like polysaccharides, proteins, and nucleic acids. TMVs have the potential to specifically target cancer cells. Originally, the EV immunizations were intended to guard against pathogenic conditions such as Bordetella pertussis, Tuberculosis and diphtheria. [324] Later, scientists learned that EV vaccines may be altered for use in the management of tumors. According to research, the antigens are transferred to DCs by the MVs produced by Listeria monocytogenes-infected macrophages, which activates defensive T lymphocyte and thymocyte immunity. Dong et al. suggest, it may be possible for ileal epithelial cells to absorb an oral TMV-based anti-cancer vaccination. One possible explanation is that TMVs activate nucleotide-binding oligomerization domain two and downstream MAPK and NF-κB, which in turn induce IECs to create chemokines that attract CD103+ and CD11c+ DCs. Alternatively, IECs may transport them to the basolateral area, where DCs may extract the TMV's cross-presenting antigen. Hence, antigen may be delivered to gut mucosal DCs via TMVs. To induce the discharge of antigen fragments, UV light was administered to tumor cells containing DNA and TMVs. Once TMV DNA was taken up by DCs, it triggered the cGAS/STING route, which in turn resulted in the production of IFN-I. MHCII and CD86 were all upregulated by IFN-I, which in turn encouraged DC maturation. Following the "armed" DCs' activation of tumor-specific T lymphocytes, the tumor cells were lysed. Zhang et al. [194] proposed that TMVs would make ideal candidates for creative and effective tumor vaccine development. By applying TMVs generated from C6 glioma cells, both irradiation and non-irradiated, Pineda et al. [325] immunized rats against glioblastoma. The group that received irradiation TMV treatment (IR-TMVs) discharged more TMVs carrying the specific antigen than the control group, which received non-irradiation TMV inoculation. Furthermore, by promoting effector T cell development, the IR-TMVs enabled tumor-invading cells to activate the immune system and eliminate tumor cells. Scientists have used TMVs from mouse prostate cancer cells to create an oral cancer vaccine, and when paired with cyclophosphamide and granulocyte-macrophage colony stimulating factor, they assessed the immunization's anti-tumor effectiveness in vivo. Combining the vaccination with two medications resulted in a substantial decrease in Tregs in vivo, suggesting a potent anti-tumor impact. TMV vaccination for tumor-bearing mice was administered by Pack et al. using TMVs from 4T1 tumor cells combined with the immunostimulatory protein B7-1, either in isolation or in conjunction with a monoclonal antibody against the anti-cytotoxic T-lymphocyte-associated protein 4. Both lung metastases and mouse survival were improved by the combined therapy. Moreover, it was shown that the vaccinations' mechanism of action was CD8+ T cell immunity that was specific to tumors.

2. Biomarker: - TMVs isolated from the body fluids of cancer patients have the potential to provide diagnostic information due to their stability [326]. According to Hou et al. [327], pyruvate kinase M2 was discovered in the plasma of HCC patients. According to this research, PKM2 in TMVs may be applied as a possible HCC diagnostic indicator. ARF activation and tumor development were shown to be directly correlated by Muralidharan-Char et al., raising the possibility that one indication of an illness is ARF. An experimental investigation by Smalley et al. [328] eight cancer-related proteins were found in TMVs. These included retinoic acid-induced protein 3, resisting, five proteins linked to the EGFR pathway, and the α-subunit of the GsGTP-binding protein. Therefore, the protein profile of these TMVs may be advantageous for earliest bladder cancer identification. Breast cancer has high HER-2/neu levels along with other associated carcinomas, such as cancer of the stomach and ovarian cancer. 1,848–1,149 plasma samples from patients with gastric cancer expressed higher amounts of HER-2 and c-MET than did control samples, according to Baran et al. The prospect that these particular proteins could be utilized as pathological indicators is increased by this discovery. Zhong et al. introduced EMMPRIN as a unique possibility cancer indicator that may be applied to adjust treatment plans and assess the prognosis of individuals with early-stage malignant cancers, thereby improving the standard of living for patients with cancer. The cancer-causing gene c-Myc is amplified in TMVs, recommending that the genetic code inside them might be an asset for cancer biomarkers (Balaj et al., 2015). TMVs that were isolated and examined from the saliva of lung cancer patients were found to contain the proteins Ras GTPase-activating-like protein, MUC5B, and BPI fold-containing family A member 1, which play a role in the progression of pulmonary fibrosis, the innate immune response, and the growth of tumor cells and transformation, respectively. Sun et al. Lung cancer may be non-invasively detected using these indicators. A worse survival rate and unfavorable clinicopathological features are linked to elevated levels of saliva MVs (SMVs) in patients with squamous cell carcinoma of the oral cavity (OSCC). According to Zhong et al.'s research, SMVs from OSCC may serve as possible indicators of the onset of malignancy.

3. Other Clinical Applications: -

To alter the tumor immunosuppression microenvironment, tumor repopulating cell-like stem cells (TRCs) are necessary. TMVs carrying anti-tumor drugs have been shown by Ma et al. to potentially overcome medicine resistance in Stem-cell-like cancer cells, or TRCs. TRC mortality resulted from TRCs' superior absorption of TMVs containing anteroposterior medications and their greater flexibility as compared to established cancer cells. In order to effectively and without the typical side effects eliminate tumor cells in mouse models, Tang et al. [329] filled TMVs with chemotherapy medicines so they could be retrieved and used. It has been shown via research that TMVs are highly accepted in medical environments and possess objective clinical effectiveness in treating lung cancer patients. Numerous therapeutic agents, including antigens, nucleic acids, antibodies, and oncolytic adenovirus, have been transported to the targeted region in order to treat related tumors. Once MVs made from autologous tumor cells and therapeutic medicines were shown to be safe and well-tolerated, they were authorized as a novel kind of gene therapy to treat cancerous tumors. TMV was a potent transport system that might transfer oncolytic adenovirus into neoplasm and perform highly potent osmotic lysis, according to Ran et al. (2017). This oncolytic adenovirus delivery method utilizing TMVs has several advantages. Initially, it had no antiviral effects on the host. Secondly, it was very effective as the virus could enter tumor cells without being impeded by the need for a receptor particular to the virus. Lastly, oncolytic adenovirus may be delivered by TMVs to the stem-like TRCs and tumor cell nuclei. The possibility of using MVs made from prostate cancer cells as paclitaxel carriers was addressed by Saari et al. providing medications with a greater cytotoxic impact to the target cells by endocytosis. According to Krishnan et al. [330] the morphologies and amounts of CD138+/CD41a-derived myeloid leukemia cells (MVs) may serve as markers of the disease status and prognostic value of treatment for individuals with multiple myeloma.

According to their research, TMV has the potential to be used as an innovative prognostic biomarker for

tracking cancerous cells. A membrane biotinylating technique supported by donor cells was presented by Chen et al. [331] as a means of labeling TMVs with quantum dots that is biocompatible. Short interfering RNAs were added, transforming the TMVs into functionalized carriers that functioned as intercellular mediators for combined tumor-targeted therapy and bioimaging. Their research may have revolutionized the sector by offering a new concept for the synthesis of nanocarriers and revolutionizing the analysis and use of TMVs.

10.6 Discussion: -

Exosomes and micro vesicles have been linked to the advancement of cancer due to their capacity to transport bioactive substances, including proteins, lipids, and nucleic acids, among cells. Numerous facets of cancer biology, such as angiogenesis, migration, invasion, proliferation, and immune evasion, may be modulated by these substances. Furthermore, it has been shown that both kinds of EVs contribute to the formation of the pre-metastatic niche and the promotion of metastasis by getting far-off locations ready for dispersed tumor cells to colonize. Even while exosomes and micro vesicles have some functional similarities, new research indicates that they could possibly play different functions in cancer. Research has shown that there may be differences in the cargo content of exosomes and micro vesicles, with certain chemicals being bundled into one form of EV more than the other. Furthermore, there may be differences in the absorption methods and downstream signaling pathways that exosomes and micro vesicles activate, which might have differing impacts on recipient cells. Furthermore, a number of variables, including as tumor stage, grade, and microenvironmental circumstances, might affect the quantity and make-up of exosomes and micro vesicles in the tumor microenvironment. Understanding the relationship between exosomes and micro vesicles in cancer may be clinically relevant, as shown by the associations between changes in the levels of certain EV populations and the prognosis and course of different cancer types. Overall, exosomes and micro vesicles have unique qualities and roles that call for further research, even though they also have some functional commonality in cancer. Understanding the intricate interactions between these two EV types and how they contribute to the development of cancer may help researchers create new diagnostic and treatment approaches that focus on EV-mediated intercellular communication in the disease.

CHAPTER SIX
CONCLUSION

11. Conclusion

Finally, the link between exosomes and micro vesicles in cancer highlights their complicated role in numerous facets of tumor biology and development. While both kinds of extracellular vesicles have comparable biogenesis, secretion, and cargo composition, they also have unique properties and roles that impact cancer growth and metastasis.
Exosomes and micro vesicles, which transport bioactive chemicals between cancer cells and the tumor microenvironment, have been shown in studies to have important roles in driving tumor growth, invasion, angiogenesis, metastasis, and therapeutic resistance. However, growing data indicates that they may have diverse impacts on recipient cells due to changes in cargo content, absorption methods, and downstream signaling cascades. Understanding the relationship between exosomes and micro vesicles in cancer has important implications for both research and therapeutic treatment. It provides prospects for biomarker discovery, allowing the development of new diagnostic and prognostic markers for cancer detection, patient stratification, and therapy response prediction. Furthermore, understanding the functions of these extracellular vesicles in cancer growth sheds light on possible treatment targets for the development of more effective anticancer methods. Moving ahead, further study is required to understand the complicated connection between exosomes, micro vesicles, and cancer biology. This involves looking into the variety of tumor-derived EV populations, understanding their interactions within the tumor microenvironment, and examining their potential as diagnostic and therapeutic tools in cancer. Overall, the link between exosomes and micro vesicles in cancer demonstrates their complex functions in tumor growth and their potential as crucial actors in cancer detection, prognosis, and therapy. By improving our knowledge of these extracellular vesicles, we may develop better cancer diagnosis, treatment, and patient outcomes.

CHAPTER SEVEN REFERENCE