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Exosome-loaded nanoradiosensitizers in radiotherapy for preventing post-irradiation tumor recurrence: mechanisms, preclinical evidence, and translational challenges

Daniel Ejim Uti[1,2*], Josephine E. Egbung[3], Bassey Omeh Anthony[4], Ogar Rose Omari[4], Wilson Achu Omang[11], Waheeb Sami Aggad[5], Hailah M. Almohaimeed[6], Neeraj Bainsal[7], Sandeep Kumar Shukla[8], Kranti Kiran Reddy Ealla[9] and Esther Ugo Alum[2,10]

*Correspondence:
Daniel Ejim Uti
dan4uti@gmail.com; daniel.ejimuti@kiu.ac.ug; daniel.uti@fuhso.edu.ng

Full list of author information is available at the end of the article

Abstract

Radiotherapy remains a cornerstone in cancer treatment, yet its therapeutic efficacy is often limited by radioresistance, tumor heterogeneity, and recurrence post-irradiation. Nanotechnology has emerged as a promising avenue to enhance radiosensitization, improve tumor selectivity, and reduce off-target toxicity. Among these, exosome-loaded nanoradiosensitizers represent an innovative strategy, leveraging the natural biocompatibility, tumor-homing ability, and immunomodulatory properties of exosomes to deliver radiosensitizing agents with precision. This review examines the design principles, fabrication strategies, and functional mechanisms of exosome-based nanoradiosensitizers, highlighting how they enhance DNA damage, modulate the tumor microenvironment, and overcome intrinsic and acquired radioresistance. We discuss the molecular mechanisms underlying radiosensitization, including reactive oxygen species (ROS) amplification, cell-cycle modulation, hypoxia alleviation, and immune system engagement. Furthermore, we explore in vitro and in vivo preclinical evidence demonstrating the efficacy of these systems in reducing tumor recurrence post-irradiation. Challenges such as scalable exosome production, cargo loading efficiency, targeted delivery, and regulatory considerations are critically evaluated. Finally, the review outlines translational perspectives, potential combinatorial therapies, and key considerations for clinical trial design. By integrating insights from nanomedicine, radiobiology, and exosome biology, this review aims to provide a comprehensive framework for the development of next-generation exosome-based nanoradiosensitizers, ultimately enhancing radiotherapy outcomes and minimizing tumor relapse in resistant cancers.

Keywords Exosome-based nanocarriers, Radiosensitization, Tumor recurrence, Radiotherapy enhancement, Tumor microenvironment

1 Introduction

Radiotherapy has continued to play a role as a focus of treatment in oncology, playing a role in curative as well as palliative therapy in a host of solid and hematologic malignancies [1]. The therapeutic action largely hinges on the fact that it causes irreversible destruction of the cancer cell DNA and oxidative stress, which is aided by significant progress in treatment planning, image guidance, and accuracy in dose delivery [2]. Nonetheless, the improvements are only able to achieve durable tumor control in most patients due to the influence of biological factors in the tumor that may decrease radiation responsiveness. Specifically, radioresistance, intratumor heterogeneity, and post-treatment recurrence remains a thorn in the side of the clinical outcomes and limit the full therapeutic efficacy of radiotherapy [3].

These obstacles are intricately tied. The population of resistant tumor cells are able to endure radiation by repairing DNA more effectively, antioxidant protection, altering cell-cycle control, and pro-survival signaling [4]. Simultaneously, heterogeneous tumor structure and microenvironment variations (such as hypoxia), leave patches of diverse radiosensitivity. Collectively these characteristics permit the residual malignant cells to continue existing after the treatment and provide contributions to the local relapse or the more malignant regrowth. These biological constraints have thus been one of the key focus areas in the evolution of next-generation radiosensitization approaches.

Top effective DNA damage recognition and repair mechanism, elevated antioxidative defense, modified cell cycle kinetics, and anti-apoptotic postulation are often combined to mediate intrinsic radioresistance [5]. Tumor cells often enhance essential cell repair mechanisms, such as non-homologous end joining and homologous recombination, to overcome radiation-induced double-strand breaks, to be able to survive near-lethal dose radiation exposures [5]. At the same time, the improved ability of redox buffering facilitated by glutathione systems, superoxide dismutases, catalase, and other enzymes counteracts reactive oxygen species caused by ionizing radiation that reduces oxidative stress to DNA, proteins, and cellular membranes [6]. Subpopulations of cancer stem-like cells further increase the resistance through integration of quiescence, high repair abilities, and pro-survival signals which allow them to regenerate the tumor after radiotherapy [7]. Acquired resistance occurs when repeated irradiation is preferentially selective of the more fit clones, or is associated with adaptive responses, i.e., epithelial mesenchymal transition, re-programming of metabolic state and evasion of immune responses [8]. In total, all the processes narrow the therapeutic window wherein an increase in dose leads to an increase of collateral damage on the normal tissues without necessarily ensuring an eradication of tumors.

There is an additional complexity created by a tumor heterogeneity. Neoplasms are not usually heterogeneous collections of identical cells; rather, they are represented by spatially and temporally disparate subclones possessing different genotypes, phenotypes and microenvironmental niches [9]. This heterogeneity is indicated by different radiosensitivity: in an individual lesion, the most interesting part of it can respond well and yet there are clones that are not eradicated but are ready to survive [10]. An indispensable factor in this intra-tumor heterogeneity is the tumor microenvironment, in particular, hypoxic areas [11]. Hypoxic areas show a lowered sensitivity to radiotherapy since oxygen elevates radiation effects of causing damages to DNA by repairing the radical lesion as permanent damage [12]. Lack of perfusion and abnormal vasculature produces

dynamic oxygen differences, making foci of hypoxia to be present where adjoining tissue has good oxygen [12]. Besides the oxygenation, stromal communication, extracellular matrix density, and immune constitution may be used to optimize radiation effects by affecting inflammation, repair programs, and the post-irradiation remodelling [1]. This means that although radiotherapy delivery may be optimum under all technical conditions, biological non-uniformity may be a hindrance to full tumor control.

The final clinical presentation of the mechanisms described above is often recurrence. The remaining viable cells (covered by radiotherapy) especially the resistant subclones and stem-like cells may have residual options to seed the regrowth, and local recurrence is observed [13, 14]. Additionally, radiotherapy is capable of stimulating wound-related roles of healing, which will be followed by bone activity to restructure the tumor environment, sometimes forming environments that enable tumor repopulation [15]. Post irradiation increases production of inflammatory mediators, growth factors, and pro-angiogenic signals and gives the surviving cells an environmental signal that favors cell proliferation, invasion or metastasis [16]. Notably, recurrence is not just a failure in the treatment process, but can be also a manifestation of evolutionary selection, i.e., the post-radiotherapy tumor is more aggressive and resistant to further treatment [16]. Therefore, the problem of improving radiosensitivity without reducing the normal tissue sparing is a pressing translational issue.

Nanotechnology has become an attractive platform thus to overcome these drawbacks by enabling the delivery of therapeutic agents in a targeted fashion whereby their release is controlled and they have the ability to multiply their roles as multifunctional [17]. Nanoparticles may be optimized with regards to size, shape, surface chemistry, and composition in order to improve the accumulation and cellular uptake in tumors, increase circulation time, and decrease off-target toxicity [17, 18]. Since radiotherapy uses radiomaterials, nanomaterials have been used as radiosensitizers (1) to enhance local dose delivery with high-atomic-number elements that establish increased production of secondary electrons, (2) to activate ROS generation to enhance oxidative stress, and (3) to deliver molecular inhibitors that prevent the repair or pro-survival signaling of DNA [19–21]. Furthermore, chemotherapeutics, immunomodulators or hypoxia flourishing agents can be internalized within nanocarriers, establishing combinatorial, removing radioresistance and multi-level heterogeneity [19, 22].

Simultaneously, exosomes, a type of trans-membrane extracellular vesicles (NCVs) released by most cell types, have found interest as a delivery system suitable in oncology with means of being stealth since, by definition, they are nanoparticles with dimensions less than 1 mm [19]. These exosomes are naturally involved in the movement of proteins, lipids, messenger RNA, and non-coding RNA among cells, thus determining intercellular communication in tumors and in the microenvironment around them [23, 24]. Their natural derivation comes with a number of benefits to drug delivery, namely, high level of biocompatibility, lesser immunogenicity than synthetic systems, inherent stability in biological fluids and the ability to penetrate physiological barriers [25]. Exosomes can be genetically engineered or engineered to be tissue-tropic, and their membranes can be coated with tracking ligands to increase their effectiveness as vectors of therapeutic RNAs (e.g., siRNA or miRNA) as well as small molecules and proteins with interest in the regulation pathways of radiosensitivity [26, 27].

Nanotechnology and exosome biology have reached their intersection, and this presents an excellent reason to use exosome-based nanoradiosensitizers. Theoretically, nanoparticles enhance physicochemical potency, i.e., dose amplification, ROS catalysis, and stimulus stimulated release, whereas exosomes are biologically optimised delivery to immune evasion, long-term stability, and cellular internalisation [28, 29]. Combining all these complementary strengths, exosome loaded nanoradiosensitizers may lead to improved tumor selective radiosensitization and decrease collateral destruction of normal tissues. Mechanically, these systems can be used to respond to radioresistance, whereby the DNA repair inhibitor or redox modulators are targeted to the resistant subclones, to enhance damage caused by radiotherapy in the location where this damage is most harmful. To address heterogeneity, they can enhance penetration into poorly perfused areas, as well as, by exploiting exosomes to mediate communication to access different compartments of the tumor. Moreover, they can inhibit recapitulation by damaging stem-like populations and reconditioning the post-irradiation microenvironment by RNA cargo which blocks repopulation cues or boosts anti-tumor immunity (Fig. 1).

Altogether, the existence of clinical challenges of radioresistance, heterogeneity, and recurrence supports the view that next generation radiosensitizers should be sought further. Exosome based nanoradiosensitizers are a strategic methodology that avoids the use of additional radiation to enhance radiation effect and additionally uses exosomal delivery methodology to enhance specificity, uptake, and tolerability. This system is, therefore, poised to be a high potential avenue to expand the therapeutic window of radiotherapy and provide lasting and at least tumor control in the currently hard-to-treat cancers.

To avoid terminological ambiguity, we use "exosome-loaded nanoradiosensitizer" here to mean a hybrid construct in which a bona fide exosome serves as the carrier and a radiosensitizing nanomaterial or radiosensitizing cargo is loaded within the vesicle lumen and/or incorporated into its membrane while preserving exosomal identity and delivery function. This should be distinguished from exosome-coated nanoparticles, in which a synthetic nanoparticle remains the primary core and exosomal membrane is used mainly as an external camouflage or targeting shell; from exosome-mimetic vesicles, which are artificially generated vesicles designed to resemble exosomes but are

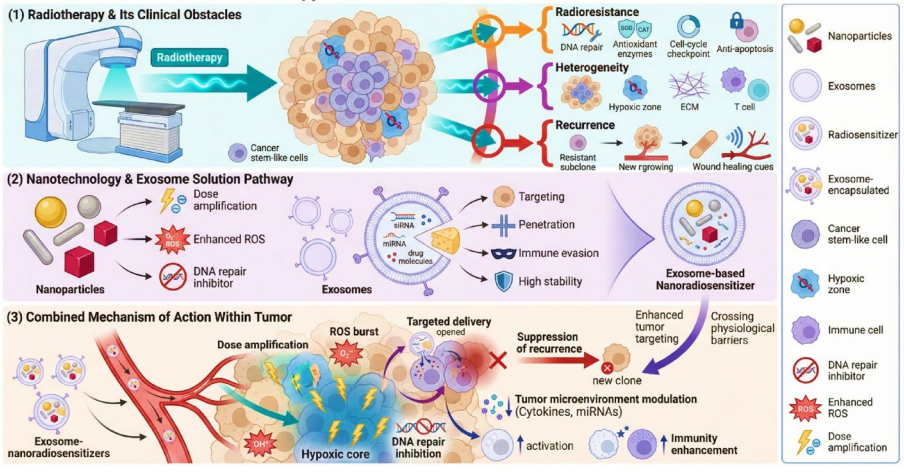

Fig. 1 How exosome-loaded nanoradiosensitizers overcome radioresistance, intratumoral heterogeneity, and recurrence-associated survival pathways

not naturally secreted endosomal extracellular vesicles; and from broader exosome-mediated delivery of radiosensitizing cargos, a wider category that includes delivery of siRNA, drugs, proteins, or signaling molecules by exosomes even when no nanoparticle component is present. In this review, the core focus is the first category true exosome-based hybrid radiosensitizer platforms while the other three are discussed as related but conceptually distinct comparator or neighboring strategies.

1.1 Evidence status, unresolved questions, and scope of this review

A number of elements in this field have been well established. Firstly, radioresistance, heterogeneity of tumors, hypoxia, and post-radiation recurrence are known biological predictors of radiotherapy failure [30, 31]. Secondly, the use of internalizing nanoradiosensitizers, especially high-Z and multi-functional nanomaterials, is already documented to boost radiation responses due to local dose amplification, ROS generation, and destabilization of DNA repair [32, 33]. Thirdly, exosomes are built biological vesicles having endogenous membrane composition, with intercellular cargo-transfer capacity, and possible use as therapeutic carriers [34].

However, some of the most appealing arguments on exosome-loaded nanoradiosensitizers are tentative, as opposed to definitive. These are better intratumoral penetration, more effectual delivery to resistant subclones, improved hypoxic or stromal niche functioning, improved immune compatibility subsequent to repeating delivery, and meaningful suppression of post-irradiation recurrence. Above all, wide supremacy over traditional nanoradiosensitizers and liposomal formulations, polymeric carriers, or free radiosensitizers has not yet been demonstrated in a consistent and clinically relevant manner.

To this end the essential question of translation research should not be the conceptual feasibility of exosome-based nanoradiosensitizers, but whether exosome-based nanoradiosensitizers can provide meaningful value compared to current delivery approaches. This involves separating intrinsic radiosensitizing potency, delivery-related benefit, identifying where the literature is contradictory, and distinguishing between those bottlenecks that are generic to all nanoradiosensitizers and those that are peculiar to hybrid exosome-based systems.

2 Methodology

The article is a narrative review aimed at synthesizing and carefully criticizing the extant literature on exosome-loaded nanoradiosensitizers and their use in optimizing the effects of radiotherapy and preventing recurrence of tumors. In sharp contrast to systematic reviews, thematic and conceptual approach, which focuses on multidisciplinary evidence (nanomedicine, radiobiology, and exosomes biology) was followed in this study; their selection was not guided by the PRISMA guidelines or the predetermined protocol.

2.1 Review framework and structure

The review has a narrative style with a logical progression of: Principles in radiotherapy resistance and tumor biology, Biological and functional properties of exosomes, Design concepts and processes of nanoradiosensitizers, Strategies for exosome loading and functionalization, Preclinical studies and mechanism understandings, and Translational

issues, and clinical attitudes. This organization facilitates a unified approach to the biological mechanisms coupled to engineering strategies and clinical implication; reflecting the interdisciplinary nature of the field.

2.2 Comparative analytical framework

To enhance critical interpretation to more than descriptive summary, exosome-loaded nanoradiosensitizers are compared to four classes of comparator platforms; conventional nanoradiosensitizers without exosomes encapsulation, liposomal formulation, polymeric nanocarrier and free radiosensitizing agent. The comparison is done based on the following criteria: intrinsic radiosensitization mechanism, delivery efficiency, tumor selectivity, ability to load into multiple cargos, evidence maturity, in vitro/3D/in vivo systems, manufacturability, and translational bottlenecks. This framework is supposed to draw the line between what has been verified to be beneficial and what is merely a speculative argument, as well as to be able to set the contexts where exosome loading can really provide an added value.

2.3 Literature search strategy

A literature searches through specific scientific data bases, such as PubMed, Scopus, Web of Science and Google Scholar, was performed to boost transparency and rigor. The search centered on published articles within the previous 10–15 years with a special emphasis on recent progress. Keywords and combinations were: exosomes" OR extracellular vesicles, nanoradiosensitizers" OR nanoparticle radiosensitization, radiotherapy resistance" OR tumor recurrence, drug delivery systems and cancer nanomedicine.

2.4 Inclusion and exclusion criteria

Studies were included on the basis of relevance to the scope of the review as opposed to systematic criteria. Included studies were, Peer-reviewed original research articles, and reviews, Studies on exosome biology, nanoparticle-based radiosensitization, or combined systems, Preclinical (in vitro and in vivo) and translational studies related to radiotherapy.

Filtered studies were: Studies which were not relevant to radiosensitization or exosome based delivery, abstract only papers or non-peer reviewed literature, and retracted literatures.

2.5 Data mining and summarization

Appropriate findings were synthesized qualitatively as opposed to quantitative analysis. Among the main themes identified were: Mechanisms of radiosensitization (e.g., ROS amplification, DNA damage, hypoxia modulation), Engineering strategies of nanoparticle and exosome integration, Functional outcomes (e.g., tumor control, recurrence prevention). Data were framed and contextualized to bring out the emerging trends, mechanistic convergence, and gaps in the knowledge.

2.6 The constrained nature of the approach

As a narrative review, this methodology is inherently subject to selection bias and does not provide a statistically reproducible synthesis of evidence. However, it allows for flexibility, conceptual depth, and integration of diverse evidence, which is particularly

important in rapidly evolving and interdisciplinary fields such as exosome-based nanomedicine.

3 Exosome biology and therapeutic potential

Exosome biology biogenesis, composition and tumor-homing Exosomes are 30–150 nm in diameter extracellular vesicles, which are mostly produced through the endosomal pathway (Fig. 2). The endosomal membrane is budged inwards to produce intraluminal vesicles in multivesicular bodies (MVBs); MVBs are then fused with the plasma membrane releasing the vesicles to form exosomes [35, 36]. Their composition contains an identity of the donor cell plus its physiologic condition in general a lipid bilayer rich in cholesterol and sphingolipids, membrane proteins (tetrapansins, CD9, CD63, CD81), adhesion molecules, and cytosolic cargo including enzymes and signaling proteins, and nucleic acids (mRNA, miRNA, lncRNA and DNA fragments) [37]. Notably, exosomes may have homing properties regulated by surface glycans, integrins and receptor-ligand interactions favoring that specific tissues or cell types are targeted [38]. In oncologic settings, tumor exosomes are often concentrated in the tumour microenvironment, as well as in the tumour metastatic niches, partially because of leaky vasculature, inflammatory adhesion factors, and presence of receptors on receiver cells (tumour cells, fibroblasts, endothelial cells, macrophages) [19, 39, 40]. The ability to target cells biologically makes exosomes attractive delivery vehicles; aptly positioned to exploit endogenous biodistribution pathways to deliver therapeutic payload to compartments of interest such as hypoxic or stromal that cannot otherwise be targeted using conventional therapeutics.

Intercellular communication through exosomes during tumours Exosomes are also high-efficiency communication agents in tumours whose remodelling of non-malignant as well as malignant compartments (Fig. 3). They enhance proliferation, survival, invasion, angiogenesis and therapeutic resistance by transferring miRNAs, oncogenic proteins, metabolites and signaling receptors [19, 41]. As an example, stressed or irradiated tumour cells release exosomes that in a population-wide adaptive response can carry cargo into DNA repair, antioxidant responses or pro-survival signalling in adjacent cells, reflecting the release of exosomes locally [19, 42]. Exosome-mediated tumour-stroma crosstalk Tumour-associated fibroblasts are also capable of delivering metabolites and

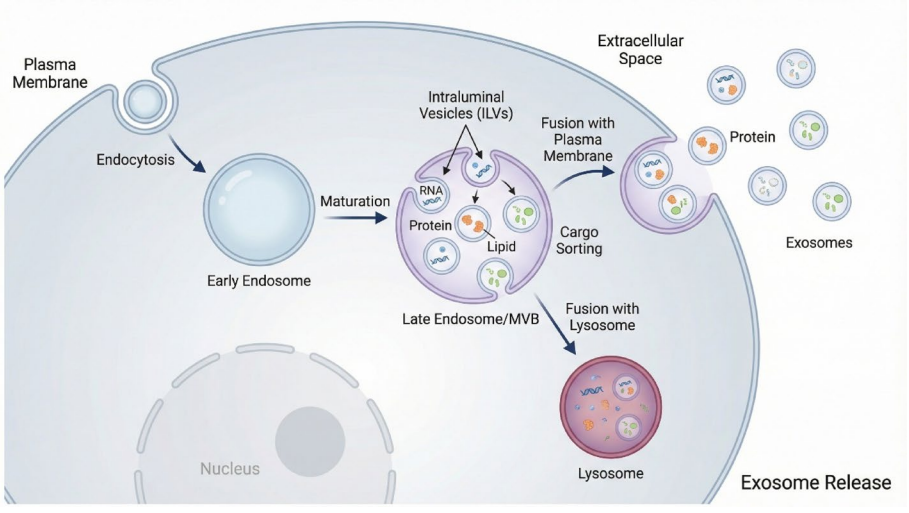

Fig. 2 Biogenesis and extracellular release of exosomes through the endosomal multivesicular-body pathway

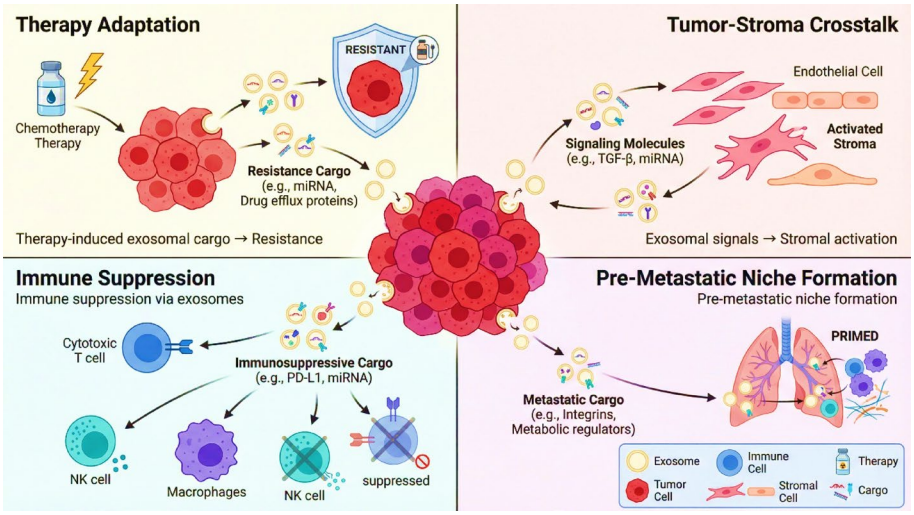

Fig. 3 Exosome-mediated communication within the tumor microenvironment promotes therapy adaptation and stromal–immune remodelling

regulatory RNAs into the tumour which rewire tumour metabolism, and tumour exosomes can reprogramme fibroblasts to pro-tumour phenotypes and induce tumour invasion by driving extracellular matrix remodelling [43]. Exosomes also have an immunologic effect, altering antigen presentation, cytokine signalling and checkpoint pathways, potentially facilitating immune suppression through the actions on macrophage polarisation and T-cell functions [19, 44]. They also help to form pre-metastatic tissues niche conditioning distant tissues, modifying vascular permeability, establishing bone-marrow-cells and altering immune tone, prepare the soil in which the disseminated tumour cells proliferate [45–47]. This central part in tumour evolution makes exosomes potentially useful not only as indicators of disease condition, but also in treatment as a specific target, or more precisely an agent of treatment, especially when focused on reprogramming of resistant tumour ecosystems instead of the direct elimination of the cancer cells herself.

3.1 Why exosomes are superior delivery systems compared to synthetic delivery systems?

The exosomes provide a biologically optimised delivery system in comparison with a lot of synthetic carriers (liposomes, polymeric nanoparticles). First, they are endogenous to the membrane, increasing their biocompatibility and, in most cases, decreasing immunogenicity, allowing a repeated dose to be administered with a weaned inflammatory reaction [48]. Second, exosomes exhibit a strong resistance against instability in physiological fluids, and they have the capacity to avoid enzymatic breakdown of delicate cargo, particularly RNAs [19]. Third, cellular uptake is effective as recipient cells identified exosomal surface proteins and lipids, which stimulates endocytosis or membrane fusion and enhances cytosolic delivery compared to synthetic systems which can be trapped in endo-lysosomal structures. Fourth, exosomes may penetrate some biological barriers more easily than larger and more foreign nanoparticles, therefore, enhancing access to recalcitrant locations [19]. Fifthly, they can be engineered: surface ligands may be added to propagate tumour targeting and the interior may be loaded with siRNA/miRNA, small molecule inhibitors, proteins or even nanoparticle radiosensitisers [19]. Lastly, exosomes can have a role to play in reducing off-target toxicity by exploiting the

natural biodistribution and cell-specific uptake distributions [19, 49]. Significant logistical challenges are still open such as scalable production, batch-to-batch uniformity, purification, and normalised potency assays, yet mechanistically, exosomes have seriously been found to make, bundling stealth, storage, and targeting properties, which have challenged nanocarriers of equivalent size made with purely synthetic materials.

3.2 Comparative appraisal of exosomes versus synthetic and free-agent radiosensitizer platforms

Despite these common claims that exosomes are superior delivery systems, existing evidence lends much more support to a conditional and not an unconditional superiority (Table 1). Their natural membrane composition, surface proteins and biological bloodstream delivery may be advantageous in certain conditions, but such advantages have to be traded off with the simplicity of manufacturing, enhanced composition control and the higher level of translational maturity of synthetic or free-agent systems [50].

Exosome-loaded systems do not have the likelihood of offering more substantial intrinsic radiation properties as compared to the conventional nanoradiosensitizers on their own. They could take advantage of their biologically informed delivery. High-Z nanoparticles already exhibit efficient local dose and ROS generation, but face inherent limits in their efficiency due to heterogeneous tumor uptake, reticuloendothelial clearance, and lack of penetration into stromal-or-resistant and other unfriendly spaces [50]. Exosome loading can thus be value-adding to the mechanical mechanism of the nanoparticle, but cannot substitute its physical action, but can enhance cellular delivery and space distribution [33].

Exosomes have the potential to be more endogenously tropic and can be more efficient to deliver cargo like nucleic acids and biologically active drugs than liposomes, especially with cytosolic delivery. Nevertheless, liposomes still have significant practical benefits, such as scalable composition, easier quality control, and a broader familiarity with the regulations. In such a way, exosomes cannot be discussed as categorically superior to liposomes, but may be possibly better in case of biological targeting, communication between cells or RNA co-delivery in the treatment plan [51].

Polymeric carriers can be used as attractive translational carriers, which have high level of formulation control, tunable release kinetics and reasonably reproducible production [52]. Exosomes can bypass polymeric systems in biological interfacing, immune compatibility and tissue specific uptake whereas exosomes come with additional heterogeneity (depending on the donor) and challenging batch characterization in addition to more complicated regulatory classification [23].

The major value added to exosome-loaded nanoradiosensitizers is more evident, when compared to free radiosensitizing agents: enhanced tumor-targeting, cargo protection, and simultaneous capacity to incorporate multiple activities in a single platform, including but not limited to high-Z dose enhancement, RNA silencing, redox regulation and immunomodulation [53]. This implies that exosome loading would be best where the free agents have erratic pharmacokinetics, general systemic toxicity or failure to penetrate the resistant tumor niches.

On the whole, exosome-loaded nanoradiosensitizer specifically has not been shown to show universal superiority, but the potential to integrate known nanoparticle

radiosensitization agents with biologically informed delivery and reprogramming of the microenvironment in complex environments in which simpler platforms are insufficient.

3.3 Standardization and gold-standard exosome characterization

The general guideline in Minimal Information for Studies of Extracellular Vesicles (MISEV 2018) [33] is that the extracellular vesicle (EV) research needs to be conducted with rigorous, transparent and reproducible procedures to ensure that the identity, purity and functionality of the EVs reported is plausible. First, researchers should use the term extracellular vesicle unless they can prove a specific subtype such as exosomes, since subtype assignment is often difficult. Second, the report must contain all pre-analytical variables, such as the origin of the sample, its collection, treatment, preservation, and the conditions of its culture as well as the possible contaminants, since they have a drastic impact on the recovery and composition of the EVs. Third, there is no single gold-standard isolation method; the separation approach to be used should be appropriate to the scientific question, all the technical details should be presented. Fourth, EV preparations must be characterized by multiple complementary methods. This involves quantitative reporting of the source, quantification of EVs, evidence of positive EVs and non-EV contaminants. Imaging techniques or single-particle are also advised to verify the characteristics of the vesicles. Fifth, functional experiments are required to demonstrate that the biological effects are actually related to EVs and not to soluble/co-isolated materials. In general, MISEV2018 encourages nomenclature standardization, thorough reporting, multimodal characterization, purity evaluation and cautious functional validation of EV research to foster comparability, rigor, and reproducibility among EV research.

When applied to the exosome-based delivery of radio sensitizers, these criteria are also significant as they offer criteria by which vesicle identity, purity and functional similarity can be established as compared to other studies [33]. Best practices involve ensuring markers of the vesicles, assessing the lack or low concentration of non-vesicular contaminants, and documenting isolation and storage conditions in a way that would enable reproducibility. To be used as a translational application, these principles must be applied to such batch-level qualities as cargo-loading consistency, colloidal stability, sterity and functional potency of radio sensitization assays. These characterization practices would enhance batch to batch consistency, enable comparisons between exosome preparations made using different donor cell or loading strategies and enhance the validity of future clinical and preclinical studies.

4 Nanoradiosensitizers: design and mechanisms

Different categories of nanomaterials used as nanoradiosensitizers Engineered nanostructures that enhance the therapeutic effect of ionizing radiations are the nanoradiosensitizers, which increase radiation-induced injury in malignant tissues selectively (Fig. 4). The most studied type of system is metals and inorganic systems, especially high-atomic number (high-Z) nanoparticles like gold, hafnium oxide, bismuth, tantalum, platinum and tungsten, etc [63, 64]. They also produce more dose deposition locally and secondary electrons during irradiation by their high density and atomic properties and increased micro damages to tumour cells through their micro-dosimetric effects [64].

Table 1 Comparative positioning of radiosensitizer delivery platforms: established strengths, limitations, and likely best-use contexts

Platform/design class	Established strengths	Main limitations	Evidence status	Specific added value relative to others	Key translational bottleneck	Best-fit use case	References
Free small-molecule radiosensitizers	Chemically defined; simplest formulation; easier dosing	Low tumor selectivity; systemic toxicity; short half-life; weak multi-cargo flexibility	Established for some agents, but often limited by PK/toxicity	Baseline comparator with lowest formulation complexity	Off-target toxicity and poor tumor exposure	When formulation simplicity and established pharmacology are priorities	[54]
Free molecular DNA-repair inhibitors	Directly target repair pathways; mechanistically precise	Systemic exposure may limit dose; no targeting to resistant niches	Established mechanism, variable clinical usability	Strong pathway specificity without carrier complexity	Therapeutic window	Tumors with defined DDR vulnerability	[54]
Conventional high-Z nanoradiosensitizers	Strongest physical dose enhancement; robust ROS/DNA damage amplification	Uptake heterogeneity; RES clearance; limited biological targeting	Strong preclinical evidence; some early translational maturity	Best intrinsic radiation-physics effect	Biodistribution and intratumoral heterogeneity	Tumors where physical dose amplification is the main objective	[55]
Polymeric nanocarriers carrying radiosensitizers	Tunable release; reproducible chemistry; multi-payload design	Less endogenous tropism; possible endosomal trapping; synthetic surface effects	Strong nanomedicine precedent	Good formulation control and release engineering	Scale-up, batch consistency, in vivo delivery efficiency	Controlled release and combination therapy designs	[56]
Liposomal radiosensitizer formulations	Clinically familiar platform; scalable; good small-molecule encapsulation	Limited intrinsic tropism; less efficient cytosolic delivery for some cargos	High translational familiarity	Practical clinical development route	Stability, leakage, circulation-versus-release balance	Reformulating existing radiosensitizers with better PK	[57]
Hybrid lipid–polymer nanocarriers	Combine stability and loading flexibility	More complex formulation; still synthetic; not biologically active by themselves	Moderate to strong preclinical support	Balanced engineering control	Manufacturing complexity	Multi-drug or image-guided combination systems	[58]
Exosome carriers without nanoparticle radiosensitizer	Biocompatible; endogenous trafficking; good RNA/protein delivery potential	Weak intrinsic radiosensitization unless carrying active cargo; donor-source variability	Strong biologic rationale, limited therapeutic standardization	Biological delivery and intercellular communication	Source standardization and EV characterization	RNA-based modulation of radioresistance pathways	[59]
Exosome-loaded high-Z nanoradiosensitizers	Combine physical radiosensitization with biologically informed delivery	Complex manufacturing; loading may damage vesicles; superiority not yet proven	Early, promising, mostly preclinical	Potential integration of physics + delivery + TME targeting	Maintaining vesicle identity while achieving sufficient nanoparticle load	Resistant tumors where both delivery failure and radiosensitizer potency are issues	[60]

Table 1 (continued)

Platform/design class	Established strengths	Main limitations	Evidence status	Specific added value relative to others	Key translational bottleneck	Best-fit use case	References
Exosome-loaded multi-cargo systems (nanoparticle + siRNA/drug)	Multi-mechanism design; may target DNA repair, redox balance, and immune pathways simultaneously	Highest design complexity; difficult attribution of mechanism; potency assays difficult	Conceptually strong, still speculative in many contexts	Most flexible platform for combined radiosensitization and recurrence control	Mechanism attribution and release-standardization	Tumors needing combined physical and molecular radiosensitization	[61]
Engineered/targeted exosome nanoradiosensitizers	Potentially improved tumor tropism and compartment-specific delivery	Surface modification may alter native tropism; added CMC and regulatory burden	Early preclinical	Possible niche-specific superiority in hard-to-reach tumor compartments	Consistent targeting performance and regulatory classification	Hypoxic, stromal-rich, or recurrence-prone tumors where selective delivery is crucial	[62]

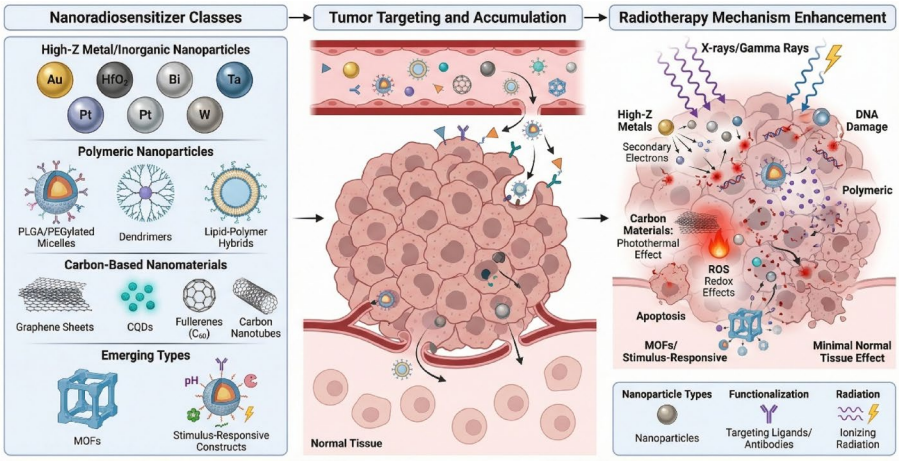

Fig. 4 Major nanoradiosensitizer platforms and their tumor-selective functions during radiotherapy enhancement

Polymeric nanomaterials, such as poly(lactic -co -glycolic acid) (PLGA), PEGylated nanomadamacelles, dendrimers and lipidpolymer hybrid nanoparticles are desirable due to their biocompatibility, tunable release properties and multimodal payload integration (radiosensitizer, chemotherapeutic agent, imaging contrast) [65, 66]. Tumour accumulation and internalisation is enhanced by surface functionalization by targeting ligands (e.g., peptides, antibodies) [67]. Carbon accentuated materials like graphene derivatives, carbon quantum dots, fullerenes and carbon nanotubes provide large surface area and Pi-pi interaction that may be utilized in drug loading, and their redox and photothermal characteristics may be utilized in combination with radiotherapy [68]. New modalities are metal-organic frameworks (MOFs) and stimulus-controlled nanomaterials that can respond to pH, enzyme activity, redox state or radiation itself, overcoming the problem of deep tumour penetration, local activation and localised systemic exposure [69, 70]. The main aim of design in all material classes is comparable: to achieve the maximum of tumour-selective maximisation of radiotherapeutic effects at minimal systemic toxicity and allowing easy production of scalable and reproducible formulations.

ROS generation, DNA damage amplification, and hypoxia modulation Three mechanisms are often interrelated and produce the radiosensitizing effect of nanomaterials (Fig. 5). (i) Reactive oxygen species (ROS) amplification: Radiolysis mediated by Ionizing radiations: Radiolysis of water results in the production of free radicals; numerous nanoparticles act as catalysts or enablers and lead to an increase in active hydroxyl radical and other ROS that oxidize nucleic acids, proteins and cell membranes [71, 72]. Some nanomaterials are also capable of nanozyme activity utility, which resembles peroxidase enzymes and continues under oxidative stress beyond the irradiation window.

(ii) DNA damage amplification: The High-Z nanoparticles enhance local dose deposition by interaction via photoelectric electrons and Compton interaction and generate secondary electrons, such as Auger electrons [73]. The resulting events cause thick, localised ionisation tracks that cause complicated DNA lesions, especially clustered, two strand breaks, which is hard to repair, altering cells into mitotic catastrophe or apoptosis [73]. Nanocarriers could also interfere with DNA repair signaling/responses by delivering inhibitors of important repair proteins like PARP, ATM/ATR or DNA-PKcs that directly silence the repair ability of the cell [74, 75].

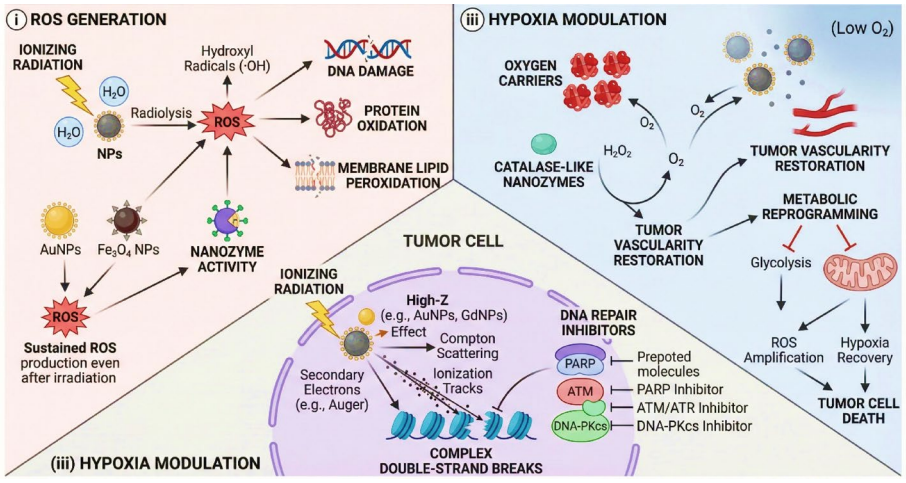

Fig. 5 Core radiosensitization mechanisms triggered by nanomaterials: dose enhancement, ROS amplification, and hypoxia modulation

(iii) Hypoxia achievement: Radiation induced lesions must be fixed by oxygen to cause the greatest cytotoxicity / Hypoxia is also one of the most notable autocrats of radioresistance. Nanoradiosensitizers may overcome hypoxia by acting as oxygen carriers, catalysing H 2 O 2 to release oxygen locally, restoring tumour vascularity or minimising cellular oxygen requirement by metabolic reprogramming. Some platforms combine the amplification of ROS and hypoxia recovery which effectively solves the hypoxia problem itself and its effects on radical fixation [76].

Synergy with chemotherapy or immunomodulation One of the main advantages of the nanoradiosensitizers is a possibility to create multi-modal synergies especially when they are used in conjunction with chemotherapeutic agents and immunotherapeutic measures [19, 77]. In the case of chemotherapy, nanoparticles facilitate co-delivery of radiosensitizing agents, e.g. DNA cross-linking reagents, antimetabolites or topoisomerase inhibitors or allow optimized sequencing (e.g. pre-loading tumour cells with replication stress and then irradiating them) [78]. Spatially and temporally localized delivery of drugs localizes the drug exposure to the maximal possible fraction of the radiotherapy dose, to create the peak dose and eliminate repair mechanisms and off-target toxicity [79]. Whereas the aim of immunomodulation is to turn radiotherapy into a useful in situ vaccine. Radiation enhances antigen release and danger-associated molecular patterns; combined with the checkpoint blockade, STING agonists, cytokine therapies or macrophage-reprogramming signals, radiation augmenting antigen presentation and cytotoxic T-cell infiltration and abating immunosuppression [80, 81]. Localised delivery of immunostimulatory payloads is possible prior to localised production of adverse events in the system through the aid of nanoparticles [82]. More importantly, it is not just additive cytotoxicity, but ecosystem-level reprogramming, such as inhibition of resistant clones (by impaired repair and redox) and stromal remodelling (by physical barrier bypass) and immune rebalancing (to an anti-tumour phenotype). Combination strategies rationally developed, i.e. radiotherapy + nanoradiosensitizers + therapeutic payloads, are thus increasingly developed as a means of reducing recurrence and obtaining local control over time [83, 84].

5 Methods for exosome loading and functionalization

Endogenous versus exogenous cargo loading approaches Exosome loading of cargo is an essential part of building exosome-based nanoradiosensitizers and has a direct positive impact on therapeutic performance, targeting accuracy, and reproducibility (Fig. 6). Endogenous loading takes advantage of the inherent exosomal biogenesis route: the donor cells are genetically altered or treated to add therapeutic content—e.g. small-molecule radiosensitizers, siRNA or CRISPR components—during the formation of vesicles [85, 86]. By doing so, the integrity of the vesicles is maintained and surface proteins that mediate tumourounding can be retained to ensure biocompatibility and the lack of immunogenicity. However, endogenous loading is often restricted by a small cargo capacity and batches variation due to cells heterogeneity [87]. Exogenous loading, in contrast, loads cargo into secluded exosomes following secretion, using physical or chemical methods, e.g. electroporation, sonication, extrusion, or permeabilization [88]. Exogenous techniques provide fine control on the concentration of the cargo, whereby it is possible to include more massive or chemically diverse agents that are not normally packaged [88]. Nevertheless, the procedures can harm the membrane integrity, cause aggregation and/or modify the surface-protein structure, which might reduce the targeting efficiency [19]. The most effective strategies are therefore usually the ones that incorporate both modalities i.e. create exosomes that have inherent targeting properties via an endogenous route and then exogenously load them with other therapeutic cargo, i.e. balancing efficiency, stability, and activity.

Surface modification for targeted delivery The exosome-based nanoradiosensitizers need total surface engineering which is fundamental to the realisation of tumour-specific navigation, reduction of off-target effects, and radiosensitisation. Exosomes also have membrane proteins such as integrins, tetra spanins and ligands, which give it a tissue tropism and this property can be enhanced through chemical or genetic modifications of the surface [89]. Genetic methods use the approach of transfection of donor-cells to produce exosome membranes containing targeting ligands, peptides, or antibodies allowing the recognition of tumour specific receptors like EGFR or the folate receptor [90]. Selective attachment to regional cells via chemical modification as against aptamers

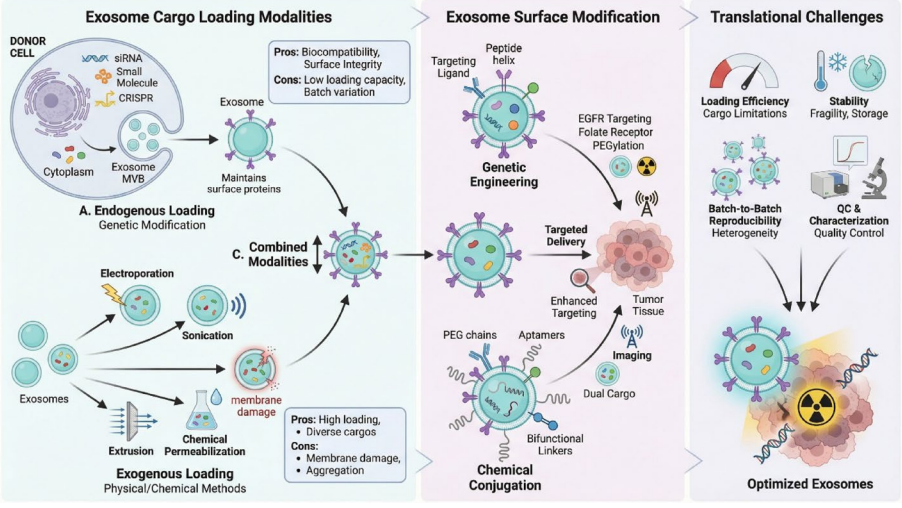

Fig. 6 Strategies for exosome loading and surface engineering, with key manufacturing constraints affecting delivery performance

simplified- malignant cells, PEGylation, or conjugation is achieved by chemical modifications, which improve circulation volume and immune evasion. Surface modification also permits dual function; both targeting and imaging probes or other therapeutics [91, 92]. These modifications should be so fundamentally directed that they do not compromise vesicle stability, should not conceal intrinsic cues used in homing and they must be biocompatible. Under optimal conditions, surface-engineered exosomes can exhibit a superior accumulation in tumour tissue, a higher rate of penetration into tumour microenvironment, and stronger delivery of Radiosensitizers, which results in increased DNA damage, hypoxia modulation, and finally reduces post-irradiation tumour recurrence [19].

Challenges in loading efficiency, stability, and batch reproducibility Although exosome-based nanoradiosensitizers have potential efficacy as therapeutics, they face considerable and major manufacturing and translational difficulties. The procedure of loading depends on type of cargo or exosome, origin of exosome, and the selected loading procedure; hydrophilic or big-molecular cargo is usually poorly incorporated hindering its usage as a functional payload [2, 19]. Exogenous techniques including electroporation will increase loading at the expense of aggregate or partial membrane damage and endogenous techniques have a limit (maximum loading) related to the natural packaging capacity of the cell [19]. Stability is a second important issue: exosomes are fragile to storage and freeze-thaw conditions as well as surface modifications, which can all disrupt membrane integrity, cargo retention or targeting ligands [93]. Clinical translation requires long and fundamental stability of the shelf. The problem of batch-to-batch reproducibility is a bottleneck, especially in the upscaling of preclinical/clinical trials; size, cargo content and bioactivity heterogeneity is added by donor-cell variation, culture regime or isolation methodology, such as ultracentrifugation, size-exclusion chromatography or tangential flow filtration [94, 95]. Cellular procedures, high level of quality control (identity, purity, potency and sterility), and improved characterization methods including nanoparticle tracking, flow cytometry and high-resolution microscopy are required to overcome these problems and reach clinically reliable exosome-based radiosensitizers [19]. Table 2 is a concise summary of exosome loading approaches, surface modifications, and associated challenges.

5.1 Cargo and efficiency: physical constraints in nanoparticle loading

Exosome-based delivery systems are very efficient in nucleic acids (e.g., siRNA, miRNA) and small-molecule therapeutics; however, the addition of high-atomic-number (High-Z) metal nanoparticles leads to unique physical and structural constraints [96]. Metallic nanoparticles are relatively large and rigid compared to small and flexible cargos which can be encapsulated into the exosomal lumen or membrane whereas the latter are said to be inherently limited to a certain size (30–150 nm) [96].

One matter of concern is the trade-off that exists between loading efficiency and exosome integrity. Radiosensitization potential can be increased by increasing the size or concentration of nanoparticle; but, too much loading may disrupt membrane curvature, cause deformation at the vesicle, or affect membrane protein activity, which is required to target and to get taken up by the cell [97]. As an example, vigorous loading methods like sonication or extrusion which is frequently needed to entrust nanoparticles may be associated with destabilizing membranes and aggregation of these membranes

Table 2 Comparison of exosome cargo loading strategies, surface modifications, and key challenges for nanoradiosensitizer delivery

No.	Loading approach	Method/technique	Cargo type	Surface modification	Key challenges	References
1	Endogenous	Donor-cell transfection	siRNA, miRNA, small-molecule radiosensitizers	Genetic expression of tumor-targeting ligands	Limited cargo capacity, cell variability	[109, 110]
2	Endogenous	Chemical stimulation of donor cells	Chemotherapeutics, radiosensitizers	Native exosome membrane proteins leveraged	Batch heterogeneity, low yield	[111]
3	Exogenous	Electroporation	siRNA, CRISPR-Cas RNP	Optional antibody/peptide conjugation	Membrane disruption, aggregation	[112]
4	Exogenous	Sonication	Small molecules, nanoparticles	PEGylation or aptamer conjugation	Vesicle instability, functional loss	[113]
5	Exogenous	Extrusion	Nanoparticles, metallic radiosensitizers	Ligand attachment for receptor targeting	Altered vesicle morphology	[114]
6	Exogenous	Permeabilization (saponin, mild detergents)	Hydrophilic drugs	Surface PEGylation	Partial cargo leakage, immunogenicity	[19]
7	Combined approach	Endogenous + Exogenous	Multimodal cargo: siRNA + radiosensitizer	Dual targeting + imaging labels	Complexity, reproducibility issues	[115]
8	Surface engineering	Genetic ligand display	Peptides, antibodies	Tumor-specific receptor targeting	May mask native tropism	[116]
9	Surface engineering	Chemical conjugation	Aptamers, PEG, fluorophores	Targeting + immune evasion	Reduced circulation stability if overmodified	[117]
10	Optimization strategies	Controlled loading protocols	Any cargo	Combination targeting & protective coatings	Ensuring batch-to-batch consistency	[118]

and partial loss of biological identity [97]. Smaller cargos, like siRNA or low-molecular-weight drugs, on the other hand, have a higher encapsulation efficiency with small perturbations of vesicle structure, with no effects on exosome stability or biodistribution properties [97]. This gap points to a fundamental constraint of the approach: ensuring the utmost radiosensitization by High-Z nanoparticle payloads should be approached at the cost of structural integrity of exosomes and biologic operation.

Moreover, loading result could be affected by the nanoparticle surface chemistry, propensity to aggregate, and density [98]. Substantial local concentrations can cause interparticle interactions which inhibit effective dispersion within vesicles, further decreasing functional delivery. As a result, hybrid or modular design strategies are more favored to maximize the performance of nanoparticles, with smaller nanoparticles or nanoparticle clusters or nanoparticle-drug complexes balancing vesicle stability and payload potency

[99]. In sum, to achieve clinical translation of exosome-loaded nanoradiosensitizers, the trade-off between loading and its bioactivity should be optimized because too much loading can counteract the natural benefits of exosomal delivery, such as biocompatibility, specific targeting, or immune evasion.

6 Preclinical evaluation of exosome-loaded nanoradiosensitizers

It is not possible to underestimate the role of preclinical testing to determine the therapeutic potential and translational viability of the exosome-laden nanoradiosensitizers. In vitro models, such as conventional two-dimensional monoculture and three-dimensional tumour spheroids, can provide unique information on cellular uptake, radiosensitisation potential, DNA damage generation, and cytotoxicity pattern [100, 101]. There are advanced co-culture systems, which include stromal and immune constituents, which can be used to interrogate exosome interactions in the tumour microenvironment involving immune modulation and extracellular matrix infiltration [101]. In vivo studies using xenograft, orthotopic and humanised mouse tumors can be used to study biodistribution, tumour accretion, radiosensitisation performance, and post-irradiation recurrence mitigation under physiologically relevant conditions [102, 103]. The main endpoint areas include tumour growth inhibition, endpoints of DNA damage, cell-cycle arrest, immune activation and survival in general.

The most significant translational limitations of exosome-loaded nanoradiosensitizers is the lack of consistency of the findings when using 2D monolayer cell models versus 3D spheroids and organoids, as well as in vivo systems [104]. Although 2D cell lines can be handy in a preliminary screening of cytotoxicity, ROS generation, DNA damage, and clonogenic survival, they often overestimate therapeutic efficacy, due to their lack of recapitulating the spatial confinement, extracellular matrix density, oxygen gradients, stromal interactions, immune context, and vascular heterogeneity in tumors in vivo [105]. Conversely, diffusion limits, heterogeneous penetration of nanoparticles, hypoxic niches, and variable exosome uptake across tumor subpopulations is more accurately captured in 3D organoids and spheroids thus reflecting the true performance of radiosensitizers. Such difference is especially relevant in the case of exosome-based nanoplatforms, in which intrinsic radiosensitizing potency is not the only factor that predetermines the biological activity; biodistribution, intratumoral penetration, cargo release, retention and stromal and immune compartment interaction are also important.

These model-discrepancies can similarly be used to understand why a high rate of nanoradiosensitizers showcased as incredibly successful in preclinical assays do not progress to Phase I/II clinical trials. In many cases, early clinical failure is not because of a lack of mechanistic promise, but because of a failure to accumulate in human tissues, because of the rapid clearance by the mononuclear phagocyte system, because of variability in manufacturing, non-uniform loading of the cargo or its target, and because of differences in biology between murine and human tumors [105]. Moreover, radiation programs in animals are simplified and may not be representative of fractionation, toxicity limit, or heterogeneity of patients as occurs in treatment. Thus, preclinical success must be viewed with a degree of caution and greater emphasis should be on orthotopic models, patient-derived organoids, humanized systems, quantitative biodistribution studies and standardized translational endpoints that are more reflective of clinical performance. Table 3 summarises exemplary preclinical models, exosomal cargo,

Table 3 Expanded preclinical assessment of exosome-loaded nanoradiosensitizers: models, cargos, efficacy, tumor context, exosome source, and radiation regimen

No.	Model type	Specific model	Tumor type	Exosome source	Exosome cargo/ nanoparticle composition	Radiation regimen	Experimental endpoint	Key findings	References
1	In vitro	2D tumor cell lines	NR	NR	siRNA radiosensitizer	NR	DNA damage, clonogenic survival	Enhanced radiosensitivity	[119]
2	In vitro	3D spheroids	NR	NR	Chemotherapeutic + radiosensitizer	NR	Tumor cell viability, hypoxia modulation	Increased penetration, improved radiosensitization	[120]
3	In vitro	Co-culture with fibroblasts	NR	NR	Metal nanoparticle radiosensitizer	NR	Cell–cell interaction, ROS generation	Stromal cells modulate exosome uptake	[121]
4	In vitro	Co-culture with immune cells	NR	NR	siRNA + small molecule radiosensitizer	NR	Cytokine profiling, apoptosis	Immune activation enhanced	[19]
5	In vivo	Subcutaneous xenograft	NR	NR	Gold nanoparticle-loaded exosomes	NR	Tumor growth inhibition	Significant radiosensitization	[122]
6	In vivo	Orthotopic tumor model	NR	NR	siRNA-loaded exosomes	NR	Tumor volume, survival	Targeted delivery, prolonged survival	[19]
7	In vivo	Humanized mice	NR	NR	Immunomodulatory exosomes + radiosensitizer	NR	Immune infiltration, recurrence suppression	Reduced recurrence, enhanced immune response	[123]
8	In vitro	3D co-culture spheroids	NR	NR	CRISPR-Cas-loaded exosomes	NR	DNA damage, cell viability	Improved radiosensitization in hypoxic regions	[124]
9	In vivo	Orthotopic pancreatic cancer	Pancreatic cancer	NR	siRNA + chemotherapeutic exosomes	NR	Tumor growth, metastasis	Decreased metastasis post-irradiation	[125]
10	In vitro	Tumor organoids	NR	NR	Small molecule radiosensitizer	NR	Apoptosis, cell-cycle arrest	Preserved tissue architecture, enhanced effect	[126]
11	In vivo	Glioblastoma xenograft	Glioblastoma	NR	Gold nanoparticle exosomes	NR	Radiosensitization, survival	Increased survival, reduced tumor recurrence	[127]

Table 3 (continued)

No.	Model type	Specific model	Tumor type	Exosome source	Exosome cargo/ nanoparticle composition	Radiation regimen	Experimental endpoint	Key findings	References
12	In vitro	2D glioma cell lines	Glioma	NR	siRNA + radiosensitizer	NR	ROS levels, clonogenic assay	Efficient uptake, DNA damage amplification	[128]
13	In vivo	Lung cancer orthotopic model	Lung cancer	NR	Metal nanoparticle + siRNA	NR	Tumor regression, toxicity evaluation	Tumor-specific accumulation, minimal off-target effects	[19]
14	In vitro	Co-culture with endothelial cells	NR	NR	Chemotherapeutic exosomes	NR	Angiogenesis markers	Inhibited angiogenesis, improved radiosensitivity	[129]
15	In vivo	Humanized breast cancer model	Breast cancer	NR	Immunomodulatory + radiosensitizer	NR	Recurrence, immune response	Reduced post-irradiation recurrence, enhanced T-cell infiltration	[130]

NR, not reported in the cited source as presented in this review or not stated clearly enough in the manuscript for confident extraction. Because donor-cell variation, cargo-loading heterogeneity, and simplified or inconsistently described radiation programs remain important limitations in the preclinical literature, direct cross-study comparison should be interpreted cautiously

experimental outcome, any salient discoveries and relevant citations, therefore providing a broad understanding of the current strategies involved in terms of augmenting exosome mediated radiosensitisation and inhibit tumour recurrence.

6.1 Contradictions and unresolved issues in the preclinical literature

The overall conflicting aspect of the field, however, is the fact that exosomes have been suggested as therapeutic carriers and at the same time, used as mediators in adaptive therapy and immune suppression, and in forming metastatic niches [106]. It implies exosome source, donor-cell state, and cargo composition is not a minor technical factor but a significant determinant of exosome biology being therapeutic or tumor-promoting.

The second paradox is the loading-efficiency: loading nanoparticles to the maximum level required to optimize radiosensitization could result in impaired (target) vesicle integrity, membrane protein activity, and native targeting [33]. Consequently, the designs might become the most exosomes-like in theory, the least exosomes-like in reality.

The third contradiction involves the pattern of discrepancy in simplified 2D systems when compared to more clinical (3D/in vivo) models. Numerous recipes seem to be effective in monolayer cultures, but in places where the effects of stromal barriers, hypoxia gradients, immune environment, and physiological biodistribution are added, they appear less so, in terms of penetration, more variable uptake, or advantageous over other established systems [107].

Mechanism attribution is another problem that is left unresolved. In most preclinical disclosures, enhancement of therapeutic outcomes following exosome administration may not be well attributable to the exosome itself, the nanoparticle, the concomitant

delivered load (RNA or drug) or an interplay within these. This makes it complicated when it comes to asserting platform specific superiority [108].

7 Mechanistic Insights

Exosome-loaded nanoradiosensitizers as molecular modulators Exosome-filled nanoradiosensitizers have the modulation effect on a range of molecular pathways controlling the survival and radiosensitivity of tumour cells. These artificial exosomes can be loaded with radiosensitizing cargos including siRNAs, chemotherapy agents or metal nanoparticles and bypass the immune system to enter tumoral cell with precise molecular targeting [131]. Some of the important signalling axes impacted are PI3K/Akt/mTOR pathways, which is involved in cellular survival and proliferation, MAPK/ERK cascade, which is often involved in radioresistance and NF -aka pathway that is the major mediator of inflammatory and stress responses [132]. Exosome-based radiosensitizers are able to silence pro-survival signals, maximise apoptosis and sensitize resistant tumour populations by modulating these signalling nodes [133]. Moreover, the mechanisms that regulate repair of DNA like the ATM/ATR-mediated signalling tend to be impaired, making the tumours increasingly vulnerable to cytotoxicity by radiation [134]. All these molecular perturbations are coordinated to activate a multi-pronged attack on tumour resilience and are given mechanistic rationalisation to be employed as next generation radiosensitizers and as the basis of combinatorial therapy with targeted inhibitors or immunotherapeutics opportunities abound.

DNA damage response modulation, amplification of oxidative stress and cell cycle arrest One of the fundamental overviews of exosome-mediated radiosensitization is the amplification of the DNA damage and oxidative stress, which increase the vulnerability of tumour cells to the ionising radiation [135]. Nanoradiosensitizers increase the reactive oxygen species (ROS) in tumoral cells, facilitating oxidative lesions and double-strand breaks. This incorporates DNA repair apparatus, including non-homologous end joining (NHEJ) as well as homologous recombination (HR) pathways, to an overloaded condition. As a result, tumour cells will accumulate lesions of the DNA that cannot be repaired, which results in apoptosis or mitotic catastrophe. In parallel, exosomal cargos may induce cell-cycle arrest at radiosensitive stages like G2/M which increased cytotoxicity further [136]. This interaction between augmentation of ROS, dysfunctional DNA repair, and tactical cytostatic regulation maximises the radiosensitisation and reduced the collateral damage to the adjacent healthy tissues. Preclinical studies indicate that such processes contribute to enhanced tumour growth inhibition and lessened post irradiation recurrence and the significance of cargo engineering to achieve optimal therapeutic responses [83].

It is important to note that the efficiency of reactive oxygen species (ROS) formation and resultant DNA damage highly relies on the physicochemical characteristics of a particular material with high atomic number (high-Z) [137]. As an example, radiosensitization by gold nanoparticles (AuNPs) is done mostly by the strong photoelectric absorption and release of the low-energy secondary electrons that cause local ionization and yield hydroxyl radical (OH) production. Conversely, bismuth based nanoparticles (BiNPs) due to their increased atomic number and X-ray attenuation coefficient

have better radiation absorption and dose enhancement ability, which could lead to a higher production of ROS under specific conditions of irradiation. Also, systems containing bismuth have been reported to enable persistent oxidative stress due to catalytic or redox-active surfaces which might extend the ROS-mediated damage past the irradiation window [138]. The above material-related dissimilarities underscore the need to rationalize the choice of nanomaterials in maximizing radiosensitization efficiency and specific therapeutic response. The degree of oxidative stress and DNA damage is also dependent on the kind of nanomaterial used, with High-Z materials, like gold and bismuth, showing varied electron emission studies and ROS generation efficiencies, which then variably adjust the degree of radiosensitization.

Tumour microenvironment reprogramming and immunomodulatory effects In addition to direct tumour cell targeting, exosome-based nanoradiosensitizers have a tremendous impact on tumour microenvironment (TME), which promotes tumour radiosensitisation and immunomediated tumour control. Exosomes have the capacity to change stromal and immune compartments, such as fibroblasts, endothelial cells, and tumour-infiltrating lymphocytes, through changes in the secretion of cytokines and the composition of an extracellular matrix. To illustrate, by attracting radiosensitiser to exosomes, vascular normalisation, alleviation of hypoxia, and diffusion of ROS are promoted, which increase radiation activity [139]. Besides, immune-modulatory cargos (i.e. siRNAs directed to immunomodulatory checkpoints or immunostimulatory nanoparticles) can stimulate cytotoxic T lymphocytes and reconfigure suppressive myeloid populations, which strengthens anti-tumour immunity after irradiation [140]. Such dual tumour cell cytotoxicity and TME reprogramming make exosome-based nanoradiosensitizers an effective platform in combination with radiotherapy against immunotherapy and targeted intervention to achieve a reduced recurrence rate and better long-term outcomes [141]. Table 4, and Fig. 7 is the summary of the exosome loaded nanoradiosensitizer mechanisms, molecular pathways, endpoints, and therapeutic effects.

8 Translational challenges and manufacturing considerations

Strong, scalable and repeatable manufacturing systems are required to translate clinical laboratory bench projects into clinical composite nanoradiochemotherapy using exosome loaded nanoradiosensitizers [153]. Mass productions of exosomes when optimized systems of donor cells are involved that include bioreactors and perfusion-based culture and growth platforms that ensure the standardisation of yield and phenotype of the vesicles [154]. Examples of such methods of purification can include ultracentrifugation, size-exclusion chromatography (SEC), and tangential flow filtration (TFF) that offer unique capabilities of scalability, purity and vesicle integrity [155]. Clinical-grade production is being increasingly preferenced with SEC and TFF because they are reproducible, and cause less vesicle damage, as well as being compatible with the restricted workflows of the good manufacturing practice (GMP), improving the translational feasibility [156, 157]. Standard quality control is a prerequisite to guarantee the safety, consistency and therapeutic fidelity of exosome based nanoradiosensitizers. The standards of release normally include identity (surface markers, size profiling), purity (protein and lipid contaminants), and potency (functional radiosensitization assays), sterility, endotoxin-levels and residual DNA / RNA content [157]. Functional bioassays, proteomics,

Table 4 Molecular and immunological mechanisms of exosome-loaded nanoradiosensitizers in radiosensitization and tumor recurrence suppression

No.	Mechanistic category	Molecular pathway/process	Exosome cargo type	Biological effect	Therapeutic outcome	References
1	Survival signaling inhibition	PI3K/Akt/mTOR suppression	siRNA, metal NPs	Reduced pro-survival signaling	Increased radiosensitivity	[142]
2	Stress response modulation	MAPK/ERK pathway inhibition	Small-molecule radiosensitizers	Decreased adaptive radioresistance	Enhanced apoptosis	[142]
3	Inflammatory signaling	NF-κB pathway inhibition	siRNA, immunomodulators	Reduced inflammatory survival signalling	Tumor growth inhibition	[143, 144]
4	DNA repair disruption	ATM/ATR signaling suppression	CRISPR/siRNA cargos	Impaired DNA repair capacity	Accumulated DNA damage	[83]
5	DNA damage amplification	ROS overproduction	Metal nanoparticle radiosensitizers	Oxidative DNA lesions, DSBs	Tumor cell death	[145]
6	Repair pathway blockade	NHEJ/HR inhibition	Gene-silencing cargos	Failed DNA repair	Mitotic catastrophe	[146]
7	Cell-cycle modulation	G2/M checkpoint arrest	siRNA + drugs	Radiosensitive phase trapping	Enhanced radiotherapy efficacy	[147]
8	Hypoxia modulation	HIF-1α suppression	Nanoparticles + siRNA	Reduced tumor hypoxia	Improved radiation response	[148]
9	TME reprogramming	Stromal cell modulation	Immunomodulatory exosomes	Altered cytokine signalling	Reduced tumor support niche	[149]
10	Immune activation	$CD8^+$ T-cell activation	Immune-stimulatory cargos	Enhanced anti-tumor immunity	Recurrence suppression	[150]
11	Immune checkpoint modulation	PD-L1 silencing	siRNA exosomes	Immune checkpoint blockade	Synergistic radioimmunotherapy	[83]
12	Vascular normalization	Endothelial modulation	Radiosensitizer-exosomes	Improved perfusion/oxygenation	Increased radiosensitivity	[151]
13	ECM remodeling	Fibroblast reprogramming	siRNA cargos	Reduced fibrosis/barrier effects	Enhanced tumor penetration	[16]
14	Immunogenic cell death	ROS + DAMPs release	Nanoradiosensitizers	Tumor antigen presentation	Long-term immune memory	[83]
15	Recurrence prevention	TME + immune synergy	Multi-cargo exosomes	Suppressed residual tumor niches	Reduced post-irradiation recurrence	[152]

flow cytometry, and nanoparticle tracking analysis are examples of advanced analytical tools that are necessary to perform batch validation [158]. Relevant Regulatory approval and clinical translation in this regard, it is especially important to establish reproducible potency assays that are connected to the mechanism of radiosensitization, e.g., reactive oxygen species (ROS) amplification or DNA damage [159].

Though SEC and TFF have become more accepted as more convenient purification methods compared to traditional ultracentrifugation, their extensive use has to be considered due to their cost and accessibility. TFF has some significant manufacturing benefits, such as closed process, less manual handling, faster processing times, and ability to concentrate large amounts of fluid, which can be used to reduce labour requirements and enhance batch reproducibility as the scale-up [160]. But this comes at the cost of special instrumentation requirements, validated membrane systems, regular cleaning

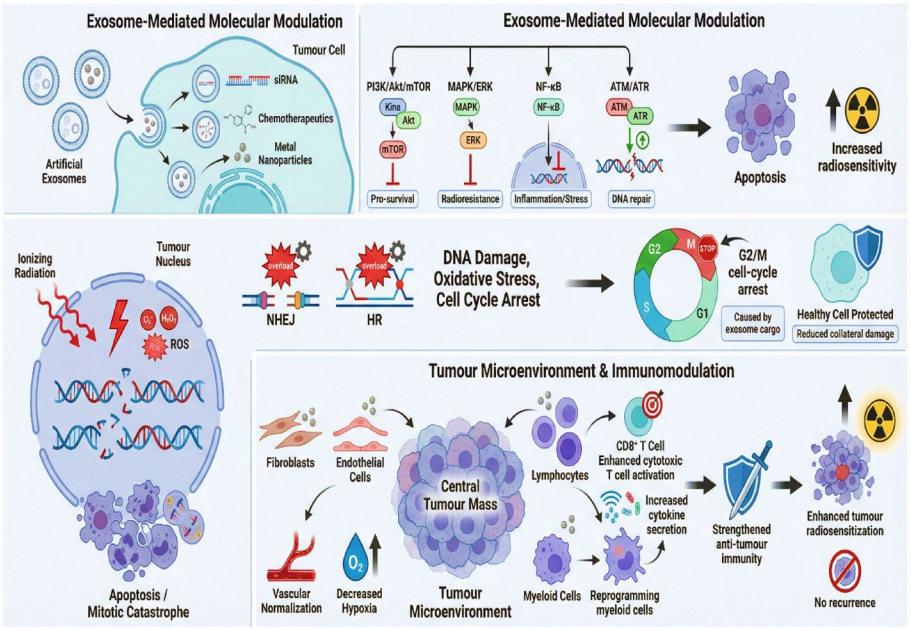

Fig. 7 Molecular and microenvironmental mechanisms by which exosome-loaded nanoradiosensitizers enhance tumor response to radiation

or consumable single usage and trained technical personnel all of which raises the initial and operating expenses [161]. SEC is also quite user-friendly due to relatively mild effects on vesicle integrity and the ability to enhance separation of exosomes and soluble protein contaminants, although its large-scale utilization might be limited due to column capacity, resin cost, fractionation time and requirement to run repetitively or in parallel to produce at high throughput [161]. Therefore, although both TFF and SEC are better suited to Good Manufacturing Practice (GMP) production than the outdated ultracentrifugation, they are not universally available particularly in low resource translational environments or early manufacturing research and development. An example of a more plausible route to more widespread clinical use is standardized hybrid operations, like TFF to bulk concentration, and SEC to polish, with automation and closed-system platforms, and low-cost disposable elements to enhance reproducibility but reduce per-batch production overhead.

Combinations of biologic nanoradiosensitizers with exosomes form complex product due to which specific regulatory challenges can occur [19]. These interfaces combine self-assembled biological vesicles, engineered nanomaterials, and therapeutic cargos which requires mutual agreement on regulatory pathways having to traverse biologics, gene therapy and nanomedicine platforms [19, 162]. These should include regulatory assessment of product characterization, consistency in manufacturing, biodistribution, the safety in the long term, immunogenicity and off-target effects. In order to promote the approval of regulatory procedures, strict classification rules, uniform laboratory-manufacturing methodology, and assays of potency connected to the mechanism are needed [163]. The adaptive regulatory models and early interactions with regulation should be relevant to boosting clinical translation and safeguard patient welfare and therapeutic predictability. Currently exosome-based radiosensitizers seem to be mostly preclinical. Although exosome therapeutics are beginning to have early phase therapeutic trials such as MSC-derived exosomes carrying KRASG12D siRNA in pancreatic

cancer, these are not radiotherapy-specific radiosensitizer platforms [164]. Clinical studies in radiotherapy more often evaluate exosomes as biomarkers of treatment response rather than as therapeutic radiosensitizers. This highlights why better regulatory-classification and translational-pathways must be clarified before exosome-loaded nanoradiosensitizers can be subjected to human trials.

8.1 Which translational bottlenecks are generic, and which are unique to exosome-loaded systems

All translational barriers are not identical. Others as heterogeneous tumor uptake, mononuclear phagocyte clearance and the distance between animal and human biodistribution is generic to nanoradiosensitizers [165]. With liposomal and polymeric systems, others such as sterility, stability during storage, release testing and process scale-up are common [166]. Non-nonetheless, exosome-based nanoradiosensitizers encounter even greater bottlenecks, including donor-cell-dependent variability, ambiguity in determining which extracellular vesicles make up the exosomes, potential loss of functional activity of native membranes in loading nanoparticles, and whether a hybrid biological nanomaterial product is regulated like a biomacromolecule [33, 167]. It is these exosome specific barriers and not proof-of-concept efficacy alone that are likely to dictate the success of clinical translation of the platform.

9 Clinical perspectives and trial design

Exosome based nanoradiosensitizers have shown significant clinical potential in a range of malignancies with intrinsic or acquired radioresistance (e.g. glioblastoma, pancreatic cancer, non-small-cell lung cancer, head/neck cancer and locally advanced breast and prostate cancer) [168]. Their modular design allows logical combination with established radiotherapy, chemotherapy, immunotherapy, and targeted modalities of therapy and hence generates synergistic tumor suppression. Immune checkpoint inhibitors or hypoxia -modulators/DNA repair inhibitors can also be used in combination and complement how effective therapy is [169]. These combinations have aided in designing individualistic radiotherapeutic plans that have enabled the dose de-escalation, greater tumor selectivity and reduced post-irradiation recurrence. Translationally, dose de-escalation concept is backed up by the preclinical results carried out that nanoradiosensitizers may boost the effectiveness of radiation at lowered dosing levels in a significant manner [170]. Various in vitro and in vivo studies reviewed here demonstrate that radiosensitization approaches especially with high-Z nanoparticles and exosome-based delivery can be able to reduce radiation dose by an average of about 20–50% of the original dose with a similar level of tumor control depending on tumor model, nanoparticle composition and delivery efficiency [84]. This is demonstrated by increased production of ROS, amplification of DNA damage and improved targeting of tumors, which have been shown to increase radiosensitivity enough to lower the dose of radiation needed to ameliorate therapeutic effects [84]. These estimates are model-dependent and, even though they should be validated in clinical trials, illustrate that exosome-loaded nanoradiosensitizers have potential to increase the therapeutic window by reducing toxicity of normal tissues without reducing tumor control.

Effective clinical translation of exosomes based radiosensitizers require effective patient stratification schemes to exclude the cohorts that are least likely to benefit. The

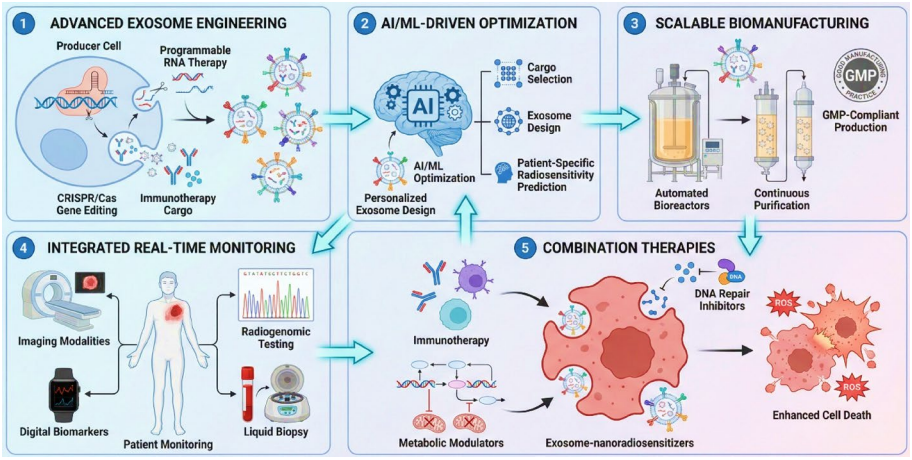

Fig. 8 Future directions for exosome-loaded nanoradiosensitizers: precision design, scalable manufacturing, and clinically guided translation

indicators reported as candidate biomarkers include tumor hypoxia signatures, activity of DNA repair-capability, ability to counter reactive oppose gas signals, and immune infiltration or preventing capabilities. Responsiveness to radiosensitization could be predicted by molecular biomarkers of ATM/ATR status, HIF-1 expression, PD-L1 levels as well as tumor-microenvironment immune composition [171, 172]. Non-invasive methods such as liquid biopsies, circulating exosome profiling and radiogenomic signatures can be used to offer real-time monitoring [173]. Designs based on biomarkers will increase precision in therapy, reduce non-responder exposure, and predictability of clinical outcome.

The safety, tolerability, and biologically relevant activity must be prioritized as first-in-human research. An integrated comparison of pharmacokinetic parameters, biodistribution evidence, and radiobiologic outcomes ought to be incorporated, instead of the old-fashioned determination of the maximum tolerated dose to be used in dose-escalation [174]. Immunogenic potential, off-target accumulation, and fundamental retention as well as the toxicity of nanomaterials should be viewed as critical safety issues [175]. Imaging-based tracking, whole immune-profiling, and all the molecular biomarkers denoting damaged DNA and oxidative stress should be introduced in real-time monitoring protocols [176]. Superior to this, adaptive trial designs in conjunction with the use of longitudinal safety surveillance will be critical in establishing safe dosing regimens as well as supporting successful clinical translation of exosome loaded nanoradiosensitizers.

10 Future directions

The exosome-loaded nanoradiosensitizers that could be investigated in the future are likely to move along the line of precision nanomedicine, synthetic biology, and even computational oncology (Fig. 8). The emerging technologies involve combining gene-editing systems, including the CRISPR/Cas systems, programmable RNA therapy, and versatile immunotherapy cargos, thus creating intelligent adaptive radiosensitization systems [177]. It is predicted that artificial intelligence and machine-learning algorithms will guide the choice of the cargo, optimize the exosome engineering, and predict the patient-specific profiles of radiosensitivity, serving to make a genuinely personalized

course of radiotherapy [178]. The expectation of overcoming the current translational barrier is presented by the development of scalable biomanufacturing directed by automated bioreactors, continuous purification processes, GMP-compliant manufacturing lines and so on. Further, dynamic therapeutic scheme responses, surveillance of responses, will be combined through imparting real-time imaging modalities, radiogenomic testing, digital biomarkers, and liquid biopsy technological [179]. Combination with modalities of immunotherapy, DNA repair inhibitors and metabolic modulators will expand the therapeutic horizon [180]. Together, these innovations will transform exosome-based nanoradiosensitizers, an experimental product, into a clinically feasible, precision-guided radiotherapeutic system, which will revolutionize the sphere of oncology practice.

11 Conclusion

Exosome loaded nanoradiosensitizers represent the future-oriented, well integrated paradigm of modern radiotherapies, integrating biological targeting, biomathematical nanotechnology and molecular radiosensitization in one distinctive therapeutic package. These systems also evade intrinsic and acquired resistance against radiation by increasing radiation-induced DNA damage, elevation of oxidative stress, reduction of/ regulation of cell-cycle checkpoints, and re-educating the tumor microenvironment. The fact that they can sensitize tumor cells directly, as well as remodel immune and stromal components of the tumor niche, places them among the powerful agents to prevent post-irradiation tumor recurrence. Notably, their modular and personalized structure allows an easy fit with immunotherapy, targeted therapy, and precision oncology models. However, exosome-loaded nanoradiosensitizers must be considered a high-potential but yet to be validated hybrid platform but not an all-purpose solution. They do not have the theoretical capability to overcome the inherent radiosensitizing potentials of traditional nanoparticles, but in coupling those physical effects to biologically informed delivery, stromal and immune homeostasis, and multi-cargo fusion. These benefits are yet to be demonstrated in standardized and clinically relevant conditions to be converted into clinically meaningful better agents compared to existing liposomal, polymeric, or free-agent strategies. Overall, exosome-based nanoradiosensitizers are the next frontiers of radiotherapy enhancement, although their ultimate clinical usefulness will be determined based on their ability to demonstrate demonstrable additional value over current systems, and to surmount the unique synthesis and regulatory challenges of hybrid exosome-based systems.

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Declarations

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Declaration of generative AI and AI-assisted technologies in the manuscript preparation process
During the preparation of this work the author(s) used Grammarly & AI to frame the grammar and English editing. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, Federal University of Health Sciences, Otukpo, Benue State, Nigeria
[2]Department of Biochemistry, Research and Publications, Kampala International University, P.O. Box 20000, Kampala, Uganda
[3]Department of Public Health Science, Faculty of Allied Medical Sciences, University of Calabar, Calabar, Nigeria
[4]Department of Medical Laboratory Science, College of Health Technology Calabar, Calabar, Cross River State, Nigeria
[5]Division of Anatomy, Department of Basic Medical Sciences, College of Medicine, University of Jeddah, 23890 Jeddah, Saudi Arabia
[6]Department of Basic Science, College of Medicine, Princess Nourah bint Abdulrahman University, P.O. Box 84428, 11671 Riyadh, Saudi Arabia
[7]University Institute of Pharma Sciences, Chandigarh University, Mohali, Punjab, India
[8]Sharda School of Pharmacy, Sharda University, Greater Noida, India
[9]Department of Oral and Maxillofacial Pathology, Malla Reddy Institute of Dental Sciences, Malla Reddy Vishwavidyapeeth, Suraram, Hyderabad, Telangana 500055, India
[10]Department of Biochemistry, Faculty of Sciences, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria
[11]Department of Science Laboratory Technology, Faculty of Biological Sciences, University of Calabar, Calabar, Cross River State, Nigeria

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