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Extracellular vesicles and interventional radiotherapy (modern brachytherapy): a translational gap analysis of dose-gradient-driven biological responses

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Abstract Extracellular vesicles (EVs) play a key role in radiation-induced biological responses and intercellular signaling. While extensively studied in external beam radiotherapy, their interaction with interventional radiotherapy (IRT, modern brachytherapy), characterized by steep and localized dose gradients, remains poorly explored. A qualitative literature-based gap analysis was conducted using PubMed and Scopus to identify studies investigating EVs in clinical or experimental settings involving IRT. Eligible publications included any form of EV analysis in patients or models treated with IRT alone or in combination with other therapies. Studies were qualitatively categorized according to treatment modality, EV characterization strategy, biological samples, and study endpoints. Only a limited number of original studies were identified. When IRT was delivered as a standalone treatment, localized irradiation was sufficient to induce measurable modulation of EV populations and cargo. In combined treatment settings, EVs were more commonly investigated as systemic biomarkers of treatment response or immune modulation, without isolating the specific biological contribution of IRT. Across studies, EV characterization approaches and clinical contexts were highly heterogeneous, and spatial dose–response relationships intrinsic to IRT were rarely addressed. Despite its intrinsic physical advantages, IRT remains underutilized as a platform for investigating EV-mediated radiation responses. The available evidence suggests that IRT can induce distinct EV modulation, but its biological potential is largely unexplored. Integrating EV analysis with IRT-specific dosimetry and spatial dose gradients represents a relevant translational opportunity for future radiobiological research.

1 Introduction

Extracellular vesicles (EVs) are increasingly recognized as central mediators of intercellular communication and biological stress responses. Released by virtually all cell types, EVs carry bioactive cargo including nucleic acids, proteins, lipids, and metabolites, reflecting the physiological or pathological state of the cell of origin. In oncology, EVs have emerged as key players in tumor progression, immune modulation, therapy resistance, and as promising candidates for liquid biopsy applications. Their accessibility in biological fluids further positions EVs as attractive tools for translational research, enabling the connection between molecular mechanisms and clinically measurable biomarkers [1, 2].

In the context of radiation oncology, a growing body of evidence indicates that EVs actively participate in radiation-induced biological processes rather than acting as passive byproducts of cellular damage. EV-mediated signaling has been implicated in DNA damage response, bystander effects, immune activation or suppression, and long-term tissue remodeling following irradiation. These processes align closely with the classical radiobiological framework of the "six Rs" of radiotherapy (RT), which describes the complex cellular and tissue-level responses to ionizing radiation [3]. Importantly, much of this evidence originates from preclinical

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models and external beam radiotherapy (EBRT) settings, highlighting the critical role of experimental systems in elucidating EV-mediated mechanisms with potential translational relevance.

Interventional Radiotherapy (IRT, also referred to as modern brachytherapy) represents a fundamentally distinct radiation modality characterized by localized source placement and steep dose gradients over short spatial scales. This intrinsic physical heterogeneity enables the controlled induction of non-uniform radiation-induced physical stress within confined volumes and closely reflects the dose distributions delivered during routine clinical practice. From a preclinical perspective, these characteristics provide a powerful and reproducible framework for investigating heterogeneous radiation responses under well-defined physical conditions. From a translational standpoint, the ability of IRT to replicate clinically realistic dose distributions makes it particularly suitable for bridging experimental observations with patient-oriented applications [4].

Importantly, beyond their physical and dosimetric characteristics, the steep dose gradients inherent to IRT may also have relevant biological implications. Within millimetric distances from the radiation source, cells are exposed to markedly different dose levels, leading to the coexistence of highly irradiated regions and adjacent low-dose compartments within the same microenvironment. This spatial heterogeneity is expected to result in differential cellular stress responses, including DNA damage, oxidative stress, and activation of adaptive pathways depending on the local absorbed dose [3]. In this context, extracellular vesicles may represent potential mediators of intercellular communication. Previous evidence suggests that EVs can be involved in radiation-induced bystander effects, contributing to the transfer of stress-related signals from irradiated to non-irradiated or less-irradiated cells through the delivery of proteins, nucleic acids, and other bioactive cargo [5, 6]. It is therefore plausible that cells exposed to higher doses may release EVs enriched in damage-associated signals, while neighboring cells in lower-dose regions may exhibit distinct EV profiles associated with sublethal or adaptive responses. Such conditions could support the establishment of a gradient-dependent paracrine signaling network between compartments experiencing different radiation intensities. This scenario may differ from conventional external beam radiotherapy models, in which irradiation is typically delivered more uniformly across the target volume and may not fully capture the complexity of intercellular communication arising from spatial dose heterogeneity [3]. In this perspective, IRT may provide a suitable experimental and translational platform to further investigate how dose gradients influence EV biogenesis, cargo composition, and intercellular signaling dynamics.

The limited exploitation of IRT in EV research does not appear to stem from methodological constraints. Advanced EV isolation, molecular profiling, and functional characterization techniques are well established and widely applied in radiation oncology research, particularly in preclinical EBRT-based models. At the same time, IRT benefits from a mature dosimetric framework, with validated models routinely used for dose calculation in both experimental and clinical settings. Together, these elements create favorable conditions for the development of preclinical IRT models that are directly translatable to the clinic, a key requirement for meaningful biomarker discovery and mechanistic validation.

Despite the rapid expansion of EV-focused literature, existing reviews have largely concentrated on EV biology in cancer, EVs as diagnostic or prognostic biomarkers, or EV-based therapeutic strategies, without explicitly addressing their interaction with specific RT modalities. Reviews dedicated to EVs in uveal melanoma, prostate cancer, and other oncological settings provide comprehensive overviews of EV roles in tumor biology and liquid biopsy applications, yet they do not examine the potential of IRT as a driver of EV-mediated radiation responses [1, 2, 7]. In particular, the translational value of integrating preclinical IRT models with EV analysis has not been systematically discussed.

Against this background, the aim of the present work is to critically examine the existing literature at the intersection of IRT and EVs, with a specific focus on translational relevance. This work seeks to assess how IRT has been used, or overlooked, as a platform to induce radiation-related physical stress and to investigate EV-mediated biological responses in both preclinical and clinical contexts. By framing the available evidence as a gap analysis, this review highlights the scarcity of studies that explicitly leverage IRT as a translational model system. Addressing this gap could substantially advance the understanding of radiation-induced stress responses and support the development of EV-based biomarkers and mechanistic insights that are directly applicable to IRT.

2 Methods

The literature review was designed as a qualitative gap analysis with the aim of evaluating how IRT has been explored as a source of radiation-induced physical stress and to what extent EVs have been investigated as biological mediators or reporters of such stress.

The literature search was performed using the PubMed and Scopus databases, selected to ensure comprehensive coverage of biomedical, translational, and technical research. Searches were conducted using combinations of the keywords “Brachytherapy” OR “Interventional Radiotherapy” AND “Extracellular Vesicles,” including related terms such as “exosomes” and “EVs.” No restrictions were applied regarding year of publication, study design, or experimental model, including in vitro, in vivo, and clinical studies, in order to capture the full extent of available evidence.

All retrieved records were screened based on title and abstract to assess relevance to the scope of the review. Eligible studies were those that explicitly involved IRT and included any form of extracellular vesicle analysis, whether biological, molecular, functional, or methodological. Studies investigating EV responses to ionizing radiation delivered exclusively via EBRT were considered for contextual comparison, particularly when used to highlight methodological maturity or conceptual differences relative to IRT-based research.

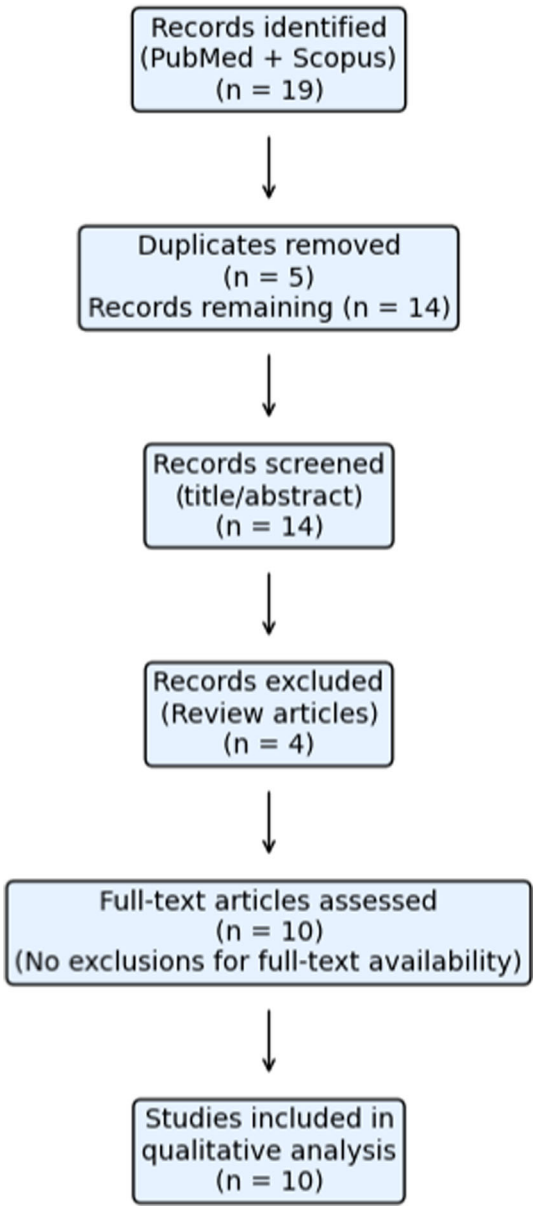

Fig. 1 PRISMA-inspired flow diagram of the study selection process

The database search yielded a total of 19 records. After removal of duplicates (n = 5), 14 records were screened based on title and abstract. Of these, 4 articles were excluded as review papers. The remaining 10 full-text articles were assessed for eligibility, and all met the inclusion criteria. No articles were excluded due to unavailability of full text. Following full-text evaluation, a final set of 10 studies was included in the qualitative analysis. The study selection process is summarized in a PRISMA-inspired flow diagram (Fig. 1).

Following screening, full-text articles were assessed and categorized according to several qualitative dimensions, including cancer site or biological context, role of IRT within the treatment protocol (primary treatment, combined modality, prior treatment, or not included), type of IRT, biological sample analyzed, level of EV characterization, and primary study endpoint. Particular attention was paid to whether extracellular vesicle analyses were explicitly linked to IRT-induced radiation effects or whether IRT was treated as a background clinical variable. A qualitative assessment of the level of evidence was also performed based on study design, type of analysis, and presence of clinical or functional validation. Studies were qualitatively categorized as Preliminary when based on descriptive or limited analyses without clear clinical or mechanistic correlation; as Moderate when reporting clinical or molecular data with exploratory or associative findings; and as Moderate-High when including functional validation and/or robust associations with clinical outcomes.

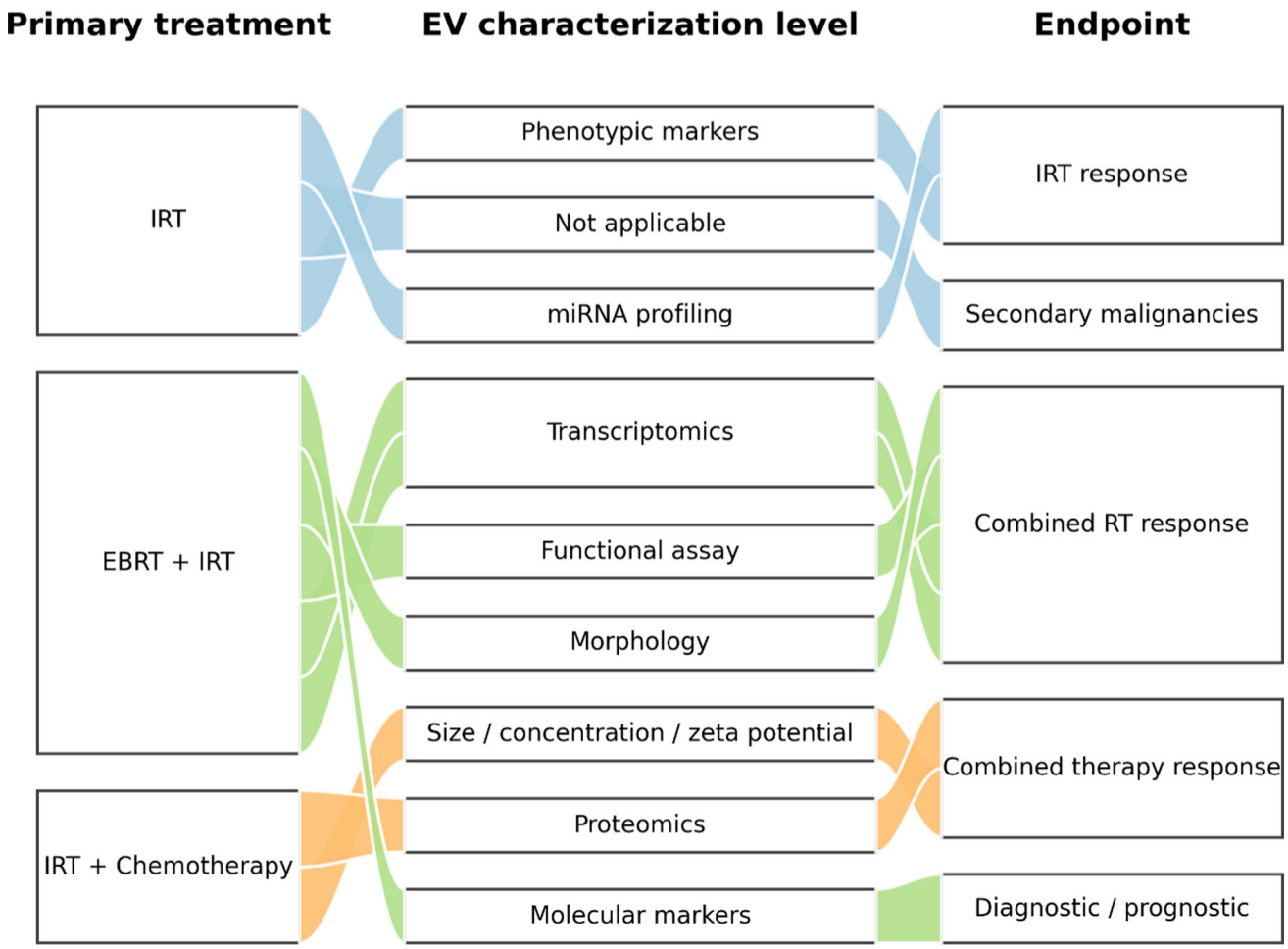

Fig. 2 Alluvial diagram illustrating the relationships between primary treatment, EV characterization level, and study endpoint in the included studies

3 Results

The literature search identified a limited number of studies investigating EVs in clinical or experimental settings involving IRT. After removal of duplicate records and review articles, a total of 10 original publications met the inclusion criteria and were included in the qualitative analysis.

An overview of the main characteristics of the included studies is provided in Tables 1 and 2, which summarize the clinical or biological context, the primary treatment modality, the role of IRT within the therapeutic pathway, the type of IRT employed, the biological samples analyzed, the level of EV characterization, and the primary study endpoints. Figure 2 presents an alluvial plot summarizing the relationships between treatment modality, EV analysis strategy, and evaluated outcomes.

As reported in Table 1, studies in which IRT was delivered as the sole treatment modality were conducted in uveal melanoma and prostate cancer cohorts. Table 2 includes studies performed in cervical cancer, hepatocellular carcinoma, uveal melanoma, and prostate cancer, in which IRT was administered in combination with EBRT and/or chemotherapy. All included investigations were conducted in clinical patient cohorts. The qualitative assessment of the level of evidence highlighted the predominance of studies with Moderate or Moderate-High evidence, mainly based on exploratory clinical analyses and in some cases, supported by functional validation. A smaller proportion of studies were classified as Preliminary.

Across the included studies, the role of IRT varied from primary definitive treatment to a component of combined treatment protocols. IRT was delivered in conjunction with EBRT in cervical cancer cohorts treated with EBRT + HDR-IRT [11–14, 17], while plaque IRT [8, 16] and LDR IRT [9, 10] were used as standalone radiation modalities in uveal melanoma and prostate cancer, respectively.

The biological materials used for EV analysis included plasma [11–13, 15–17], serum [10], whole blood [14], aqueous humor [8], and tissue specimens [9]. EV characterization approaches ranged from phenotypic marker and morphology-based analyses [8, 14] to transcriptomic, proteomic, molecular, and functional assays, as summarized in Table 1 and Table 2.

Table 1 Summary of studies examining EVs in clinical or experimental settings with IRT as the sole treatment

Paper	Year	Authors	Nation	Cancer site/Context	Primary Treatment	IRT Type	Biological Sample	EV characterization level	Endpoint	Main EV-related finding	Level of evidence
[8]	2024	Sirivolu et al	United States	Uveal melanoma	IRT	Plaque	Aqueous humor	Phenotypic markers (CD9, CD63, CD81)	IRT response	IRT modulates specific EV subpopulations	Moderate
[9]	2022	Zhao et al	United States	Prostate cancer	IRT	LDR	Tissue	Not applicable	Secondary malignances	Not applicable	Preliminary
[10]	2021	Iinuma et al	Japan	Prostate cancer	IRT	LDR	Serum	MiRNA profiling	IRT response	Sustained downregulation of EV miR-93 after IRT	Moderate

Table 2 Summary of studies examining EVs in clinical or experimental settings involving IRT combined with EBRT and/or chemotherapy

Paper	Year	Authors	Nation	Cancer site/Context	Primary Treatment	IRT Type	Biological Sample	EV characterization level	Endpoint	Main EV-related finding	Level of evidence
[11]	2021	Cho et al	Korea	Cervical cancer	EBRT + IRT	HDR	Plasma	Transcriptomics	Combined RT response	EV mRNAs correlate with immune decay and survival	Moderate
[12]	2022	Cho et al	Korea	Cervical cancer	EBRT + IRT	HDR	Plasma	Transcriptomics	Combined RT response	EV transcriptome linked to aggressive disease	Moderate-High
[13]	2022	Ren et al	China	Cervical cancer	EBRT + IRT	HDR	Plasma	Functional assay	Combined RT response	EVs mediate macrophage polarization	Moderate-High
[14]	2020	Mamaeva et al	Russia	Cervical cancer	EBRT + IRT	HDR	Whole blood	Morphology	Combined RT response	Radiation induces dynamic RBC-bound vesicular changes	Preliminary
[15]	2024	Egerer et al	Germany	Hepatocellular carcinoma	IRT + Chemotherapy	HDR	Plasma	Size, concentration, zeta potential	Combined therapy response	EV features predict survival and response	Moderate-High
[16]	2022	Khan et al	United States	Uveal Melanoma	IRT + Chemotherapy	Plaque	Plasma	Proteomics	Combined therapy response	EV protein cargo reflects targeted therapy exposure	Moderate
[17]	2019	Adeleke et al	United Kingdom	Prostate cancer	EBRT + IRT	HDR	Plasma	Molecular markers	Diagnostic/prognostic	Exosomes support disease restaging	Moderate

The primary endpoints reported across studies included IRT response [8, 10], combined RT response [11–15], diagnostic or prognostic evaluation [17], and associations with survival outcomes. EV-related findings were reported in relation to these endpoints according to the specific EV features assessed in each study.

Overall, the included studies describe extracellular vesicle analyses performed in oncological patient cohorts treated with IRT, either as a standalone treatment or in combination with EBRT and/or chemotherapy, using heterogeneous biological samples and EV characterization strategies. Table 1 and Table 2 summarize individual study characteristics, while Fig. 2 illustrates the distribution of treatment modalities, EV characterization, and evaluated endpoints.

EV analyses in the context of IRT were reported across heterogeneous oncological settings, either with IRT delivered as a standalone treatment or as part of combined therapeutic regimens. When IRT was administered alone, localized radiation exposure was sufficient to induce measurable EV modulation. In uveal melanoma patients treated with plaque-based IRT, selective changes in tetraspanin-defined EV subpopulations were detected in the aqueous humor, indicating localized vesicular responses within the tumor microenvironment [8].

Similarly, in prostate cancer patients undergoing LDR IRT, serum EV-associated microRNA profiling revealed sustained post-treatment molecular modulation detectable at the systemic level [10]. In combined treatment settings, EVs were more frequently investigated as mediators of treatment-induced intercellular signaling. In cervical cancer cohorts treated with EBRT plus HDR IRT and concurrent chemotherapy, plasma-derived EVs exhibited transcriptomic signatures associated with immune suppression, inflammatory signaling, disease aggressiveness, and survival [11, 12].

Functional studies further demonstrated that EVs isolated after combined RT could actively modulate macrophage polarization, providing direct evidence of EV-mediated immune signaling following irradiation [13]. Additional analyses of whole blood samples revealed radiation-associated vesicular alterations linked to red blood cell morphology, highlighting dynamic systemic EV responses during treatment [14].

Other studies investigated EV-related features as indicators of treatment response in multimodal oncological settings involving IRT. In hepatocellular carcinoma and uveal melanoma cohorts, circulating EV size, concentration, surface charge, and proteomic cargo exhibited longitudinal changes associated with clinical outcomes, reflecting therapy-induced systemic biological modulation [15, 16]. However, in these studies, the specific biological contribution of IRT could not be independently isolated from that of EBRT or systemic therapies.

Finally, some investigations included IRT-treated cohorts without directly addressing EV-mediated biological effects. Tissue-based analyses described long-term pathological changes following brachytherapy in prostate cancer without EV characterization [9], while prospective imaging studies integrated blood sampling for exploratory exosome analyses without reporting EV-related biological endpoints [17]. Together, these studies illustrate the heterogeneity of current approaches and underscore the limited and inconsistent biological exploitation of IRT for extracellular vesicle research.

4 Discussion

This work was conceived to assess whether IRT can be considered a suitable yet underutilized platform for investigating radiation-induced physical stress and its biological consequences through extracellular vesicles (EVs). The underlying rationale is that EVs act not only as sensitive reporters of cellular stress but also as active mediators of intercellular signaling, capable of propagating radiation-induced responses across irradiated and non-irradiated compartments at both local and systemic levels. While this conceptual framework is well established in radiation oncology, the results of the present literature-based analysis demonstrate that its application to IRT remains remarkably limited.

Importantly, this work is not intended to be a systematic review of the literature. Rather, it represents a focused revision of available studies aimed at contextualizing existing evidence and above all, highlighting a clear gap in the biological exploitation of IRT, particularly with respect to EV-mediated radiation responses and intercellular signaling mechanisms.

As emerging from the studies summarized in Tables 1 and 2, EV analyses are increasingly incorporated into clinical and translational investigations involving RT-treated patients. However, in most cases, EV modulation is interpreted in relation to systemic therapies or combined radiation exposure, with IRT being considered a prior, concomitant, or complementary intervention rather than a modality subjected to direct biological interrogation. In particular, studies involving IRT in combination with EBRT and/or chemotherapy (Table 2) predominantly analyze EVs at a systemic level, without accounting for the spatially heterogeneous dose distributions that are intrinsic to IRT [11–17]. Even when IRT represents a substantial component of the delivered treatment, its specific physical contribution to EV release, cargo modulation, and downstream signaling is rarely isolated or discussed.

Conversely, a limited number of proof-of-principle studies summarized in Table 1 indicate that IRT delivered as a standalone treatment is sufficient to induce measurable and persistent changes in EVs. Modulation of EV subpopulations following plaque-based IRT and sustained alterations in EV-associated microRNA cargo after LDR prostate IRT provide direct evidence that localized irradiation can generate distinct EV signatures [8, 10]. These findings support the biological plausibility of using IRT-induced physical stress as a trigger for EV-mediated signaling. Nevertheless, the available studies remain largely confined to temporal comparisons before and after treatment and do not integrate dosimetric parameters, spatial dose heterogeneity, or distance-dependent effects

relative to the radiation source. As a result, the role of EVs as vectors of intercellular signaling driven by non-uniform radiation exposure remains largely unexplored.

The scarcity of IRT-focused EV studies does not appear to be attributable to methodological or technical limitations. As reflected by the broader EV literature and by studies included in this work, advanced EV isolation methods, high-resolution molecular profiling, and functional characterization pipelines are already widely available and routinely applied in EBRT-based radiation research, including transcriptomic, proteomic, and functional approaches [7, 9, 16]. In parallel, extensive evidence demonstrates that EVs actively participate in key radiobiological processes traditionally summarized by the "six Rs" of RT, including DNA damage repair, cell cycle redistribution, repopulation, reoxygenation, intrinsic radiosensitivity, and immune reactivation [3]. Through the transfer of bioactive cargo such as proteins, lipids, DNA fragments, and regulatory RNAs, EVs mediate intercellular signaling pathways that propagate radiation-induced stress signals, modulate survival mechanisms, and reshape immune and microenvironmental interactions following irradiation.

Notably, these radiobiological mechanisms are highly sensitive to radiation-induced physical stress and spatial dose heterogeneity. However, they have been predominantly investigated using homogeneous irradiation models, which may overlook critical EV-mediated signaling events arising from clinically relevant non-uniform dose distributions. This methodological mismatch underscores the need to integrate EV-based analyses into irradiation paradigms that better reflect the spatial complexity of clinical RT.

In this context, IRT offers several intrinsic advantages that make it a particularly powerful experimental and translational model for studying EV-mediated intercellular signaling. The physical nature of IRT is characterized by steep and well-defined dose gradients generated directly by the radioactive source, enabling the controlled induction of heterogeneous physical stress over millimetric distances within the same biological system. Such conditions are difficult to reproduce using conventional EBRT techniques and provide an ideal framework for investigating how localized radiation exposure shapes EV release, cargo composition, and signaling toward neighboring and distant cells as a function of local absorbed dose and spatial proximity to the source.

Furthermore, IRT is supported by a mature and robust dosimetric framework, with well-established dose calculation models routinely applied in experimental and clinical settings, including TG-43-based approaches and more advanced model-based algorithms [18, 19]. The availability of these tools enables accurate reconstruction of dose distributions even in complex geometries, supporting quantitative correlations between physical dose metrics and EV-mediated biological and signaling responses.

The experimental versatility of IRT is further enhanced by the increasing adoption of three-dimensional printing technologies. As highlighted by studies included in this work, customized applicators, phantoms, and irradiation setups can be rapidly designed to tailor source positioning, source-to-target distance, and dose gradients, enabling highly controlled and reproducible irradiation conditions [20]. Importantly, 3D printing allows not only accurate reproduction of patient-specific geometries but also deliberate modulation of dose delivery through customized layouts, multilayer structures, and high-density shielding [21]. This capability opens the possibility of designing experimental IRT setups in which radiation-induced physical stress is spatially shaped in a targeted manner, thereby enabling systematic investigation of how defined dose gradients drive EV-mediated intercellular signaling. Building on these considerations, a simplified in vitro experimental configuration can be envisioned (Fig. 3), in which a 3D-printed applicator is used to precisely position a multiwell cell culture system at defined distances from the radiation source, generating a spatially resolved dose gradient across the platform. This approach enables the identification of high- and low-dose regions within the same experimental setup, allowing site-specific EV collection and direct comparison of dose-dependent variations in EV release and cargo composition. Notably, the use of 3D-printed setups enhances both the precision and conformability of dose delivery, as source positioning, geometry, and shielding can be tailored to reproduce IRT-like dose heterogeneity under controlled and reproducible conditions. This is particularly relevant for in vitro studies, where conventional irradiation approaches often lack the spatial resolution required to mimic clinically relevant dose gradients.

Despite these intrinsic advantages, the results of this work clearly show that studies explicitly exploiting IRT to induce and investigate radiation-induced physical stress through EV-mediated signaling remain extremely limited. This disconnect represents a true gap in the literature rather than a marginal or emerging trend. Given the increasing clinical use of HDR IRT and the central role of EVs in radiation response and cell–cell communication, addressing this gap could provide novel mechanistic insights and support the development of EV-based biomarkers and signaling models specifically tailored to IRT.

Some limitations of the available evidence should nonetheless be acknowledged. The number of studies directly addressing EVs in the context of IRT remains small, limiting the possibility of drawing modality-specific or quantitative conclusions. Moreover, the included studies are highly heterogeneous in terms of tumor sites, treatment protocols, biological samples, EV isolation methods, and analytical endpoints, which constrains cross-study comparability. A further relevant source of variability specifically relates to extracellular vesicle isolation techniques, which may significantly influence EV yield, purity, size distribution, and molecular composition. Different methodologies, including differential ultracentrifugation, precipitation-based approaches, and size-exclusion chromatography, are known to isolate partially overlapping but non-identical EV populations, potentially affecting both quantitative and qualitative results. For instance, ultracentrifugation, although widely used, may co-isolate protein aggregates and lipoproteins, whereas precipitation-based methods can increase yield at the expense of purity. In contrast, size-exclusion chromatography generally provides higher purity but may result in lower recovery. This methodological heterogeneity may therefore contribute to differences in EV cargo and concentration reported across studies included in this analysis, particularly in terms of protein, lipid, and nucleic acid profiles. Importantly, EV subpopulations obtained through different isolation strategies may reflect distinct biological states or cellular origins, thereby introducing an additional layer of variability in downstream analyses. These aspects have been extensively

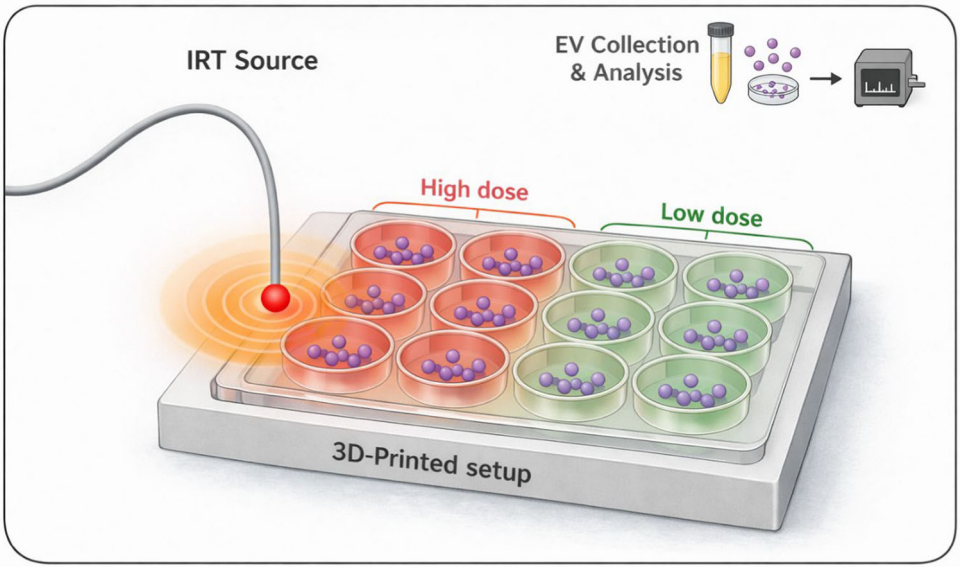

Fig. 3 Conceptual schematic of a simplified 3D-printed in vitro IRT experimental setup for spatially resolved investigation of EV-mediated signaling

discussed in recent guidelines and methodological reviews, which emphasize the need for standardized and well-characterized EV isolation workflows to ensure reproducibility and comparability across studies [22, 23]. In addition, experimental evidence supports the impact of isolation strategies on EV biochemical profiles, showing that different purification approaches can lead to measurable differences in vesicle composition and associated molecular signatures [24]. Taken together, these observations highlight isolation protocols as a potential confounding factor when interpreting EV-related findings.

In many cases, IRT is delivered as part of combined treatment regimens, complicating the isolation of its specific biological contribution. Finally, dosimetric information is often reported at a global level, without spatial resolution, preventing detailed analyses of dose- and distance-dependent EV-mediated signaling responses. In this context, the present work prioritizes conceptual synthesis and gap identification over quantitative aggregation, an approach that is appropriate given the exploratory nature of the field.

Overall, the evidence emerging from the analyzed studies suggests that IRT should be regarded not only as a therapeutic modality but also as a powerful experimental platform to induce controlled radiation-induced physical stress and to investigate EV-mediated intercellular signaling. The systematic integration of EV analyses with IRT-specific dosimetry, spatial dose mapping, and customizable irradiation setups—particularly those enabled by three-dimensional printing—represents a logical and timely direction for future radiobiological and translational research [3, 4, 7].

5 Conclusions

EVs are increasingly recognized as central mediators of radiation-induced biological stress; however, their investigation in the context of IRT remains limited. Despite the intrinsic physical advantages of IRT, including the presence of steep and highly localized dose gradients, this modality has rarely been exploited as a platform to investigate EV-mediated radiation responses. The available evidence indicates that IRT is capable of inducing measurable modulation of EV populations and cargo, yet most studies consider it as a background clinical component rather than as a modality subjected to biological interrogation. This underutilization reflects a clear translational gap rather than a technical or methodological limitation. To address this gap, a structured roadmap for future research can be outlined, including: the integration of spatially resolved dosimetric information with site-specific EV sampling; the development of controlled preclinical IRT models enabling investigation of dose gradient-dependent responses; and the adoption of standardized EV characterization and functional validation strategies. Together, these elements may support the transition from observational findings to mechanistic and translational investigations, ultimately enabling a more comprehensive understanding of IRT-induced biological effects and associated signaling pathways.

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Data availability The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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