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ExRNA as theranostic agents in cancer: current progress and future perspectives

Nik Mohd Afizan Nik Abd Rahman[1,*], Che Nur Mazadillina Che Zahari[1], Mohd Azuraidi Osman[1], Noorjahan Banu Mohamed Alitheen[1], Nur Akmarina Mohd Said[2], Shazreen Shaharuddin[3] and Putri Cahaya Situmorang[4]

[1] Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang, Selangor 43400, Malaysia
[2] Department of Pharmaceutical Life Sciences, Faculty of Pharmacy, Universiti Malaya, Kuala Lumpur 50603, Malaysia
[3] Department of Physiology, Faculty of Medicine and Defence Health, University Pertahanan Nasional Malaysia, Kuala Lumpur 57000, Malaysia
[4] Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Sumatera Utara 20155, Indonesia

* Correspondence author; E-mail: m.afizan@upm.edu.my.

Highlights:

- ExRNAs are "Theranostics" for Cancer: These tiny bits of RNA, especially those in exosomes, are super promising because they can both diagnose and treat cancer.
- ExRNAs are great for "liquid biopsies" (like blood tests) because they are stable and can tell us a lot about a tumor without needing a tissue sample.
- ExRNAs can also help predict how a cancer might behave, if it will come back, or a patient's survival chances.
- Exosomes, which carry exRNAs, are like natural delivery cargo for cancer drugs.
- ExRNAs are good for tracking if cancer treatments are working or if the cancer is becoming resistant to drugs.
- The future involves combining exRNA info with other biological data (multi-omics), using AI to find patterns, and creating "smart exosomes" that precisely deliver therapies.

Abstract: Cancer remains a leading cause of mortality worldwide despite the development of novel, precise, and less invasive strategies for diagnosis, prognosis, and treatment. Encapsulated within different extracellular vesicles (EVs), especially exosomes, extracellular RNAs (exRNAs) have become important players in disease pathogenesis and intercellular communication. They are perfect candidates for liquid biopsies because of their stability, ubiquity in biofluids, and capacity to replicate the physiological and pathological conditions of parental cells. The growing role of exRNAs as "theranostic agents" in cancer, combining diagnostic, prognostic, and therapeutic capabilities, is thoroughly explored

in this review. We investigate their potential as non-invasive biomarkers for tumor classification, early detection, and disease progression or recurrence prediction. Furthermore, we discuss the expanding therapeutic potential of exRNAs, which is frequently made possible by engineered exosome platforms and ranges from delivering therapeutic RNA molecules to inhibiting oncogenic exRNAs. Finally, we also highlight the current challenges in exRNA research, such as targeted delivery, standardization, isolation, and characterization, and we outline potential future directions for integrating exRNA-based theranostics into standard clinical practice for better cancer patient care.

Keywords: exRNA; cancer theranostics; liquid biopsy; exosomes; biomarkers

1. Introduction

Uncontrolled cell growth, invasion, and metastasis are hallmarks of the complex disease known as cancer, which poses a serious threat to global health [1]. Despite recent advancements in immunotherapy, radiotherapy, chemotherapy, and surgery, problems persist, such as severe side effects, treatment resistance, and late-stage diagnosis. A promising paradigm shift towards personalized and precision medicine in oncology is provided by the idea of "theranostics," which combines therapeutic and diagnostic approaches on a single platform [2]. By delivering targeted therapies, predicting treatment response, and identifying biomarkers for early disease detection, this strategy aims to improve patient outcomes.

Because of their crucial functions in intercellular communication, as well as their potential as novel disease biomarkers and therapeutic delivery vehicles, extracellular vesicles (EVs), and exosomes in particular, have garnered a lot of attention in recent years [3]. Almost every type of cell secretes exosomes, which are nanoscale (30–150 nm) lipid bilayer vesicles [4]. They transport a wide range of biomolecules, such as proteins, lipids, DNA, and different types of RNA, which are referred to as extracellular RNAs (exRNAs) [5]. These exRNAs consist of messenger RNAs (mRNAs), transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), and, prominently, non-coding RNAs (ncRNAs) such as microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) [6].

Exosomes are frequently secreted by cancer cells, and the molecular environment of the parent tumor is reflected in the exosomal cargo [7]. Tumor-derived exRNAs are very desirable candidates for liquid biopsies because of this special quality, which offers a non-invasive method to evaluate tumor presence, heterogeneity, and treatment limitations. Exosomes' capacity to transport functional exRNAs to recipient cells presents previously unheard-of possibilities for therapeutic interventions, such as modifying gene expression in cancer cells or the tumor microenvironment, in addition to their diagnostic applications [8].

This review aims to provide a comprehensive overview of the current understanding and emerging applications of exRNAs as theranostic agents in cancer (Figure 1). We will discuss their potential as prognostic and diagnostic biomarkers, explore using them therapeutically, and address the major obstacles that need to be eradicated before they can be successfully integrated into clinical applications.

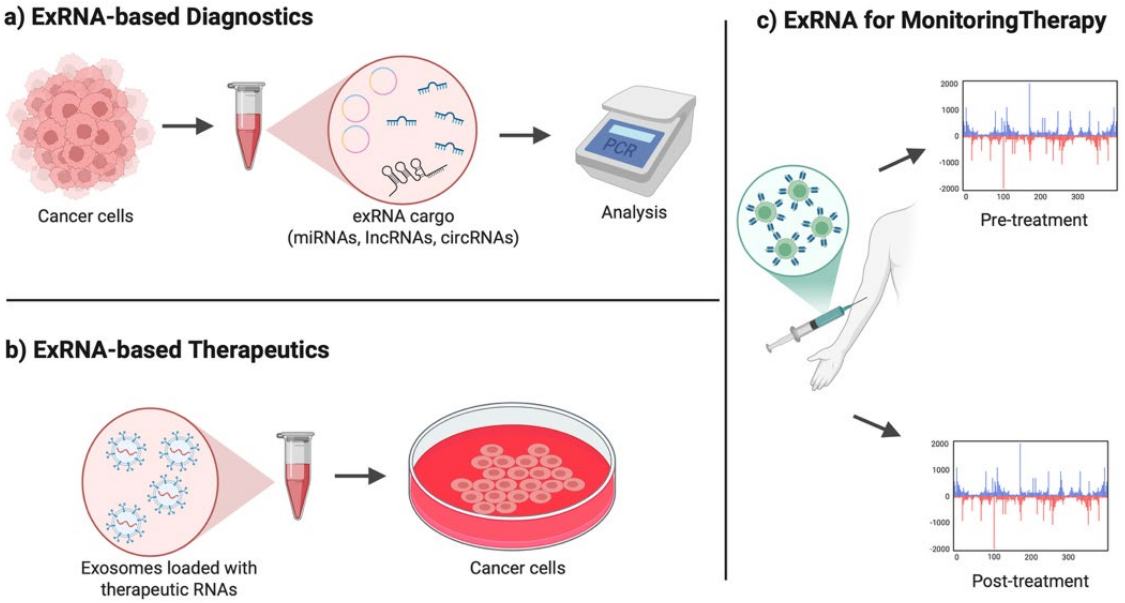

Figure 1. Overview of exRNA-based strategies. The multifaceted applications of exRNA in cancer, including **(a)** diagnostics, **(b)** therapeutics, and **(c)** monitoring of therapy.

2. ExRNA biogenesis and cargo packaging

The endosomal pathway is the source of exosomes. Exosomes originate from the endosomal pathway by the formation of the early sorting endosome (ESEs), late sorting endosome (LSEs), and ultimately the multivesicular bodies (MVBs) [9]. In particular, early endosomes develop into late endosomes, which subsequently undergo inward budding of their limiting membrane to form intraluminal vesicles (ILVs) inside MVBs [10]. MVBs, now referred to as exosomes, have the ability to either fuse with lysosomes for breakdown or dock and fuse with the plasma membrane, releasing ILVs into the extracellular environment [11].

The packaging of specific RNAs into exosomes is not a random process but rather a highly regulated and selective mechanism, although the precise molecular machinery is still being fully elucidated. This non-random packaging is evidenced by several proposed mechanisms that suggest a selective loading process for exosomal RNA cargo (Table 1). These mechanisms include: i) RNA-binding proteins (RBPs), ii) ceramide-mediated budding, iii) lysosomal-associated multivesicular body protein (LAMP), and iv) direct budding from the plasma membrane:

Table 1. Mechanisms of RNA packaging into extracellular vesicles.

Mechanism	Description	Key players	References
RNA-binding proteins (RBPs)	Specific RBPs are known to bind to particular RNA sequences and facilitate their encapsulation into forming vesicles within the cell.	Ago2, hnRNPA2B1, Y-box protein 1 (YB-1), heterogeneous nuclear ribonucleoproteins (hnRNPs)	[12–14]
Ceramide-mediated Budding	The lipid ceramide plays a role in the inward budding of endosomal membranes, which can influence which RNAs are included in the vesicles.	Ceramide	[15–18]
LAMP2B Association	Some RNAs may associate with specific LAMP proteins found on the surface of multivesicular bodies (MVBs) to be packaged into exosomes.	Lysosomal-associated multivesicular body protein (LAMP) 2B	[19–21]
Direct budding from the plasma membrane	While less common for typical exosomes, some vesicles can bud directly from the cell's outer membrane, carrying a unique cargo.	Plasma membrane	[3,22–24]

Once released, exosomes can pass through bodily fluids and be absorbed by distant recipient cells through phagocytosis, endocytosis (caveolin-mediated, clathrin-mediated), or direct membrane fusion [25]. After internalization, exosomal cargo, including exRNAs, is discharged into the recipient cell's cytoplasm, where it can alter gene expression and cellular functions, affecting angiogenesis, immune evasion, metastasis, and cancer progression [26].

3. ExRNA as diagnostic biomarkers in cancer

To improve patient outcomes, non-invasive, highly sensitive, and specific biomarkers for early cancer detection and diagnosis must be identified. ExRNA, especially those taken from liquid biopsies, can be used instead of traditional tissue biopsies because they are stable, easy to access, and can change over time to reflect tumor biology [27].

The desirability of exRNAs over other liquid biopsy components like circulating tumor DNA (ctDNA) or proteins stems from several key advantages: ExRNAs, particularly those encapsulated within exosomes, exhibit remarkable stability in biofluids, making them less prone to degradation compared to naked RNA or even some forms of ctDNA [28]. This inherent stability ensures better detectability and a more accurate representation of the tumor's molecular landscape. For instance, circular RNAs (circRNAs) are highly stable due to their closed-loop structure, providing a significant benefit for detection as biomarkers [29]. Furthermore, exosomes are actively secreted by cancer cells, and their cargo, including exRNAs (such as miRNAs, lncRNAs, and circRNAs), directly reflects the real-time molecular environment and physiological or pathological conditions of the parental tumor cells [30]. These features

offer an ever-evolving and comprehensive snapshot of tumor presence, heterogeneity, and evolution that might not be fully captured by analyzing static DNA mutations or transient protein expression alone [31]. The dynamic responsiveness means exRNA profiles can change in response to tumor progression, the efficacy of therapeutic interventions, and the development of drug resistance. This makes them exceptionally valuable for real-time monitoring of treatment response, early detection of resistance, and predicting disease recurrence, thereby enabling adaptive and personalized clinical decision-making [32]. Finally, exRNAs contained within exosomes are crucial mediators of intercellular communication, actively influencing processes like angiogenesis, immune evasion, metastasis, and overall cancer progression by altering gene expression in recipient cells [33]. This functional aspect provides unique insights into the tumor's active signaling with its microenvironment, a dimension often not directly revealed by isolated DNA or protein analysis.

3.1. MicroRNAs (miRNAs)

Exosomal miRNAs have been widely studied and show great promise as diagnostic biomarkers in cancer. Their abnormal levels are well-reported across various types of cancers, and because we can find them in easily accessible bodily fluids, they're highly attractive candidates for new diagnostic tests [34]. For early detection, exosomal miRNAs are proving valuable in identifying cancer at its earliest stages when treatment is most effective [35]. For instance, higher levels of miR-21, miR-145, and miR-210 in the blood plasma of lung cancer patients have shown promise for early diagnosis, even if they are found in the initial stages of the disease [36,37]. In pancreatic cancer, serum exosomal miR-21, miR-107, and miR-210 have been highly effective at identifying the difference between patients with pancreatic cancer, healthy individuals, and those with benign pancreatic conditions [38,39]. Similarly, specific combinations of exosomal miRNAs, such as the miR-21 cluster, miR-92a, and miR-223, found in plasma or stool samples are being explored for non-invasive screening in colorectal cancer [40,41]. Beyond just early detection, these specific exosomal miRNA signatures can also help us distinguish between different cancer types or subtypes, which is crucial for tailoring personalized treatment plans. For instance, the levels of exosomal miR-223 can help doctors differentiate between neuroendocrine tumors and other gastrointestinal cancers [42].

3.2. Long non-coding RNAs (lncRNAs)

Similar to miRNA, exosomal long non-coding RNAs (lncRNAs) are also increasingly recognized as powerful diagnostic biomarkers, primarily due to their unique tissue-specific expression and significant roles in cancer development and progression. For instance, exosomal lncRNA H19 and GAS5 measurements in plasma have great potential for diagnosis; elevated H19 levels and decreased GAS5 levels are often indicative of gastric cancer [43]. While lncRNA PCAT1 and exosomal PCA3 (prostate cancer gene 3) are being actively studied for non-invasive detection of prostate cancer, they may have higher specificity than conventional PSA tests [44]. Furthermore, exosomal lncRNAs HEIH and HOTAIR have been found to be useful biomarkers for precisely identifying the stage of hepatocellular carcinoma (HCC) as well as for cancer diagnosis [45].

3.3. Circular RNAs (circRNAs)

CircRNAs are very stable because they're closed-loop RNA molecules, which makes them especially useful as biomarkers in bodily fluids compared to linear RNAs that quickly break down. In fact, this stability provides a significant benefit for detection. For instance, exosomal S100A9 has been found to be substantially elevated in the blood plasma of patients with colorectal cancer (CRC), suggesting that they may be used as diagnostic markers [46]. According to a clinical study, circRNAs like circ_0067934 that are present in urine exosomes may provide a non-invasive means of detecting bladder cancer [47]. Furthermore, a promising new diagnostic biomarker for non-small cell lung cancer has been discovered: exosomal circ-IARS [48].

4. ExRNA as prognostic biomarkers in cancer

ExRNAs are proving to be immensely useful for prognosis, which goes beyond just diagnosing cancer. They provide information about how a disease might progress, the possibility that it will recur, and even a patient's chances of survival [49]. For clinical decision-making and patient treatment plan adaptation, this information is completely important.

4.1. MicroRNAs (miRNAs)

Pertaining to miRNAs, high levels of exosomal miR-105 from breast cancer cells have been associated with a worse prognosis and a higher risk of metastasis, in part because it increases blood vessel permeability [50]. Furthermore, exosomal miR-21 is consistently linked to a lower overall survival rate for a number of cancer types [51]. Exosomal miR-211 has been linked to BRAF inhibitor resistance in melanoma, which aids in identifying patients who may not respond well to treatment [52]. Additionally, higher exosomal levels of miR-145 and miR-200a are associated with metastases, more advanced stages, and shorter survival times for ovarian cancer [53].

4.2. Long non-coding RNAs (lncRNAs)

Long non-coding RNAs (lncRNAs) can serve as powerful indicators for predicting how a disease, particularly cancer, is likely to behave in a patient. For instance, exosomal lncRNA UCA1 has been found to be a predictor of a worse prognosis and chemotherapy resistance in gastric cancer when its levels are elevated [54]. On the other hand, exosomal lncRNA MALAT1 levels in prostate cancer are linked to advanced disease and the risk of postoperative biochemical recurrence [55]. In the meantime, exosomal lncRNA PVT1 has been linked to both poor prognosis and resistance to gemcitabine, a common chemotherapy drug in pancreatic cancer [56].

4.3. Circular RNAs (circRNAs)

Lastly, circRNAs are becoming important prognostic markers as well. Exosomal circ_0029325 has been demonstrated to predict overall survival and disease recurrence in hepatocellular carcinoma (HCC) [57]. Elevated exosomal circ_0000284 is linked to larger tumors, more advanced stages, and a generally worse prognosis for colorectal cancer (CRC) [58]. These findings suggest that circRNAs could serve as

valuable biomarkers for predicting outcomes in various types of cancer. Extensive research is needed to fully understand the role of exosomal circRNAs in cancer progression and treatment response.

Area under the Curve (AUC) is one crucial indicator used to assess the operation of a binary classification model, especially when dealing with Receiver Operating Characteristic (ROC) curve. It measures the capability of a model in discriminating between positive and negative cases and thus varies between 0 and 1- where a score of 0.5 implies no discrimination and 1.0 as perfect discrimination [59]. In the context of exRNAs as biomarkers, AUC is an important variable determining the diagnostic and prognostic performance in a number of cancers [60]. Differences in AUC values of exRNA biomarkers may occur because of the following reasons sample size, variation among populations, biological variability in the expression of biomarkers, methods of measurement, complexity of models, and how the threshold is selected to divide data into categories [61]. It is possible that knowing these factors can help provide the correct interpretation of these AUC values, as it can strongly determine the validity and usefulness of diagnostic or prognostic models in the clinical setting.

5. ExRNA as therapeutic agents in cancer

A key advantage of using exosomes as organic drug delivery vehicles for cancer treatment is demonstrated by the functional transfer of exRNAs into recipient cells. Exosomes provide some of the benefits, such as low immunogenicity, high biocompatibility, the capacity to penetrate biological barriers (such as the blood-brain barrier), and intrinsic targeting capabilities [62,63].

5.1. Delivery of therapeutic RNAs

Exosomes are showing incredible promise as natural delivery systems for various therapeutic RNAs aimed at treating cancer. They can be manipulated to transport antitumor miRNAs like miR-145, miR-34a, and let-7b directly to cancer cells. Once delivered, these miRNAs can work to inhibit cancer cell growth, trigger their self-destruction, and even make them more sensitive to chemotherapy [64]. For instance, exosomes from mesenchymal stem cells, when loaded with miR-145, have effectively suppressed colon cancer by targeting cancer-promoting genes like c-Myc [65]. Similarly, exosomes from normal fibroblasts, engineered to produce more miR-34a, could inhibit lung cancer cell growth and spread [66]. Besides miRNAs, exosomes are also capable of delivering siRNAs or shRNAs to switch off genes that promote cancer. An exciting instance of this is how exosomes derived from dendritic cells, being modified with a Lamp2b-targeting peptide, were able to deliver Kras-specific siRNA to pancreatic cancer cells and successfully inhibit tumor growth in living organisms [67]. While it's less common and still presents challenges in ensuring efficient protein production, exosomes theoretically also hold the potential to deliver therapeutic mRNAs for expressing beneficial proteins in target cells.

5.2. Inhibition of oncogenic exRNAs

Directly preventing the adverse effects of oncogenic exRNAs is another promising strategy in addition to delivering therapeutic RNAs. Anti-miRNA oligonucleotides (AMOs), which are made expressly to prevent cancer-promoting exosomal miRNAs from entering recipient cells, are one method [68]. This approach is more indirect but still works; for instance, it has been demonstrated that inhibiting exosomal miR-21 effectively lowers the proliferation and metastasis of cancer cells [69]. Making exogenous RNA

"sponges" that competitively bind to oncogenic exosomal lncRNAs or circRNAs and inhibit them from interacting with their natural targets, like endogenous miRNAs, is another new therapeutic approach that aims to counteract the cancer-promoting effects of these molecules.

5.3. Engineering exosomes for enhanced therapy

Significant efforts are now being made to transform exosomes into even more effective and precise therapeutic instruments in cancer treatment. One key strategy involves adding targeting ligands such as antibodies, peptides, or aptamers to the exosome surface. This ensures that the therapeutic cargo is delivered precisely where it is needed by acting as a molecular address that directs them to specific cancer cells or specific regions within a tumor [70]. Researchers are also focused on increasing the loading efficiency of therapeutic RNAs into exosomes using methods such as electroporation, sonication, or saponin treatment while meticulously maintaining the integrity of the exosome [71]. To monitor their effectiveness in real-time, scientists are working on incorporating reporter systems within exosomes for tracking their delivery and therapeutic impact. Finally, an exciting frontier is the development of immunomodulatory exosomes, engineered to deliver RNAs that can reprogram immune cells within the tumor's immediate environment [72]. For instance, shifting M2 macrophages to their anti-tumor M1 state or activating T cells will ultimately enhance the body's own immune response against the cancer [73,74].

6. ExRNA as monitoring strategies in cancer therapy

Due to exRNAs constantly evolving, they are extremely useful elements for tracking the effectiveness of cancer treatments, anticipating when medications may prevent effective, and even identifying any disease or recurrence that may still exist [32]. This enables medical professionals to modify therapy in real time, resulting in individualized patient care. Some changes in particular exosomal miRNA levels, like miR-21 or miR-1246, have been demonstrated to be directly correlated with the means in which patients react to chemotherapy or targeted therapies for a variety of cancers, making them useful for tracking treatment response [75]. While an increase may unfortunately indicate drug resistance or the advancement of the disease, a decrease in exRNAs originating from the tumor may indicate that treatment is effective.

In addition, exRNAs are showing promise in the prediction of drug resistance. Specifically, resistant cancer cells can actually transfer their resistance to sensitive cells through exosomal circRNAs (like circ_0000092 in colon cancer) or miRNAs (like miR-21 in glioblastoma), and their elevated levels can predict treatment failure [76,77]. Clinicians can be encouraged to proactively transition to alternative therapies by monitoring these resistance-associated exRNAs. Lastly, exRNAs provide a potent method for identifying minimal residual disease (MRD). Tumor-specific exRNAs may be a sign of MRD and enable much earlier intervention to avoid a complete relapse if they are consistently detected even after primary treatment that appears to be successful [78].

7. Challenges and future perspectives

7.1. Challenges

Despite their immense promise, several challenges must be tackled before exRNA-based theranostics can fully realize their full potential in clinical practice. Firstly, isolation and characterization remain a major hurdle. Current methods for isolating exosomes, such as size exclusion chromatography and ultracentrifugation, frequently produce low yields and contamination from other vesicles or proteins [79]. More importantly, there is a serious lack of standardization, which makes it challenging to compare research findings across different labs. To guarantee repeatable research and a seamless transition to the clinic, we urgently need a global agreement on the correct methods for isolating, characterizing, and quantifying exRNAs (according to standards like MISEV) [80]. On top of that, reliable, high-throughput, and cost-effective platforms for comprehensive exRNA profiling are essential for large-scale clinical application.

Secondly, biodistribution and targeted delivery present significant challenges. It is challenging to precisely target therapeutic exosomes to cancer cells while protecting healthy tissues because systemic administration frequently results in non-specific uptake by macrophages and other cells [81]. Even though exosomes can pass through the blood-brain barrier (BBB), research is still ongoing to determine how most effectively to deliver them to brain tumors. We also need a much better understanding of the half-life, clearance, and distribution of therapeutic exosomes in the body in order to optimize dosing and administration routes [82]. Thirdly, immunogenicity and safety are paramount concerns. Exosomes are generally considered to have low immunogenicity, but using genetically modified exosomes or administering them repeatedly, especially from non-autologous (non-self) sources, may cause unintended immune reactions [83].

Furthermore, delivering therapeutic RNAs, particularly miRNAs, can have unintended off-target effects because they can regulate multiple genes. As a result, careful design and comprehensive safety profiling are absolutely essential. Lastly, scaling up for GMP (Good Manufacturing Practice) production of therapeutic exosome preparations remains a significant challenge [84]. Finally, the regulatory pathway for treatments based on exosomesis still very emerging. Clear guidelines from regulatory agencies such as the FDA and EMA are urgently needed to provide the standard procedure for their clinical approval [85].

7.2. Future perspectives

ExRNA-based theranostics has a very promising future because of a few of innovative strategies. Multi-omics integration is one important avenue in which we integrate exRNA profiles with other important biological information, including circulating tumor DNA (ctDNA), lipids, exosomal proteins, and even single-cell RNA sequencing of tumors [86]. We would be able to create far more reliable and accurate models for identifying cancer and forecasting its progression in part to this comprehensive data fusion.

The field is also expected to undergo a revolution because of machine learning and artificial intelligence. Finding complex exRNA signatures and creating predictive algorithms that can more accurately direct diagnosis, prognosis, and treatment response will require the use of these potent

computational approaches [87]. The creation of "smart exosomes" or "designer exosomes" is an inventive idea. These modified vesicles would be programmed to react to stimuli in the tumor microenvironment, such as pH variations, particular enzymes, or even light, in order to release their therapeutic cargo precisely when and where it is required, improving the spatial and temporal control of therapy [88]. For instance, Kras-specific siRNA to pancreatic cancer cells, successfully inhibiting tumor growth in living organisms [89]. This demonstrates the potential of engineered exosome platforms for targeted therapy, aligning with Figure 1's therapeutic panel (b) which illustrates exosomes loaded with therapeutic RNAs treating cancer cells. While this highlights a promising avenue, ongoing research continues to explore and establish more widespread *in vivo* efficacy outcomes and their impact on tumor reduction and survival rates.

In addition, hybrid exosomes and biomimetic nanoparticles are starting to appear. These cutting-edge innovations aim to combine the best qualities of synthetic nanoparticles, such as controllable size, scalable production, and highly precise cargo loading, with the best qualities of natural exosomes, such as their innate biocompatibility and targeting abilities [90]. In the end, preclinical research must give way to carefully planned, extensive clinical trials if these developments are to be genuinely beneficial to patients [91]. These studies are essential for firmly establishing exRNA-based theranostics' practical applicability and securing their position in standard clinical practice for better cancer patient care.

8. Conclusion

In conclusion, exRNAs have become a novel class of molecules with enormous potential as theranostic agents in cancer especially those encapsulated within exosomes. They are uniquely positioned for non-invasive liquid biopsy applications for early detection, accurate diagnosis, and prognostication due to their unmatched stability in biofluids, dynamic reflection of tumor biology, and innate capacity for intercellular communication. At the same time, a potent new paradigm for targeted cancer therapy and real-time treatment efficacy monitoring is provided by the ability to engineer exosomes to deliver therapeutic RNA molecules or block oncogenic pathways. Standardization, large-scale production, targeted delivery, and regulatory approval are still major obstacles, but these should be addressed by the quick developments in exRNA research and exosome engineering. The exciting potential to revolutionize cancer treatment and open the door to genuinely individualized and successful precision oncology lies in the incorporation of exRNA-based theranostics into standard clinical practice.

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Authors' contribution

Conceptualization, P.C.S. and N.B.M.A.; methodology, N.A.M.S. and S.S.; validation, N.M.A.N.A.R. and N.B.M.A.; formal analysis, C.N.M.C.Z.; writing—original draft preparation, N.M.A.N.A.R. and C.N.M.C.Z.; writing—review and editing, N.M.A.N.A.R., C.N.M.C.Z and M.O.A. All authors have read and agreed to the published version of the manuscript.

Conflicts of interests

The authors declare no conflicts of interest.

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