Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Review

CircRNAs in extracellular vesicles associated with triple-negative breast cancer

Ashraf Ahmed Qurtam*

Department of Biology, College of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh-11623, Saudi Arabia

Article Info

Article history:

Received: August 22, 2024
Accepted: November 22, 2024
Published: December 31, 2024

Use your device to scan and read the article online

Abstract

Triple-negative breast cancer (TNBC) is a highly aggressive cancer with distant metastasis. Accumulated evidence has demonstrated that exosomes are involved in TNBC metastasis. Elucidating the mechanism underlying TNBC metastasis has important clinical significance. Extracellular vesicles (EVs) present a promising avenue for diagnosing and treating triple-negative breast cancer (TNBC) through a technique called "liquid biopsy," offering a new wellspring of biomarkers. These tiny lipid bilayer vesicles, released by most cells, carry a diverse array of RNA molecules that can influence the behaviour of recipient cells. Among these, circular RNAs (circRNAs) have emerged as a subtype of noncoding RNAs capable of modulating gene expression by sponging microRNAs, thus playing crucial roles in various aspects of cancer development and progression, including TNBC. Despite their significance, our understanding of circRNAs involvement in TNBC remains incomplete. However, studies have shown that circRNAs are abundant in EVs, with exosomal circRNAs (exo-circRNAs) particularly influential in cancer biology. These exo-circRNAs can be taken up by neighboring or distant cells, impacting numerous aspects of their physiological states, thereby enhancing cell communication and tumor dissemination. This review provides an overview of EVs key characteristics and functions before delving into exo-circRNAs potential roles in driving or suppressing TNBC, as well as their implications for cancer diagnosis, prognosis, and monitoring.

Keywords: Extracellular vesicles, Breast cancer, circular RNA, Extracellular vesicles (EVs), Cancer diagnosis

1. Introduction

Extracellular vesicles are released by nearly all cells containing plasma and body fluids as part of their normal functioning, holding significant diagnostic and therapeutic potential for numerous diseases. The study of these vesicles enables a more precise understanding of intracellular communication between cells. There are two main types of extracellular vesicles: ectosomes and exosomes [1]. Ectosomes, ranging from 0.50 to 1 um in diameter, form directly through a budding process from the plasma membrane, while exosomes, ranging from 30 to 150 nm, originate from endosomes and are released into body fluids such as blood, saliva, breast milk, and urine [2]. Additionally, apoptotic bodies resulting from apoptosis are also considered extracellular vesicles, adding to their diversity.

The discovery of exosomes represents a revolutionary contribution to cell biology. Exosomes are enveloped by a lipid bilayer, providing stability to their contents and preventing easy destruction by RNases. They play pivotal roles in cell-to-cell communication and interaction with other cells through various mechanisms, including lectin, lipid, and integrins interactions. Moreover, exosomes are involved in immune system functions, viral replication, regulation of pathophysiological processes, and the tumor environment [3-5]. For example, tumor-derived exosomes (TDEs) contribute to the development of pre-metastatic niches [6].

Given their diverse functions, exosomes can serve as vectors for drug delivery into tissues. For instance, exosomes released by tumor cells facilitate communication with surrounding cells and can indicate cancer presence as a biomarker. Thus, the presence of exosomes holds promise for both diagnostic and therapeutic applications in various diseases.

2. Exosome biogenesis and release

The biogenesis of exosomes involves a budding process where double invagination of the plasma membrane occurs, forming a multivesicular body (MVB). This is followed by the fusion of the MVB with the plasma membrane, leading to the expulsion of exosomes in the form of intraluminal vesicles (ILVs) via exocytosis [7]. MVBs can also be degraded through lysosomal fusion. In a direct pathway, T cells and erythroleukemia cell lines release exosomes directly from the plasma membrane [8]. For the delivery of exosomal contents to recipient cells, fusion with the plasma membrane and endocytosis are involved. The exchange of exosomal contents between cells facilitates homeostasis and helps combat stress. Intracellular signaling relies on the interaction between exosome surface proteins and receptors present in the recipient cell (Figure 1). The number of exosomes secreted varies according to

* Corresponding author.
E-mail address: aaqurtam@imamu.edu.sa (A. A. Qurtam).
Doi: http://dx.doi.org/10.14715/cmb/2024.70.12.14

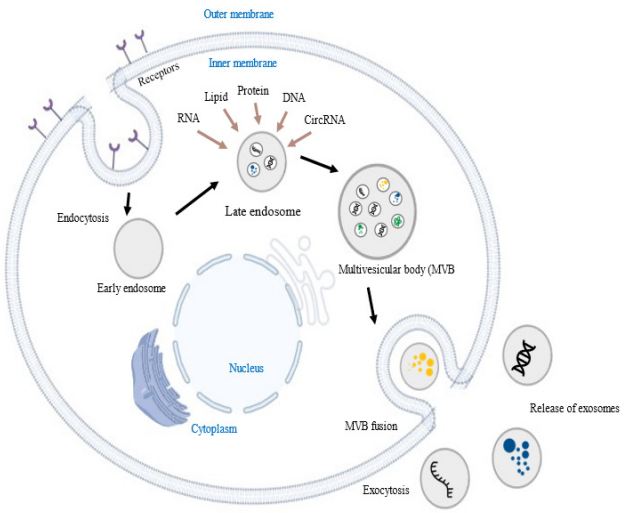

Fig. 1. Exosome biogenesis and release.

cell type; for instance, cancerous cells release more exosomes than normal cells, with breast cancer cells releasing approximately 65 exosomes per hour [9].

Various technologies, such as dynamic light scattering, atomic force microscopy, Raman spectroscopy, transmission electron microscopy, and nanoparticle tracking analysis, allow for the measurement of exosomes [10], providing information about their size, concentration, and phenotypic characteristics [11-13]. Exosome isolation methods include centrifugation, ultrafiltration, chromatographic techniques, and microfluidics for high-purity extraction [14-15].

Exosomes contain diverse cargos, including lipids, DNA, miRNA, mRNA, proteins (membrane, nuclear, cytosolic), metabolites, and circRNAs [16]. The abundance and packaging of these components are regulated selectively, leading to heterogeneity in exosomal composition. This heterogeneity extends to exosome size, which can result from irregularities in the formation process via plasma membrane invagination [17]. Furthermore, exosomes can have a heterogeneous origin. Functionally, exosomes can induce both apoptosis and cell survival.

Cargo sorting within exosomes can involve ESCRT (endosomal sorting complex required for transport) pathways which comprise a multiprotein machinery that includes both ESCRT-dependent and ESCRT-independent mechanisms [18].

2.1. ESCRT dependent pathway

The ESCRT (Endosomal Sorting Complex Required for Transport) complex plays a crucial role in cargo sorting within cells. This complex consists of class E vacuolar protein sorting (Vps) components, which include four subcomplexes: ESCRT-0, I, II, and III, working in a coordinated cascade [19]. Mono- or poly-ubiquitinylated proteins are recognized by STAM and Hrs within the ESCRT-0 subcomplex. The FYVE domain aids in cargo sorting through the clathrin vesicle machinery. While the ubiquitin-binding domain (UBD) is present in both ESCRT-I and ESCRT-II, it is absent in ESCRT-III [20].

ESCRT-I and ESCRT-II combine to form a saddle-shaped complex, which then recruits ESCRT-III, ultimately leading to the production of intraluminal vesicles (ILVs) through polymerization [21]. In many instances, ubiquitin is removed from cargo proteins by enzymes.

Additionally, alternative pathways involving ESCRT-III have been observed. These pathways can provide two auxiliary mechanisms: the ALIX-dependent pathway and the HD-PTP-dependent pathway [22]. These alternative routes offer further insight into the complex mechanisms of cargo sorting within cells.

2.2. ESCRT independent pathway

This pathway operates independently of both ESCRT and ubiquitin. Membrane lipid rafts play pivotal roles in this process, with cholesterol, ceramide, and sphingolipids, along with proteins like tetraspanins, caveolins, and flotillins, contributing significantly [23,24]. Tetraspanins, for instance, are associated with both transporting cargos to multivesicular bodies (MVBs) and compartmentalizing endosomal membranes [25]. Among tetraspanins, CD63 has garnered particular importance in tumor signaling.

Caveolins, characterized by their hairpin structure, are membrane proteins that bind cholesterol [26]. Through the formation of caveolae, caveolins mediate endocytosis processes. Flotillin proteins are also crucial in this pathway, being involved in protein sorting processes [27].

Together, these lipid rafts and associated proteins play essential roles in a pathway that operates independently of ESCRT and ubiquitin, contributing to various cellular functions and signaling mechanisms [27’28].

2.3. Proteins

The protein content inside exosomes varies between different cell types, but there are common sets of proteins consistently found among them. Many of these proteins are sorted through both the ESCRT pathways, while some are selectively sorted. Tetraspanins, notably CD81, CD63, CD82, Tsg101 (associated with ESCRT), Alix-1 (associated with MVB), and various heat shock proteins, are among the most common. For instance, Hsp90α requires Rab protein for sorting [29], with Protein Rab22a-NeoF1 and PYK2 interacting with Hsp90 for sorting. Ago2 is sorted when associated with Alix. Chaperones are also utilized for sorting, such as Hsp90 and Hsc70 for cytosolic proteins [30]. Hsc70 and LAMP2A bind to protein HIF1α for sorting in an ESCRT-independent manner. Additionally, several enzymes including peroxidase, enolase-1, lipid kinase, and GTPase are found inside exosomes.

2.4. DNA

Regarding DNA, research on its sorting into exosomes presents contrasting findings. Some studies suggest that DNA secretion from cells doesn't involve exosomes, while others propose that genomic DNA (gDNA) is sorted into exosomes. Single-stranded DNA and mitochondrial DNA were reported inside exosomes until 2014 [31-33]. In that year, Kahlert and colleagues confirmed the presence of double-stranded DNA in cancer cell exosomes using DNA digestion methods involving DNAse I [34’35]. Thakur and collaborators used an alternative method with shrimp DNAse, supporting the presence of double-stranded DNA [36]. It was initially thought that exosomal DNA originated from cytoplasmic DNA due to DNA damage or normal DNA metabolism, but this hypothesis doesn't fully explain the heterogeneity of DNA found inside exosomes [37]. The sizes of DNA found range from 100 bp to 10 Kbp.

In cancer cells, the interaction of gDNA with tetraspanin CD63 aids in sorting [38]. Mitochondrial DNA can also be sorted by an LC3/autophagy-independent mechanism.

2.5. RNA

RNA sorting inside exosomes is a selective process, with the presence of RNA in exosomes potentially resulting from absorption when exosomes circulate [39]. Various types of RNA, including non-coding RNA and miRNA, are found in exosomes. Certain miRNAs, such as miR-150 and miR-320, are prioritized during sorting. Protein involvement may also occur in the sorting of miRNAs within exosomes, with RNA binding proteins (RBPs) like YBX1, hnRNPK, FMR1, and Ago2 playing roles [40-43]. CircRNAs, like circRHOBTB3 and circNEIL3, are sorted by hnRNPA2B1, which is also involved in the sorting of miRNAs and lncRNAs [44, 45].

Circular RNA (circRNA) constitutes a significant portion of the non-coding RNA landscape. These molecules can exist freely in circulation or be enclosed within exosomes in the extracellular space. Their presence in exosomes involves various pathways. CircRNAs are abundant in eukaryotic cells and are conserved across species [46]. They have been implicated in various diseases, including cancers, autoimmune diseases, heart diseases, liver diseases, and renal diseases [47-49].

The circular closed-loop structure of circRNAs is formed by the attachment of the free 5' and 3' ends of RNA with a phosphodiester bond, creating a covalent bond [50]. Initially considered functionless, circRNAs have been found to play roles in gene expression regulation, protein interaction, and miRNA sponge activity, among others. They are produced through back splicing, distinct from linear RNA processing, which gives them protection from exonucleases and RNases, contributing to their stability and longer half-life compared to linear RNAs [51]. CircRNAs have emerged as biomarkers in various diseases and are predominantly found in the nucleus, though their transport mechanism to the cytoplasm remains unclear. Possible transportation mechanisms include ATP-dependent mechanisms and the involvement of N6-methyladenosine. Recent studies have highlighted the roles of helicase UAP56/URH49 in circRNA transport [52], with larger nucleotides transported by UAP56 and shorter ones by URH49 [53-55]. Following transportation, circRNAs may act as miRNA sponges, modulating gene expression in the cytoplasm.

2.5.1. Classification of circular RNA

CircRNAs are classified based on their splicing junctions, leading to distinct categories: exonic circRNAs (ecircRNAs), intronic circRNAs (ciRNAs), and exonic-intronic circRNAs (EIciRNAs). Additionally, a specific type called tRNA intronic circRNA (tricRNA) is formed from the splicing of pre-tRNA [56-61].

EcircRNAs mainly reside in the nucleus and consist of one or more exons. CiRNAs comprise introns, while EIciRNAs are composed of both exons and introns, primarily found in the nucleus. TricRNAs, on the other hand, are generated from pre-tRNA splicing [62-64].

CiRNAs are generated through lariat formation, where circularization occurs, and the DBR1 gene prevents debranching enzyme action. The size of ecircRNAs depends on the number of exons involved, influencing the efficiency of back-splicing. EIciRNA formation requires the presence of internal repeats, allowing parental gene transcription. This process involves U1 snRNP acting through RNA-RNA interactions.

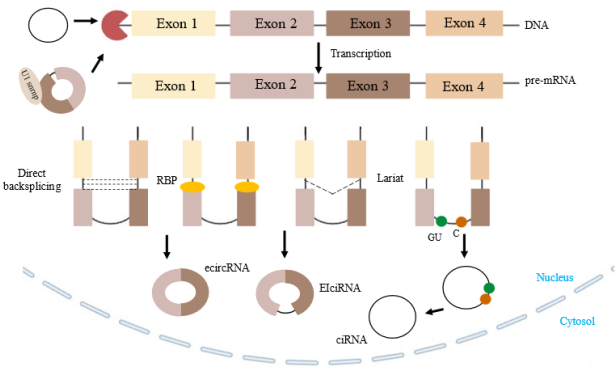

Fig. 2. Formation of different circRNAs.

2.5.2. Abundance

Studies have shown that approximately 20% of genes in the human brain produce circRNAs, whereas in the human heart, this percentage is around 9% (Figure 2). Interestingly, low proliferating cells tend to have more circRNAs compared to high proliferating cells [65-67]. Additionally, experiments have demonstrated higher expression of circRNAs in fetal tissues compared to adult tissues. Memczak et al. [68]. reported that circRNAs are particularly abundant in peripheral whole blood, and in human fibroblasts, approximately 25,000 different circRNAs have been identified [52].

2.3. TNBC

Triple-negative breast cancer (TNBC) is a prevalent global health issue. Cancer encompasses a wide array of diseases, all stemming from disruptions in the normal cell cycle, which involves processes like mitosis for cell multiplication. Regulation of these cycles is crucial, with mechanisms such as apoptosis, or programmed cell death, eliminating non-functional cells. Genetic control plays a pivotal role in these processes. In cancer, there's an aberrant, uncontrolled proliferation of these cells, forming tumors that can metastasize to other parts of the body, leading to significant health complications.

Breast cancer, one of the most common types, has garnered attention as a growing concern [69]. Globally, it constitutes 10.4% of all female cancers, ranking second only to lung cancer. In 2004, breast cancer caused 519,000 deaths worldwide. Recent data from 2018 indicates approximately 2 million new cases and around 60,000 deaths annually, with 42,260 deaths reported in the US alone in 2019 [70]. While breast cancer predominantly affects women, it also affects men, albeit less frequently, potentially due to delayed diagnosis. Breast cancer can manifest in various regions of the breast and may present as benign, such as cyst formation, a type of fibrocystic change.

Breast cancer encompasses various subtypes, with triple-negative breast cancer (TNBC) representing 10-20% of cases [71]. TNBC is associated with lower survival rates compared to other subtypes and has a higher likelihood of recurrence within a five-year prognosis [72,73]. This type

lacks expression of three hormone receptors: estrogen receptor, progesterone receptor (ER/PR), and HER2 (human epidermal growth factor receptor) [74].

Treatment for TNBC typically involves chemotherapy, utilizing agents such as anthracycline, taxane-based drugs, and platinum salts [75,76]. Additionally, PARP inhibitors and immune modulators have received approval for TNBC therapy [77,78]. PARP plays a role in DNA repair through ADP ribose transfer, making PARP inhibitors a targeted treatment option [79]. Atezolizumab, in combination with nab-paclitaxel, has been recently approved in the US for TNBC [80]. TNBC often exhibits heightened expression of growth factor receptors like EGFR, VEGFR, and FGFR [81], suggesting potential targets for inhibition with drugs like imatinib and lapatinib. However, the effectiveness of these therapies remains limited, highlighting the need for the identification of suitable biomarkers for TNBC.

Conventional biomarkers in breast cancer include CEA, CA-125, and CA15-3. Recent research has identified several circular RNAs (circRNAs) as potential biomarkers, such as hsa_circ_0068033, hsa_circ_0001785, and hsa_circ_0108942, detectable in plasma. Among these, hsa_circ_0001785 shows promise with a specificity of 75.6%. Comparative analysis with conventional biomarkers indicates that hsa_circ_0001785 has a higher area under the curve (AUC) value, suggesting its potential as a more effective biomarker for TNBC.

2.4. Exo-circRNA in TNBC

2.4.1. Function (As miRNA sponge)

In tumor malignancy, sponging plays a significant role. MiRNAs, short strands of RNA (19-25 nucleotides), are involved in post-transcriptional gene silencing. CircRNAs contain binding sites for miRNAs, and this sequestration aids in their regulation [82]. Due to these binding sites, circRNAs are known as competing endogenous RNA (ceRNA). Some circRNAs have the ability to bind more than one miRNA [83]. For instance, circRAD18 can bind both miR-208a and miR-3164, leading to the upregulation of IGF1 and FGF2, which promotes TNBC progression. CircGFRA1, with a binding site for miR-34a [84], also promotes tumor progression. Reports indicate that circ-RNA CDR1as, also known as ciRS-7 and abundantly found in the mammalian brain, acts as the first miRNA sponge containing 74 binding sites. It sequesters miR-671, inhibiting miRNA-mediated cleavage through mismatched nucleotides. Other circRNAs, such as circHIPK2 with a single binding site for miR124-2HG, circHIPK3 with 18 binding sites discovered through luciferase screening, and circSRY with 16 binding sites for miR-138 found in mouse testis, also play roles in sponging miRNAs [85]. Circ0069094 acts as a sponge for miR-591, serving as a biomarker for detecting breast cancer.

In transcription regulation and alternative splicing, alternative splicing controls gene expression. Circ-RNAs in the nucleus can regulate gene expression. Studies have shown the involvement of circRNAs in inhibiting transcription, such as circURI1, which ultimately promotes cancer. Among the four types of circRNAs, EIciRNAs, consisting of both exons and introns, are known to regulate transcription and RNA pol II. The interaction between EIciRNAs and RNA pol II allows efficient binding of the enzyme to the core promoter. Examples include circPAIP2 and circEIF3 identified in the nucleus, which increases parental gene expression through interaction with U1 snRNA and ElciRNA. CircSIRT7 and circANKRD52 are also involved in regulation, with their interaction with the RNA Pol II complex upregulating parental gene transcription [86]. A recently discovered circRNA from the insulin gene interacts with the RBP TDP-43 (RNA-binding protein) and regulates the transcription of insulin secretion-associated genes. Insulin is crucial in regulating blood glucose levels, and a decrease in its production can lead to diabetes. CircRNAs are also involved in the transcription regulation of genes in signaling pathways like Wnt/β-catenin, as seen with circRNA_069718.

2.6. Translation

While most circRNAs are typically unable to undergo translation, recent research has identified circRNAs that can efficiently be translated into proteins. The inability of most circRNAs to translate is attributed to the lack of 5' capping required for translation initiation. However, it has been discovered that circRNAs can be translated in a cap-independent manner. For instance, CircFBXW7 yields the protein FBXW7-185aa (21 kDa), although its functions remain unclear. Translation initiation is facilitated by the presence of a start codon (AUG), an open reading frame (ORF), and internal ribosome entry site (IRES) acting as templates. Examples include circMbl3 and circ-ZNF609, where IRES assists in translation [87-88]. Additionally, small peptides can be translated through m6A modification due to the presence of m6A motifs. Notably, circSMO, found in gliomas, encodes SMO-193aa, a component of the hedgehog pathway, while circPINTexon2 encodes PINT87aa, which is less abundant in glioma tissues [89]. CircAXIN1 encodes AXIN1-295aa, a participant in the Wnt pathway. Research indicates that the presence of multiple ORFs in circRNAs without stop codons facilitates translation (Table 1).

Few miRNAs are involved in translation repression by forming a complex with mRNA. CircRNA thus helps in translation by sponging miRNAs making mRNA free for initiating translation (Figure 3).

2.5.1. Protein interactions

Following miRNA sponging, another crucial function of circRNAs is facilitating protein-protein interactions, where they act as protein scaffolds or chaperones. This capacity for protein binding is facilitated by their tertiary structure. Such binding can have bidirectional effects, with proteins guiding circRNA synthesis and degradation, while circRNAs can act as protein sponges or decoys. Additionally, circRNAs are involved in protein translocation or transportation from the nucleus to the cytoplasm.

The oncogenic and tumor suppressor activities of exo-

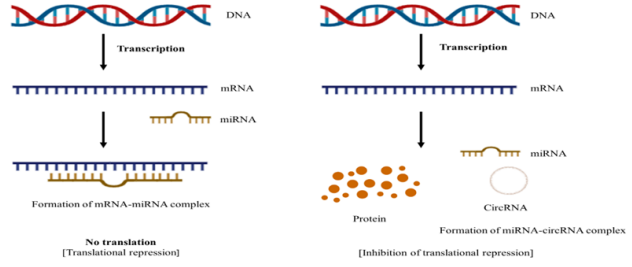

Fig. 3. CircRNA in inhibiting translational repression.

Table 1. Exosomal circRNAs that can be translated (found in TNBC).

Exosomal circRNAs that can be translated (found in TNBC)	Target/pathway involved /axis	Function	Ref.
circFBXW7	miR-197-3p/ FBXW7-185aa	Inhibition of tumor in TNBC functioning as a sponge of miR-197-3p and suppresses TNBC growth encoding the FBXW7-185aa protein.	[90]
circKIF4A	miR-375/ KIF4A	Tumor proliferation in TNBC functioning as a sponge of miR-375 and KIF4A expression is regulated.	[91]
circUBE2D2	miR-512-3p/CDCA3	Tumor proliferation in TNBC functions as a sponge of miR-512-3p regulating CDCA3 expression and promotes doxorubicin resistance	[92]
CircITCH	Wnt/β -catenin signaling pathway	Inhibition of tumor in TNBC sponging miR-214/miR-17 and increase the expression of its ITCH linear isoform and inactivation of Wnt/b-catenin signalling pathway	[93]
circSEPT9	LIF/ Stat3 signaling pathway	Promotes tumor proliferation in TNBC functioning as a sponge of miR-637 to downregulate LIF and activate LIF/Stat3 signalling pathway	[94]
circANKS1B	miR-148a-3p/miR-152-3p/ USF1	Promotes tumor migration and invasion sponging miR-148a-3p/miR-152-3p, increases the expression of USF1 transcription factor and promotes EMT	[95]

circRNAs are evident in triple-negative breast cancer (TNBC). There is an increased expression or upregulation of circRNAs in tumor formation, migration, and metastasis. For instance, circUBE2D2 in TNBC sponges miR-512-3p, leading to the upregulation of CDCA3 expression. Experiments have shown that the downregulation of miR-512-3p depletes circUBE2D2, resulting in tumor suppression. Another example is circANKS1B, which sponges miR-148A-3p and miR-152-3p, and its upregulation promotes tumor proliferation. Downregulation of circRNAs is associated with tumor suppression, where proliferation, invasion, and metastasis are inhibited. Examples include circNR3C2 and circTADA2A-E6.

CircITCH, implicated in various cancers, plays a crucial role in tumor suppression in TNBC. Its overexpression downregulates the Wnt/β-catenin pathway by sponging miR-214 and miR-1793. Additionally, circRNAs regulate functions in mitochondria. These circ-mtRNAs, such as circRNA_103809, when overexpressed, can impair miR-532-3p function and interfere with the epithelial-mesenchymal transition (EMT) pathway.

2.6. Metastasis of exosomal circRNA in TNBC

Metastasis of exosomal circRNAs in TNBC is a significant concern. Metastasis involves several complex steps ultimately leading to patient mortality. Understanding these mechanisms is crucial for improving therapies and management. Exosomal circRNA expression increases in breast cancer and contributes to miRNA sponging and tumor suppression. Certain circRNAs are associated with increased metastasis and invasion. For instance, circFBXL5 upregulation in breast and lung cancer induces SRSF6 expression through miR-660 sponging [96]. CircANKS1B promotes epithelial-mesenchymal transition (EMT) via the TGF-B1 signaling pathway. CircHMCU impacts EMT and cell cycle phase G1 [97]. Methylation and demethylation control aggressive tumor spreading in cirFECR1 (Table 2). CircBCMB1 has been observed to metastasize to the brain by sponging miR-125a and regulating the protein BRD4, leading to altered MMP9 expression [98].

Conversely, decreased expression of exosomal circRNAs is also observed in breast cancer. Microarrays have shown decreased expression of circNF1C in breast cancer (Figure 4). In some cases, a lower level of circRNA and higher miRNA expression are observed, as seen with circRNA_000554.

2.7. Exosomal circRNA in apoptosis

Apoptosis serves as a crucial mechanism in normal cells, preventing uncontrolled proliferation. CircRNAs have been observed to influence the apoptotic process, contributing to the pathogenesis of breast cancer by in-

Table 2. Expression of different exosomal circRNAs in TNBC.

Circ-RNA	Expression	miRNA	Gene targeted	Hallmark	Ref.
circUBE2D2	Upregulation	miR-512-3p	CDCA3	Migration (+) Invasion (+) Proliferation (+)	[92]
circAGFG1	Upregulation	miR-195-5p	CCNE1	Migration (+) Invasion (+) Proliferation (+) Apoptosis (-)	[99]
circRNA_069718	Upregulation	NA	Genes related to Wnt/b-catenin pathway-	Migration (+) Invasion (+) Proliferation (+) Apoptosis (-)	[100]
circSEPT9	Upregulation	miR-637	LIF	Migration (+) Invasion (+) Proliferation (+) Apoptosis (-)	[94]
circFBXW7	Downregulation	miR-197-3p	FBXW7	Migration (-) Invasion (-) Proliferation (-)	[90]
CircITCH	Downregulation	miR-214/ miR-17	ITCH1	Migration (-) Invasion (-) Proliferation (-)	[93]
circKIF4A	Upregulation	miR-375	KIF4A	Migration (+) Proliferation (+)	[91]
circRAD18	Upregulation	miR-208a/miR-3164	IGF1/FGF2	Migration (+) Proliferation (+) Apoptosis (-)	[83]
circTADA2A-E6	Downregulation	miR-203a-3p	SOCS3	Migration (-) Invasion (-) Proliferation (-)	[101]
circANKS1B	Upregulation	miR-148a-3p/miR-152-3p	USF1	Migration (+) Invasion (+) EMT (+)	[95]
circUBAP2	Upregulation	miRNA-661	MTA1	Migration (+) Proliferation (+) Apoptosis (-)	[102]
circPLK1	Upregulation	miR-296-5p	PLK1	Invasion (+) Proliferation (+)	[103]
circEPSTI1	Upregulation	miR-4753/miR-6809	BCL11A	Proliferation (+) Apoptosis (-)	[58]
CircGFRA1	Upregulation	miR-34a	GFRA1	Proliferation (+) Apoptosis (-)	[104]
hsa_circ_001783	Upregulation	miR-200c-3p	ETS1, ZEB1, ZEBI2	Migration (+) Invasion (+) Proliferation (+)	[105]
CircNR3C2	Downregulation	miR-513a-3p	HRD1, vimentin	Migration (-) Invasion (-) Proliferation (-) EMT (-)	[106]

(+) Increased activity, (-) Decreased activity.

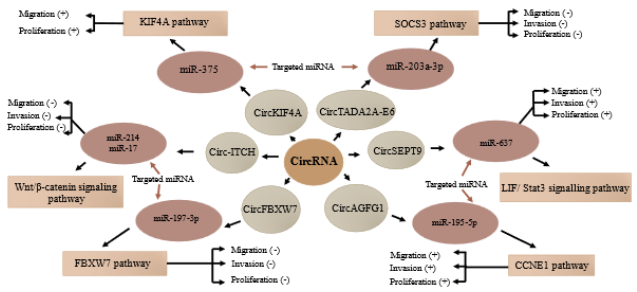

Fig. 4. Expression of different exosomal circRNAs in TNBC.

teracting with downstream signaling pathways. This interaction often involves the sponging action of miRNAs by circRNAs. For instance, circABCC4, which sponges miR-154-5p [108], enhances apoptosis when downregulated [109]. Conversely, upregulated circRNAs like circRNA_0001283 induce apoptosis by sponging miR-187. Similarly, overexpression of circRNA_000911 inhibits tumor growth by sponging miR-449a, despite its initial downregulation in breast cancer cells [110]. Another example is hsa_circ_0068033, which sponges miR-659. These circRNA-miRNA interactions also regulate different pathways, such as enhancing Notch1 and NF-κB signaling pathways [111]. The PI3K/AKT signaling pathway, crucial in apoptosis control, is regulated by the circRNA/PI3K/AKT axis, thereby inhibiting apoptosis.

2.7.1. Exo-circRNA and chemotherapeutic resistance

Exosomal circRNAs and chemotherapeutic resistance present significant challenges in breast cancer treatment [112]. Effective therapy selection is crucial among the various available options. Tamoxifen, commonly used for TNBC patients, was found to have increased sensitivity when combined with circRNA_0025202, which was downregulated in tamoxifen-resistant cells [113]. Conversely, circUBE2D2 was upregulated in tamoxifen-resistant cells, leading to resistance by sponging miR-200a-3p [114].

In TNBC patients, resistance to paclitaxel poses a major challenge. Upregulated circ-RNF111 in paclitaxel-resistant cells contributes to resistance by upregulating E2F3 through miR-140-5p sponging [115]. Conversely, downregulation of hsa_circ_0000199 increases sensitivity to paclitaxel. Similar observations were made with therapies involving gemcitabine, cisplatin, and Adriamycin [116]. Monastrol, another chemotherapeutic agent, suppresses tumors by inhibiting the mitotic kinesin Eg5 required for bipolar spindle formation. CircRNA-MTO1, upregulated in monastrol-resistant cells, can be downregulated in TNBC cells to reverse resistance to monastrol [117].

CircKDM4C, usually downregulated in cells resistant to doxorubicin, can be overexpressed to reverse resistance [92]. Additionally, downregulation of circUBE2D2 reverses resistance to doxorubicin through miR-512-3p downregulation and CDCA3 upregulation (Table 3). Similarly, hsa_circ_0092276 overexpression leads to therapy resistance via altered autophagy-related gene 7 through miR-384 sponging [119].

2.8. Challenges and limitations in exosomal circRNAs in research

Exosomal circular RNAs (exo-circRNAs) detected in triple-negative breast cancer (TNBC) can either act as tumor suppressors or promote tumor proliferation, influencing the effectiveness of chemotherapeutic drugs in TNBC therapy. Sequencing techniques have provided insights into the roles of exo-circRNAs in TNBC. Some assumptions have been made regarding exosomal circRNAs: exosomes protect circRNAs from clearance by transferring genetic information to other cells, while they may also facilitate circRNA clearance through exocytosis from the vesicle. Recently, certain circRNAs have been found to have functional roles in cancer research, making them promising biomarkers or prognostic markers for detecting TNBC in patients.

Despite these advances, further studies and validation are needed. The scarcity of circRNA in exosomes presents challenges in detection. Additionally, due to their circular structure and sequence similarity with linear counterparts, studies may lack precision. The impact of circRNAs on pathophysiological processes is under study. There is unclear research on how circRNAs are ultimately degraded and how they are enriched in exosomes during formation. According to assumptions, circRNAs plentifully found in the cytoplasm are passively incorporated into exosomes. As the current development of exo-circRNA is in its nascent stage, more advanced tools are needed to aid research in this area.

Table 3. Exo-circRNA and chemotherapeutic resistance.

Chemotherapeutic agent	circRNA	Expression	Effect	Ref.
	circ_UBE2D2	Upregulation	Resistant	[114]
Tamoxifen	circBMPR2	Downregulation	Resistant	[120]
	circRNA_0025202	Upregulation	Sensitive	[113]
	circ-RNF111	Upregulation	Resistant	[115]
Paclitaxel	circGFRA1	Upregulation	Resistant	[121]
	circ-ABCB10	Upregulation	Resistant	[122]
Monastrol	circRNA-MTO1	Downregulation	Sensitive	[117]
	circ_0085495	Upregulation	Resistant	[123]
Adriamycin	circ_0006528	Upregulation	Resistant	[124]
	circ_0001667	Upregulation	Resistant	[125]
Lapatinib	circ-MMP11	Upregulation	Resistant	[126]
5-Fluorouracil	circFBXL5	Upregulation	Resistant	[127]

3. Conclusion

The study highlights the multifaceted roles of exosomal circular RNAs (exo-circRNAs) in triple-negative breast cancer (TNBC) pathogenesis and therapy. These exo-circRNAs can either suppress or promote tumor growth and influence the effectiveness of chemotherapeutic drugs. While sequencing techniques have shed light on their functions, challenges such as detection difficulty and lack of precision in studies persist due to their circular structure and sequence similarities. Despite these obstacles, exo-circRNAs hold promise as biomarkers or prognostic markers for TNBC detection. However, further research and validation are imperative to fully understand their mechanisms of action and potential clinical applications. Additionally, advancements in tools and methodologies are needed to propel research in this nascent field forward.

Conflicts of Interest

The author declares no conflicts of interest.

References

1. Cocucci E, Meldolesi J (2005) Ectosomes and exosomes: shedding the confusion between extracellular vesicles. Trends Cell Biol 25(6):364-372. doi:10.1016/j.tcb.2015.01.004
2. Boriachek K, Islam MN, Möller A, et al. (2018) Biological Functions and Current Advances in Isolation and Detection Strategies for Exosome Nanovesicles. Small14(6). doi:10.1002/smll.201702153
3. Yu G, Jung H, Kang YY, Mok H (2018) Comparative evaluation of cell- and serum-derived exosomes to deliver immune stimulators to lymph nodes. Biomaterials 162:71-81. doi:10.1016/j.biomaterials.2018.02.003
4. Zhang Y, Wang XF (2015) A niche role for cancer exosomes in metastasis. Nat Cell Biol 17(6):709-711. doi:10.1038/ncb3181
5. Gao L, Wang L, Dai T, et al. (2018) Tumor-derived exosomes antagonize innate antiviral immunity. Nat Immunol19(3):233-245. doi:10.1038/s41590-017-0043-5
6. Yin L, Liu X, Shao X, et al. (2021) The role of exosomes in lung cancer metastasis and clinical applications: an updated review. J Transl Med19(1):312. doi:10.1186/s12967-021-02985-1
7. Kalluri R (2016) The biology and function of exosomes in cancer. J Clin Invest126(4):1208-1215. doi:10.1172/JCI81135
8. Booth AM, Fang Y, Fallon JK, Yang JM, Hildreth JEK, Gould SJ (2006) Exosomes and HIV Gag bud from endosome-like domains of the T cell plasma membrane. J Cell Biol172(6):923-935. doi:10.1083/jcb.200508014
9. Chiu YJ, Cai W, Shih YRV, Lian I, Lo YH (2016) A Single-Cell Assay for Time Lapse Studies of Exosome Secretion and Cell Behaviors. Small12(27):3658-3666. doi:10.1002/smll.201600725
10. Smith ZJ, Lee C, Rojalin T, et al. (2015) Single exosome study reveals subpopulations distributed among cell lines with variability related to membrane content. J Extracell Vesicles4:28533. doi:10.3402/jev.v4.28533
11. Kogure T, Lin WL, Yan IK, Braconi C, Patel T (2011) Intercellular nanovesicle-mediated microRNA transfer: a mechanism of environmental modulation of hepatocellular cancer cell growth. Hepatology54(4):1237-1248. doi:10.1002/hep.24504
12. Théry C, Amigorena S, Raposo G, Clayton A (2006) Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr Protoc Cell BiolChapter 3:Unit 3.22. doi:10.1002/0471143030.cb0322s30
13. Raposo G, Nijman HW, Stoorvogel W, et al. (1996) B lymphocytes secrete antigen-presenting vesicles. J Exp Med183(3):1161-1172. doi:10.1084/jem.183.3.1161
14. Chen C, Skog J, Hsu CH, et al. (2010) Microfluidic isolation and transcriptome analysis of serum microvesicles. Lab Chip10(4):505-511. doi:10.1039/b916199f
15. Wang Z, Wu H jen, Fine D, et al. (2013) Ciliated micropillars for the microfluidic-based isolation of nanoscale lipid vesicles. Lab Chip13(15):2879-2882. doi:10.1039/c3lc41343h
16. Braicu C, Tomuleasa C, Monroig P, Cucuianu A, Berindan-Neagoe I, Calin GA (2015) Exosomes as divine messengers: are they the Hermes of modern molecular oncology? Cell Death Differ22(1):34-45. doi:10.1038/cdd.2014.130
17. Wen SW, Lima LG, Lobb RJ, et al. (2019) Breast Cancer-Derived Exosomes Reflect the Cell-of-Origin Phenotype. Proteomics19(8):e1800180. doi:10.1002/pmic.201800180
18. Villarroya-Beltri C, Baixauli F, Gutiérrez-Vázquez C, Sánchez-Madrid F, Mittelbrunn M (2014) Sorting it out: regulation of exosome loading. Semin Cancer Biol 28:3-13. doi:10.1016/j.semcancer.2014.04.009
19. Winter V, Hauser MT (2006) Exploring the ESCRTing machinery in eukaryotes. Trends Plant Sci11(3):115-123. doi:10.1016/j.tplants.2006.01.008
20. Henne WM, Buchkovich NJ, Emr SD (2011) The ESCRT pathway. Dev Cell21(1):77-91. doi:10.1016/j.devcel.2011.05.015
21. Schöneberg J, Lee IH, Iwasa JH, Hurley JH (2017) Reverse-topology membrane scission by the ESCRT proteins. Nat Rev Mol Cell Biol18(1):5-17. doi:10.1038/nrm.2016.121
22. Huebner AR, Cheng L, Somparn P, Knepper MA, Fenton RA, Pisitkun T (2016) Deubiquitylation of Protein Cargo Is Not an Essential Step in Exosome Formation. Mol Cell Proteomics15(5):1556-1571. doi:10.1074/mcp.M115.054965
23. Skotland T, Hessvik NP, Sandvig K, Llorente A (2019) Exosomal lipid composition and the role of ether lipids and phosphoinositides in exosome biology. J Lipid Res60(1):9-18. doi:10.1194/jlr.R084343
24. Dawson G (2021) Isolation of Lipid Rafts (Detergent-Resistant Microdomains) and Comparison to Extracellular Vesicles (Exosomes). Methods Mol Biol2187:99-112. doi:10.1007/978-1-0716-0814-2_6
25. Perez-Hernandez D, Gutiérrez-Vázquez C, Jorge I, et al. (2013) The intracellular interactome of tetraspanin-enriched microdomains reveals their function as sorting machineries toward exosomes. J Biol Chem 288(17):11649-11661. doi:10.1074/jbc.M112.445304
26. Hurwitz SN, Conlon MM, Rider MA, Brownstein NC, Meckes DG (2016) Nanoparticle analysis sheds budding insights into genetic drivers of extracellular vesicle biogenesis. J Extracell Vesicles 5:31295. doi:10.3402/jev.v5.31295
27. Parton RG, McMahon KA, Wu Y (2020) Caveolae: Formation, dynamics, and function. Curr Opin Cell Biol 65:8-16. doi:10.1016/j.ceb.2020.02.001
28. Kwiatkowska K, Matveichuk OV, Fronk J, Ciesielska A (2020) Flotillins: At the Intersection of Protein S-Palmitoylation and Lipid-Mediated Signaling. Int J Mol Sci 21(7):2283. doi:10.3390/ijms21072283
29. Zhang S, Wang C, Ma B, et al. (2020) Mutant p53 Drives Cancer Metastasis via RCP-Mediated Hsp90α Secretion. Cell Rep 32(1):107879. doi:10.1016/j.celrep.2020.107879
30. Géminard C, De Gassart A, Blanc L, Vidal M (2004) Degradation of AP2 during reticulocyte maturation enhances binding of hsc70 and Alix to a common site on TFR for sorting into exosomes. Traffic5(3):181-193. doi:10.1111/j.1600-0854.2004.0167.x
31. Jeppesen DK, Fenix AM, Franklin JL, et al. (2019) Reassessment of Exosome Composition. Cell177(2):428-445.e18. doi:10.1016/j.cell.2019.02.029

32. Takahashi A, Okada R, Nagao K, et al. (2017) Exosomes maintain cellular homeostasis by excreting harmful DNA from cells. Nat Commun 8:15287. doi:10.1038/ncomms15287
33. Torralba D, Baixauli F, Villarroya-Beltri C, et al. (2018) Priming of dendritic cells by DNA-containing extracellular vesicles from activated T cells through antigen-driven contacts. Nature Communications9. doi:10.1038/s41467-018-05077-9
34. Balaj L, Lessard R, Dai L, et al. (2011) Tumour microvesicles contain retrotransposon elements and amplified oncogene sequences. Nat Commun2:180. doi:10.1038/ncomms1180
35. Guescini M, Genedani S, Stocchi V, Agnati LF (2010) Astrocytes and Glioblastoma cells release exosomes carrying mtDNA. J Neural Transm (Vienna) 117(1):1-4. doi:10.1007/s00702-009-0288-8
36. Kahlert C, Melo SA, Protopopov A, et al. (2014) Identification of double-stranded genomic DNA spanning all chromosomes with mutated KRAS and p53 DNA in the serum exosomes of patients with pancreatic cancer. *J*289(7):3869-3875. doi:10.1074/jbc.C113.532267
37. Thakur BK, Zhang H, Becker A, et al. (2014) Double-stranded DNA in exosomes: a novel biomarker in cancer detection. Cell Res 24(6):766-769. doi:10.1038/cr.2014.44
38. Yokoi A, Villar-Prados A, Oliphint PA, et al. (2019) Mechanisms of nuclear content loading to exosomes. Sci Adv 5(11):eaax8849. doi:10.1126/sciadv.aax8849
39. Zhang J, Li S, Li L, et al. (2015) Exosome and exosomal microRNA: trafficking, sorting, and function. Genomics Proteomics Bioinformatics 13(1):17-24. doi:10.1016/j.gpb.2015.02.001
40. Wozniak AL, Adams A, King KE, et al. (2020) The RNA binding protein FMR1 controls selective exosomal miRNA cargo loading during inflammation. J Cell Biol 219(10):e201912074. doi:10.1083/jcb.201912074
41. Groot M, Lee H (2020) Sorting Mechanisms for MicroRNAs into Extracellular Vesicles and Their Associated Diseases. Cells 9(4):1044. doi:10.3390/cells9041044
42. Hobor F, Dallmann A, Ball NJ, et al. (2018) A cryptic RNA-binding domain mediates Syncrip recognition and exosomal partitioning of miRNA targets. Nat Commun 9(1):831. doi:10.1038/s41467-018-03182-3
43. Teng Y, Ren Y, Hu X, et al. (2017) MVP-mediated exosomal sorting of miR-193a promotes colon cancer progression. Nat Commun 8:14448. doi:10.1038/ncomms14448
44. Pan Z, Zhao R, Li B, et al. (2022) EWSR1-induced circNEIL3 promotes glioma progression and exosome-mediated macrophage immunosuppressive polarization via stabilizing IGF2BP3. Mol Cancer 21(1):16. doi:10.1186/s12943-021-01485-6
45. Chen C, Yu H, Han F, et al. (2022) Tumor-suppressive circRHOBTB3 is excreted out of cells via exosome to sustain colorectal cancer cell fitness. Mol Cancer 21(1):46. doi:10.1186/s12943-022-01511-1
46. Hsu MT, Coca-Prados M (1979) Electron microscopic evidence for the circular form of RNA in the cytoplasm of eukaryotic cells. Nature 280(5720):339-340. doi:10.1038/280339a0
47. Sanger HL, Klotz G, Riesner D, Gross HJ, Kleinschmidt AK (1976) Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures. Proc Natl Acad Sci U S A 73(11):3852-3856. doi:10.1073/pnas.73.11.3852
48. Circular RNAs are abundant, conserved, and associated with ALU repeats - PubMed. Accessed April 6, 2024. https://pubmed.ncbi.nlm.nih.gov/23249747/
49. Haddad G, Lorenzen JM (2019) Biogenesis and Function of Circular RNAs in Health and in Disease. Front Pharmacol 10:428. doi:10.3389/fphar.2019.00428
50. Chen LL, Yang L (2015) Regulation of circRNA biogenesis. RNA Biol 12(4):381-388. doi:10.1080/15476286.2015.1020271
51. Ashwal-Fluss R, Meyer M, Pamudurti NR, et al. (2014) circRNA biogenesis competes with pre-mRNA splicing. Mol Cell 56(1):55-66. doi:10.1016/j.molcel.2014.08.019
52. Suzuki H, Zuo Y, Wang J, Zhang MQ, Malhotra A, Mayeda A (2006) Characterization of RNase R-digested cellular RNA source that consists of lariat and circular RNAs from pre-mRNA splicing. Nucleic Acids Res 34(8):e63. doi:10.1093/nar/gkl151
53. Li HM, Ma XL, Li HG (2019) Intriguing circles: Conflicts and controversies in circular RNA research. Wiley Interdiscip Rev RNA10(5):e1538. doi:10.1002/wrna.1538
54. Vincent HA, Deutscher MP (2006) Substrate recognition and catalysis by the exoribonuclease RNase R. J Biol Chem 281(40):29769-29775. doi:10.1074/jbc.M606744200
55. Vausort M, Salgado-Somoza A, Zhang L, et al. (2016) Myocardial Infarction-Associated Circular RNA Predicting Left Ventricular Dysfunction. J Am Coll Cardiol 68(11):1247-1248. doi:10.1016/j.jacc.2016.06.040
56. Salgado-Somoza A, Zhang L, Vausort M, Devaux Y (2017) The circular RNA MICRA for risk stratification after myocardial infarction. Int J Cardiol Heart Vasc 17:33-36. doi:10.1016/j.ijcha.2017.11.001
57. Vo JN, Cieslik M, Zhang Y, et al. (2019) The Landscape of Circular RNA in Cancer. Cell ;176(4):869-881.e13. doi:10.1016/j.cell.2018.12.021
58. Chen B, Wei W, Huang X, et al. (2018) circEPSTI1 as a Prognostic Marker and Mediator of Triple-Negative Breast Cancer Progression. Theranostics 8(14):4003-4015. doi:10.7150/thno.24106
59. Chen X, Mao R, Su W, et al. (2020) Circular RNA circHIPK3 modulates autophagy via MIR124-3p-STAT3-PRKAA/AMPKα signaling in STK11 mutant lung cancer. Autophagy 16(4):659-671. doi:10.1080/15548627.2019.1634945
60. Liu CX, Li X, Nan F, et al. (2019) Structure and Degradation of Circular RNAs Regulate PKR Activation in Innate Immunity. Cell 177(4):865-880.e21. doi:10.1016/j.cell.2019.03.046
61. Carrara M, Fuschi P, Ivan C, Martelli F (2018) Circular RNAs: Methodological challenges and perspectives in cardiovascular diseases. J Cell Mol Med 22(11):5176-5187. doi:10.1111/jcmm.13789
62. Azizzad Ranji E, Kahrizi D, Khanahmadi M, Rashidi Monfared S (2025) CircRNAs: Biogenesis, identification and expression analysis. Cell Mol Biomed Rep 5(1): 63-79. doi: 10.55705/cmbr.2025.460043.1248
63. Huang C, Liang D, Tatomer DC, Wilusz JE (2018) A length-dependent evolutionarily conserved pathway controls nuclear export of circular RNAs. Genes Dev 32(9-10):639-644. doi:10.1101/gad.314856.118
64. Conn SJ, Pillman KA, Toubia J, et al. (2015) The RNA binding protein quaking regulates formation of circRNAs. Cell 160(6):1125-1134. doi:10.1016/j.cell.2015.02.014
65. Aufiero S, Reckman YJ, Pinto YM, Creemers EE (2019) Circular RNAs open a new chapter in cardiovascular biology. Nat Rev Cardiol 16(8):503-514. doi:10.1038/s41569-019-0185-2
66. Bachmayr-Heyda A, Reiner AT, Auer K, et al. (2015) Correlation of circular RNA abundance with proliferation--exemplified with colorectal and ovarian cancer, idiopathic lung fibrosis, and normal human tissues. Sci Rep 5:8057. doi:10.1038/srep08057
67. Qu S, Liu Z, Yang X, et al. (2018) The emerging functions and roles of circular RNAs in cancer. Cancer Lett 414:301-309. doi:10.1016/j.canlet.2017.11.022
68. Kristensen LS, Andersen MS, Stagsted LVW, Ebbesen KK, Hansen TB, Kjems J (2019) The biogenesis, biology and characterization of circular RNAs. Nat Rev Genet 20(11):675-691.

doi:10.1038/s41576-019-0158-7
69. Khuwaja G, Abu-Rezq A (2004) Bimodal breast cancer classification system. Pattern Analysis and Applications - PAA 7:235-242. doi:10.1007/BF02683990
70. Siegel RL, Miller KD, Jemal A (2019) Cancer statistics, 2019. CA Cancer J Clin 69(1):7-34. doi:10.3322/caac.21551
71. Dent R, Trudeau M, Pritchard KI, et al. (2007) Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 13(15 Pt 1):4429-4434. doi:10.1158/1078-0432.CCR-06-3045
72. Onitilo AA, Engel JM, Greenlee RT, Mukesh BN (2009) Breast cancer subtypes based on ER/PR and Her2 expression: comparison of clinicopathologic features and survival. Clin Med Res 7(1-2):4-13. doi:10.3121/cmr.2009.825
73. Carey L, Winer E, Viale G, Cameron D, Gianni L (2010) Triple-negative breast cancer: disease entity or title of convenience? Nat Rev Clin Oncol 7(12):683-692. doi:10.1038/nrclinonc.2010.154
74. Venkitaraman R (2010) Triple-negative/basal-like breast cancer: clinical, pathologic and molecular features. Expert Rev Anticancer Ther 10(2):199-207. doi:10.1586/era.09.189
75. Bianchini G, Balko JM, Mayer IA, Sanders ME, Gianni L (2016) Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. Nat Rev Clin Oncol 13(11):674-690. doi:10.1038/nrclinonc.2016.66
76. Foulkes WD, Smith IE, Reis-Filho JS (2010) Triple-negative breast cancer. N Engl J Med 363(20):1938-1948. doi:10.1056/NEJMra1001389
77. Robson M, Im SA, Senkus E, et al. (2017) Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. N Engl J Med 377(6):523-533. doi:10.1056/NEJMoa1706450
78. Schmid P, Adams S, Rugo HS, et al. (2018) Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. N Engl J Med 379(22):2108-2121. doi:10.1056/NEJMoa1809615
79. Audebert M, Salles B, Calsou P (2004) Involvement of poly(ADP-ribose) polymerase-1 and XRCC1/DNA ligase III in an alternative route for DNA double-strand breaks rejoining. J Biol Chem 279(53):55117-55126. doi:10.1074/jbc.M404524200
80. Murciano-Goroff YR, Warner AB, Wolchok JD (2020) The future of cancer immunotherapy: microenvironment-targeting combinations. Cell Res 30(6):507-519. doi:10.1038/s41422-020-0337-2
81. Turner N, Lambros MB, Horlings HM, et al. (2010) Integrative molecular profiling of triple negative breast cancers identifies amplicon drivers and potential therapeutic targets. Oncogene 29(14):2013-2023. doi:10.1038/onc.2009.489
82. Weng W, Wei Q, Toden S, et al. (2017) Circular RNA ciRS-7-A Promising Prognostic Biomarker and a Potential Therapeutic Target in Colorectal Cancer. Clin Cancer Res 23(14):3918-3928. doi:10.1158/1078-0432.CCR-16-2541
83. Zou Y, Zheng S, Xiao W, et al. (2019) circRAD18 sponges miR-208a/3164 to promote triple-negative breast cancer progression through regulating IGF1 and FGF2 expression. Carcinogenesis 40(12):1469-1479. doi:10.1093/carcin/bgz071
84. Guo Z, Cao Q, Zhao Z, Song C (2020) Biogenesis, Features, Functions, and Disease Relationships of a Specific Circular RNA: CDR1as. Aging Dis 11(4):1009-1020. doi:10.14336/AD.2019.0920
85. Hansen TB, Jensen TI, Clausen BH, et al. (2013) Natural RNA circles function as efficient microRNA sponges. Nature 495(7441):384-388. doi:10.1038/nature11993
86. Zhang Y, Zhang XO, Chen T, et al. (2013) Circular intronic long noncoding RNAs. Mol Cell 51(6):792-806. doi:10.1016/j.molcel.2013.08.017
87. Pamudurti NR, Bartok O, Jens M, et al. (2017) Translation of CircRNAs. Mol Cell 66(1):9-21.e7. doi:10.1016/j.molcel.2017.02.021
88. Chen CY, Sarnow P (1995) Initiation of protein synthesis by the eukaryotic translational apparatus on circular RNAs. 268(5209):415-417. doi:10.1126/science.7536344
89. Yang Y, Fan X, Mao M, et al. (2017) Extensive translation of circular RNAs driven by N6-methyladenosine. Cell Res 27(5):626-641. doi:10.1038/cr.2017.31
90. Yang Y, Gao X, Zhang M, et al. (2018) Novel Role of FBXW7 Circular RNA in Repressing Glioma Tumorigenesis. J Natl Cancer Inst 110(3):304-315. doi:10.1093/jnci/djx166
91. Tang H, Huang X, Wang J, et al. (2019) circKIF4A acts as a prognostic factor and mediator to regulate the progression of triple-negative breast cancer. Mol Cancer 18(1):23. doi:10.1186/s12943-019-0946-x
92. Dou D, Ren X, Han M, et al. (2020) CircUBE2D2 (hsa_circ_0005728) promotes cell proliferation, metastasis and chemoresistance in triple-negative breast cancer by regulating miR-512-3p/CDCA3 axis. Cancer Cell Int 20:454. doi:10.1186/s12935-020-01547-7
93. Wang ST, Liu LB, Li XM, et al. (2019) Circ-ITCH regulates triple-negative breast cancer progression through the Wnt/β-catenin pathway. Neoplasma 66(2):232-239. doi:10.4149/neo_2018_180710N460
94. Zheng X, Huang M, Xing L, et al. (2020) The circRNA circSEPT9 mediated by E2F1 and EIF4A3 facilitates the carcinogenesis and development of triple-negative breast cancer. Mol Cancer 19:73. doi:10.1186/s12943-020-01183-9
95. Zeng K, He B, Yang BB, et al. (2018) The pro-metastasis effect of circANKS1B in breast cancer. Mol Cancer 17(1):160. doi:10.1186/s12943-018-0914-x
96. Zhou H, Tang G, Zhao M, et al. (2020) circFBXL5 promotes breast cancer progression by sponging miR-660. J Cell Mol Med 24(1):356-361. doi:10.1111/jcmm.14737
97. Song X, Liang Y, Sang Y, et al. (2020) circHMCU Promotes Proliferation and Metastasis of Breast Cancer by Sponging the let-7 Family. Mol Ther Nucleic Acids 20:518-533. doi:10.1016/j.omtn.2020.03.014
98. Fu B, Liu W, Zhu C, et al. (2021) Circular RNA circBCBM1 promotes breast cancer brain metastasis by modulating miR-125a/BRD4 axis. Int J Biol Sci 17(12):3104-3117. doi:10.7150/ijbs.58916
99. Yang R, Xing L, Zheng X, Sun Y, Wang X, Chen J (2019) The circRNA circAGFG1 acts as a sponge of miR-195-5p to promote triple-negative breast cancer progression through regulating CCNE1 expression. Mol Cancer 18(1):4. doi:10.1186/s12943-018-0933-7
100. Zhang J, Xu HD, Xing XJ, Liang ZT, Xia ZH, Zhao Y (2019) CircRNA_069718 promotes cell proliferation and invasion in triple-negative breast cancer by activating Wnt/β-catenin pathway. Eur Rev Med Pharmacol Sci 23(12):5315-5322. doi:10.26355/eurrev_201906_18198
101. Xu JZ, Shao CC, Wang XJ, et al. (2019) circTADA2As suppress breast cancer progression and metastasis via targeting miR-203a-3p/SOCS3 axis. Cell Death Dis 10(3):175. doi:10.1038/s41419-019-1382-y
102. Wang S, Li Q, Wang Y, et al. (2018) Upregulation of circ-UBAP2 predicts poor prognosis and promotes triple-negative breast cancer progression through the miR-661/MTA1 pathway. Biochem Biophys Res Commun 505(4):996-1002. doi:10.1016/j.bbrc.2018.10.026
103. Kong Y, Yang L, Wei W, et al. (2019) CircPLK1 sponges miR-296-5p to facilitate triple-negative breast cancer progression. Epigenomics 11(10):1163-1176. doi:10.2217/epi-2019-0093
104. He R, Liu P, Xie X, et al. (2017) circGFRA1 and GFRA1 act as

ceRNAs in triple negative breast cancer by regulating miR-34a. J Exp Clin Cancer Res 36:145. doi:10.1186/s13046-017-0614-1

105. Liu Z, Zhou Y, Liang G, et al. (2019) Circular RNA hsa_circ_001783 regulates breast cancer progression via sponging miR-200c-3p. Cell Death Dis 10(2):1-14. doi:10.1038/s41419-018-1287-1
106. Fan Y, Wang J, Jin W, et al. (2021) CircNR3C2 promotes HRD1-mediated tumor-suppressive effect via sponging miR-513a-3p in triple-negative breast cancer. Mol Cancer 20(1):25. doi:10.1186/s12943-021-01321-x
107. Kaczanowski S (2016) Apoptosis: its origin, history, maintenance and the medical implications for cancer and aging. Phys Biol 13(3):031001. doi:10.1088/1478-3975/13/3/031001
108. Jiang J, Cheng X (2019) Circular RNA circABCC4 acts as a ceRNA of miR-154-5p to improve cell viability, migration and invasion of breast cancer cells in vitro. Cell Cycle 19(20):2653-2661. doi:10.1080/15384101.2020.1815147
109. Circular RNA-0001283 Suppresses Breast Cancer Proliferation and Invasion via MiR-187/HIPK3 Axis - PMC. Accessed April 6, 2024. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7047918/
110. Wang H, Xiao Y, Wu L, Ma D (2018) Comprehensive circular RNA profiling reveals the regulatory role of the circRNA-000911/miR-449a pathway in breast carcinogenesis. Int J Oncol 52(3):743-754. doi:10.3892/ijo.2018.4265
111. Meng Y, Wang W, Kang J, Wang X, Sun L (2017) Role of the PI3K/AKT signalling pathway in apoptotic cell death in the cerebral cortex of streptozotocin-induced diabetic rats. Exp Ther Med 13(5):2417-2422. doi:10.3892/etm.2017.4259
112. Fisusi FA, Akala EO (2019) Drug Combinations in Breast Cancer Therapy. Pharm Nanotechnol 7(1):3-23. doi:10.2174/2211738507666190122111224
113. Sang Y, Chen B, Song X, et al. (2019) circRNA_0025202 Regulates Tamoxifen Sensitivity and Tumor Progression via Regulating the miR-182-5p/FOXO3a Axis in Breast Cancer. Mol Ther 27(9):1638-1652. doi:10.1016/j.ymthe.2019.05.011
114. Hu K, Liu X, Li Y, et al. (2020) Exosomes Mediated Transfer of Circ_UBE2D2 Enhances the Resistance of Breast Cancer to Tamoxifen by Binding to MiR-200a-3p. Med Sci Monit 26:e922253. doi:10.12659/MSM.922253
115. Zang H, Li Y, Zhang X, Huang G (2020) Circ-RNF111 contributes to paclitaxel resistance in breast cancer by elevating E2F3 expression via miR-140-5p. Thorac Cancer 11(7):1891-1903. doi:10.1111/1759-7714.13475
116. Hsa_circ_0000199 facilitates chemo-tolerance of triple-negative breast cancer by interfering with miR-206/613-led PI3K/Akt/mTOR signaling - PMC. Accessed April 6, 2024. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7906206/
117. Liu Y, Dong Y, Zhao L, Su L, Luo J (2018) Circular RNA-MTO1 suppresses breast cancer cell viability and reverses monastrol resistance through regulating the TRAF4/Eg5 axis. Int J Oncol 53(4):1752-1762. doi:10.3892/ijo.2018.4485
118. Liang Y, Song X, Li Y, et al. (2019) circKDM4C suppresses tumor progression and attenuates doxorubicin resistance by regulating miR-548p/PBLD axis in breast cancer. Oncogene 38(42):6850-6866. doi:10.1038/s41388-019-0926-z
119. Wang Q, Liang D, Shen P, Yu Y, Yan Y, You W (2021) Hsa_circ_0092276 promotes doxorubicin resistance in breast cancer cells by regulating autophagy via miR-348/ATG7 axis. Transl Oncol 14(8):101045. doi:10.1016/j.tranon.2021.101045
120. Liang Y, Song X, Li Y, et al. (2019) Targeting the circBMPR2/miR-553/USP4 Axis as a Potent Therapeutic Approach for Breast Cancer. Mol Ther Nucleic Acids 17:347-361. doi:10.1016/j.omtn.2019.05.005
121. Zheng SR, Huang Q di, Zheng ZH, Zhang ZT, Guo GL (2021) circGFRA1 affects the sensitivity of triple-negative breast cancer cells to paclitaxel via the miR-361-5p/TLR4 pathway. J Biochem 169(5):601-611. doi:10.1093/jb/mvaa148
122. Yang W, Gong P, Yang Y, Yang C, Yang B, Ren L (2020) Circ-ABCB10 Contributes to Paclitaxel Resistance in Breast Cancer Through Let-7a-5p/DUSP7 Axis. Cancer Manag Res 12:2327-2337. doi:10.2147/CMAR.S238513
123. Xie H, Zheng R (2022) Circ_0085495 knockdown reduces adriamycin resistance in breast cancer through miR-873-5p/integrin β1 axis. Anticancer Drugs 33(1):e166-e177. doi:10.1097/CAD.0000000000001174
124. Gao D, Qi X, Zhang X, Fang K, Guo Z, Li L (2019) hsa_circRNA_0006528 as a competing endogenous RNA promotes human breast cancer progression by sponging miR-7-5p and activating the MAPK/ERK signaling pathway. Mol Carcinog 58(4):554-564. doi:10.1002/mc.22950
125. Cui Y, Fan J, Shi W, Zhou Z (2022) Circ_0001667 knockdown blocks cancer progression and attenuates adriamycin resistance by depleting NCOA3 via releasing miR-4458 in breast cancer. Drug Dev Res 83(1):75-87. doi:10.1002/ddr.21845
126. Wu X, Ren Y, Yao R, Zhou L, Fan R (2021) Circular RNA circMMP11 Contributes to Lapatinib Resistance of Breast Cancer Cells by Regulating the miR-153-3p/ANLN Axis. Front Oncol 11:639961. doi:10.3389/fonc.2021.639961
127. Zhu M, Wang Y, Wang F, Li L, Qiu X (2021) CircFBXL5 promotes the 5-FU resistance of breast cancer via modulating miR-216b/HMGA2 axis. Cancer Cell Int 21:384. doi:10.1186/s12935-021-02088-3