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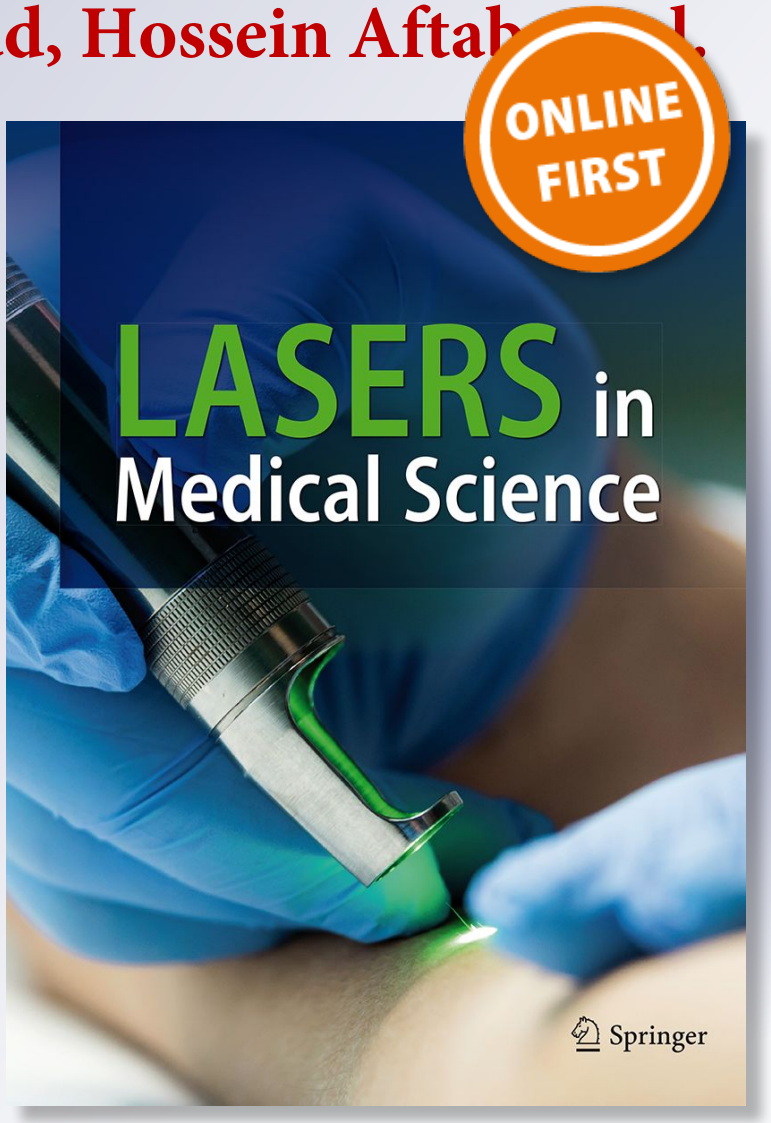

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REVIEW ARTICLE

Tumor-derived extracellular vesicles: insights into bystander effects of exosomes after irradiation

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Abstract

This review article aims to address the kinetic of TDEs in cancer cells pre- and post-radiotherapy. Radiotherapy is traditionally used for the treatment of multiple cancer types; however, there is growing evidence to show that radiotherapy exerts NTEs on cells near to the irradiated cells. In tumor mass, irradiated cells can affect non-irradiated cells in different ways. Of note, exosomes are nano-scaled cell particles releasing from tumor cells and play key roles in survival, metastasis, and immunosuppression of tumor cells. Recent evidence indicated that irradiation has the potential to affect the dynamic of different signaling pathways such as exosome biogenesis. Indeed, exosomes act as intercellular mediators in various cell communication through transmitting biomolecules. Due to their critical roles in cancer biology, exosomes are at the center of attention. TDEs contain an exclusive molecular signature that they may serve as tumor biomarker in the diagnosis of different cancers. Interestingly, radiotherapy and IR could also contribute to altering the dynamic of exosome secretion. Most probably, the content of exosomes in irradiated cells is different compared to exosomes originated from the non-irradiated BCs. Irradiated cells release exosomes with exclusive content that mediate NTEs in BCs. Considering variation in cell type, IR doses, and radio-resistance or radio-sensitivity of different cancers, there is, however, contradictions in the feature and activity of irradiated exosomes on neighboring cells.

Keywords Extracellular vesicles · Exosomes · Cancer cells · Radiotherapy · Bystander effects

Introduction

EVs, lipid bi-layer heterogeneous vesicles, are commonly released from almost normal and cancerous mammalian cells into the surrounding ECM [1, 2]. Increasing investigations have suggested the pivotal role of EVs in the establishment of intercellular communication between cells. EVs contain a large number of biomolecules including proteins, mRNAs, miRNAs, and lipids. Among EVs, exosomes have attracted significant attention due to the inevitable activity under normal and pathological conditions [3, 4, 5]. Compared to normal cells, tumor cells potentially release a large number of exosomes into ECM by which contribute to intense pathological effects such as metastasis, immunomodulation, angiogenesis, and formation of metastatic foci in remote sites [6]. Because of harboring exclusive molecular components, TDEs may provide novel approaches in cancer diagnosis and treatment [6]. Radiotherapy is one of the most common approaches to eliminate tumor cells and shrink tumor masses by targeting cell organelles [7]. Growing studies suggest that the emergence of insulting conditions including ROS

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generation, DNA break, and damage to the integrity of the cell structure is responsible for IR-induced therapeutic effects in irradiated cells [7]. In recent years, efforts have been focused on the examination of radiotherapy NTEs to assess cellular responses in non-irradiated cells [8]. NTEs could reflect the phenomenon that radiation may indirectly affect non-irradiated cells. In this context, cells receiving stress signals from irradiated cells are known BCs. It is no wonder the BCs may exhibit the same properties as irradiated cells up to certain levels [8]. Several studies showed that IR could affect the dynamic of exosome biogenesis in tumor cells exposed to irradiation. Interestingly, exosomes are capable of sharing IR-induced effects to the non-irradiated cell, thus probably affect the function of recipient cells [9]. In this review article, we highlighted the current knowledge related to exosome biogenesis and biological activities of tumor-released exosomes. In addition, we focused on exosomes kinetic under IR.

Extracellular vesicles

EVs are nano-sized phospholipid particles that are released by several cell types into ECM by using intricate sub-cellular signaling pathways [1]. Recent documents highlighted the potency of EVs in the establishment of cell-to-cell communication via the reciprocal exchange of an array of biomolecules [10]. EVs are present not only in the bloodstream but also they could be distributed in in vivo bio-fluids peculiarly milk, cerebrospinal fluid, malignant ascites, amniotic fluid, urine, and saliva [11]. According to data from various studies, EVs can be categorized into three distinct types including exosomes, MVs, and ABs. This classification is based on origin pathway and diameter size [12]. Exosomes are a subfamily of EVs, ranging from 30 to 120 nm in diameter, originated from an endocytotic pathway through complex mechanisms [13] (Fig. 1). Exosomes from various cell sources exhibit the specific molecular identity including CD9, CD63, CD81, CD82, TSG101, and ALIX [14]. TEM imaging showed that exosomes possess cup-shaped characteristic with a diameter between ~ 30 and 120 nm, and the cryoEM technique revealed the spherical morphology of exosomes [15]. Exosomes are capable of inducing/inhibiting cellular signaling pathways in recipient cells [16] (Fig. 1). The existence of heterogeneity in EV population highlighted another subtype of EVs termed MVs that are released from the cells through direct budding of the PM [10]. Under a TEM imaging, MVs are specified with ~ 100–1000 nm in diameter an irregular shape. MV generation is similar to the abscission steps as described for cytokinesis [10] as well as virus outward protrusions from the cells. Most cells release MVs bearing biomolecules the same as exosomes to maintaining cell homeostasis. The main difference is that MVs are released following action of stimulants, but exosomes could be released either in a constitutive or inducible manner [17]. The last form of EVs so-called ABs is 1000–6000 nm particles and produced by apoptotic cells [18]. Emerging evidence suggests the key role of Rho-associated kinase 1 in the formation of the heterogeneous ABs. Caspase-3 is considered as a Rho-associated kinase 1 activator with ability to phosphorylate myosin light chain and ultimately stimulates membrane segmentation [19]. It was previously clear that phosphorylation of myosin light chain and ATPase activity participates in actin-myosin cytoskeleton interaction that breaks nuclear integrity up. Consequently, chromosomal and DNA fragments were packaged into ABs blebs. As these particles encompass biological components such as whole organelles, proteins, microRNAs, and nuclear-related fragments, they could participate in the intercellular communication and may mediate the progression of several diseases [8].

Exosome biogenesis pathways

Before the discovery of exosomes, two experiments reported the ability of reticulocytes to secrete transferrin receptors into the surrounding niche via small nano-sized vesicles. After that time, numerous studies showed that several cells release vesicles to communicate with cells at proximity [20]. In common belief, exosomes are generated through the endocytotic pathway where various proteins participate to load exosome cargoes. Key studies have implied that exosomes are produced through invagination of the late endosome membrane; late endosomes named MVBs located traditionally in the cytoplasm (Fig. 1). Energetically snip off the bent side of MVB results in fall down of nascent vesicles into MVB lumen as the ILVs. Once MVBs are fused with PM, ILVs released into ECM as exosomes [21]. MVBs surrounding membrane components are responsible for sorting exosome cargo incorporation with ESCRT machinery and a mixture of molecules collaboratively attached to its membrane (Fig. 1). ESCRT machinery is made up of four complexes including 0, I, II, and III that identify and select ubiquitinated proteins into ILVs through consumption of the ATP [22] (Fig. 2). Based on current knowledge, it has been suggested that ESCRT-0 initially identifies ubiquitinated proteins through HRS subunit. In this context, HRS interacts not only with ubiquitylated proteins but also with lipid phosphatidylinositol 3-phosphate, found in the MVB membrane that triggers ESCRT-machinery activity. Then, ESCRT-0 connects to the ESCRT-I constituents which in turn causes to the participation of ESCRT-II subunits to promote the invagination of the MVB membrane (Fig. 2). In the next steps, ESCRT-III complex with the help of ESCRT-II complex free the nascent ILVs from the border of the invaginated MVB membrane. In the final step, VPS4-ATPase, an ESCRT-III subunit, discards the ubiquitin tags and also encourages the ESCRT subunits to disassemble from MVB

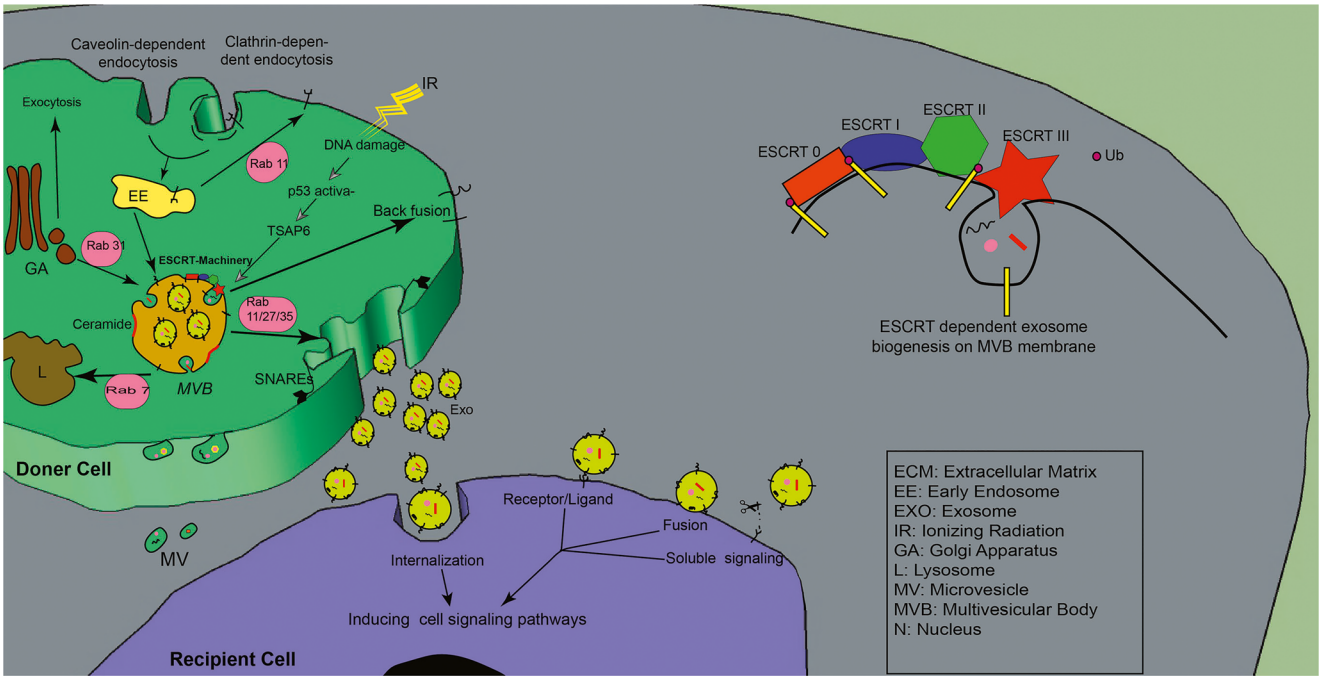

Fig. 1 Biogenesis, trafficking, and secretion of exosomes. Reverse budding of the MVB membrane into, resulting in the generation of free ILVs in MVB compartment. Exosome biogenesis was mediated by ESCRT-dependent and ESCRT-independent mechanisms. ESCRT-machinery is lied down on the cytoplasmic surface of the MVB membrane and composed of the 4 complexes including ESCRT 0, I, II, and III. Firstly, Hrs a member of ESCRT 0 identifies ubiquitinated proteins and interacts with PIP3 to set up protein sorting ESCRT machinery. In the ESCRT-independent mechanism, tetraspanins, ceramide, and etc. contribute to generating exosomes. MVB cargoes consist of molecules from endocytotic compartments, Golgi apparatus, and cytoplasm. Intracellular MVB trafficking was mediated by the Rab-GTPase family. Several Rab proteins contribute to the transportation of vehicles in different pathways. Three fates are proposed to MVB; in secretory pathway, MVBs directly fuse to the PM and shed ILVs into the ECM which now called exosomes. SNARE proteins mediate fusion of MVB membrane with the PM. In degrade pathway, MVB coalesces with the lysosome. Alternatively, MVB could back fuse to the PM and showcases some molecules on the PM. IR actively causes DNA damage which initiates downstream cell signaling cascade; results in producing more exosome through TSAPs pathway. Once secreted, exosomes contribute to remodeling target cell signaling pathways in three possible routes. In internalization route, exosomes were captured by endocytosis and phagocytosis processes. Ligand/receptor interaction is another way that exosomes participate in inducing cell signaling. In addition, cells recruit membrane fusion-related molecules to merge the exosomal membrane with the PM. Alternatively, enzymatically released soluble factors from exosomes in the ECM may activate special receptors on the target cell PM. MVs are generating from the cell via outward budding of the PM so-called shedding vesicles. MVs may contain bio-molecules and contribute to intercellular communication the same as exosomes

membrane. Of interest, some ESCRT components and accessory proteins such as TSG101, HRS, and ALIX are released with exosomes [12] (Fig. 2). Molecular insight into exosome biogenesis revealed that exosome generation could occur via ESCRT-independent mechanisms (Fig. 1). For example, inhibition of some components of ESCRT-machinery results in ILV generation and cells continues to generate ILVs in MVBs, indicating the involvement of ESCRT-independent mechanism [23]. Growing observations confirmed the association of various proteins on the exosomal membrane with exosome biogenesis. Accumulated tetraspanins on the exosome membrane are also engaged to produce exosomes via ESCRT-independent mechanism [24]. The involvement of tetraspanin-8 in exosome biogenesis was previously confirmed by the fact that tetraspanin-8 has potential to sort specific mRNAs and proteins into exosomes [25]. Trajkovic and colleague showed that lipids are also essential components in vesicular transport [26], and membrane curvature is closely related to collaboration between proteins and lipids. The impact of lipids on exosome formation was confirmed by inhibition of neutral sphingomyelinase 2 enzyme, a ceramide generator, which led to a reduction in proteolipid protein bearing exosome secretion from oligodendrocytes [26]. The inhibition of neutral sphingomyelinase 2 enzyme decreased the secretion of EGFP-CD63 positive exosomes from EGFP-CD63-engineered PC-3 cells. The formation of ceramide microdomains is a possible underlying mechanism that induces MVB membrane curvature [26]. Additional evidence for the lipid-related exosome formation comes from phospholipase D2 enzyme and protein kinase D1/2 experiment where phosphatidic acid promotes exosome biogenesis [27]. In conclusion, it is necessary to mention that cell recruits the ESCRT-dependent or ESCRT-independent mechanism to MVBs/exosome biogenesis, but the mechanisms may possibly not be exclusively isolated. Both mechanisms may synergistically be involved, and various subpopulations of MVBs could dominantly

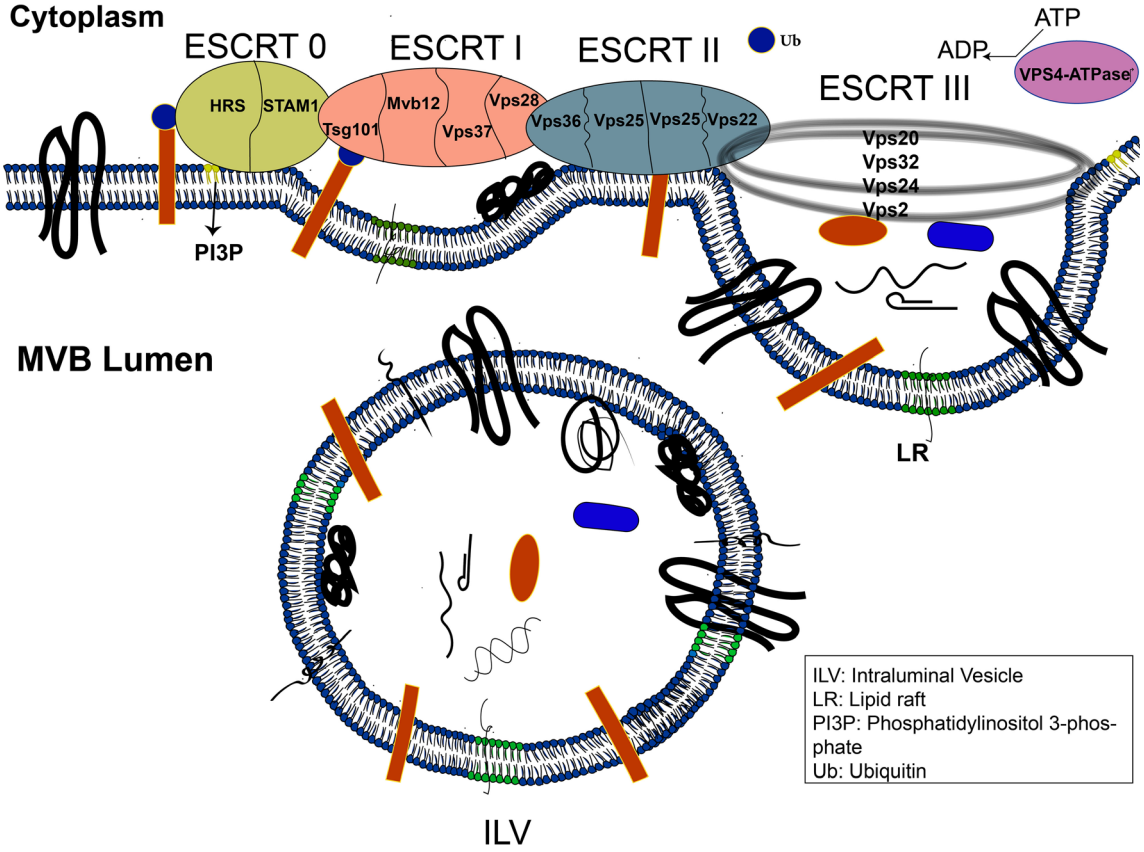

Fig. 2 The key role of ESCRT machinery in exosome formation. ESCRT machinery composed of different units including ESCRT-0, -I, -II, and -III which are located on the cytoplasmic face of MVB surrounding membrane. The ubiquitylated proteins can be captured by ESCRT machinery. First, the ESCRT-0 complex (composed of HRS and STAM1 subunits) sets up ESCRT machinery. Its HRS subunit categorizes the ubiquitylated proteins on the MVB membrane. This unit preferentially interacts with the TSG101 subunit of the ESCRT-I complex (contains Tsg101, Vps28, Vps37, and Mvb12 units), which finally recruits ESCRT-II subunits (Vps36, Vps22, and 2Vps25). ESCRT-I incorporation with ESCRT-II induces the invagination of the ILVs into the MVBs lumen. As assumed, RNAs and proteins are loaded into ILVs. After that, ESCRT-III subunits (Vps20, Vps32, Vps24, and Vps2) bind to the limped side of the budding ILVs and dismemberment them into MVBs lumen. At this moment, Doa4 (Degradation of Alpha 4) detaches ubiquitin tags from proteins. Finally, VPS4-ATPase by hydrolyzing ATP promotes ESCRTs disassemble

appear via diverse types of machinery. Furthermore, the cell origin and/or cell condition might be a vital factor to determine which machinery employed to exosome biogenesis.

Exosome trafficking and uptake

In the context of intracellular vesicle trafficking, various kinds of molecules including Rab-GTPases control the transport pathways. For instance, Rab proteins play main roles in mediating different steps of intracellular vesicular trafficking [28]. Previous investigations proposed a preferential interaction between Rab proteins in cellular vesicle network. Calling attention, Rab27 family promotes trafficking and docking of MVBs to PM [11] (Fig. 1). Furthermore, the suppression of some Rab proteins (Rab2b, Rab9a, and Rab5a) destroys exosome secretion in tumor cells [11]. Intracellular trafficking of MVBs may be controlled by Rab11 and Rab35 in other cancer cells [29]. More recently, our team confirmed the involvement of Rab27a, b and Rab8b in exosomal secretion pathway in MSCs and endothelial cells in vitro [30, 31]. Data support the idea that SNARs in collaboration with the Rab proteins fuse MVB with PM to secrete exosomes [32]. Somewhere else it is mentioned that intracellular accumulation of GFP-CD63 in induced following the inhibition of Ykt6 translation in vitro [33]. VAMP-7 controls MVB fusion with the PM to release acetylcholinesterase-loading EVs indicated in the K562 cell line [34]. SNARE proteins including VAMP-7,-8 and SNAP-23 promote the fusion of secretory lysosomes with the PM [35]. Although the suppression of VAMP-7 in renal cells could reduce lysosomal secretion, this manipulation does not affect the secretion of HSP70-enriched EVs [36]. According to the literature [37], three possible intracellular fates are represented for MVBs. MVB could fuse with the PM to secrete ILVs as exosomes into the ECM. In an alternative way, MVB may decide to choose lysosome as a home to

degrade cargoes. According to some studies, the inhibition of MVB-PM fusion may be considered as another destination to decorate PM with specific molecules. It has shown that the level of cholesterol and a lyso-bis phosphatidic acid component of MVB membrane is a determinant factor of MVB destination by which it decides to degrade or empty its cargoes into the ECM [37]. After secretion into ECM, exosomes can deliver their cargoes to target cells through three possible mechanisms (Fig. 1) [21]: (i) direct fusion, (ii) receptor/ligand interaction, and (iii) endocytosis pathway. For direct fusion, exosome membrane is connected with the PM of recipient cell the same as other membrane fusion process, thus inject cargoes into the host cytoplasm to affect the cell function. Alternatively, receptors located at exosomal membrane such as intercellular adhesion molecule 1 could easily contact with molecules located (lymphocyte function-associated antigen 1 receptor) on the target cell membrane and induce downstream cell signaling pathways [38]. Ultimately, endocytosis is a traditional way that exosomes are encapsulated to the endocytotic pathway and may trigger cell signaling. Additionally, it is proposed that exosomal membrane-related molecules may be cleavage by enzymes located at the ECM, and activating exosomal derivatives which could consequently bind to respective receptors on target cells as a ligand (Fig. 1) [39]. According to little knowledge about EV uptake, pathways independently or synergically may be recruited to uptake exosome and their friends. Determination of which mechanism is exclusively involved is difficult due to technical limitation and the heterogeneity in both EV populations and in the cell types [40]. Further advancing in the field of EVs such as EV uptake mechanism will persuade design therapeutic tools to delete communications between such EVs and normal recipient cells or to enhance the delivery potential of therapeutic EVs.

Tumor-derived exosomes

Similar to the ability of normal cells to secrete exosomes, cancer cells could release progressively oncogenic exosomes in the pathological condition [41]. TDEs exhibit aggressive feature and promote pathological aspects of cancers [42] (Fig. 3). In the tumor environment, resident cells continuously produce exosomes to communicate with other cells. The possibility that the cancer metastasis may be driven by TDEs was suggested by observations through the dynamic of fluorescent exosomes between cells. In the co-culture system, TDEs were passively delivered to endothelial, leading to the development of endothelial spheroids and sprouts and metastasis [43] (Fig. 3). Similar results were reported that TDEs can persuade lymph node metastasis [44]. In support of this claim, exosomal proteins actively contribute to promoting tumor invasion. In one study, it was revealed that oncogenic receptor EGFRvIII was transferred by microvesicles from glioma cells and promoted cancer cell invasion and proliferation rate [45]. Not surprisingly, exosomal miRNAs participate in inducing cancerous phenotype in target cells; therefore, they can mediate the formation of pre-metastatic cells. In this regard, breast carcinoma exosomes could promote metastasis potential of uncertainly metastatic cells in animal xenograft cancer models and increase the clonogenic potential at remote sites via miR-200-dependent pathway [46]. Accordingly, these exosomes are enriched with miR-105 which could inhibit ZO-1 protein expression in endothelial cells; in turn, ZO-1 inhibition leads to the vascular permeability and susceptibility to cancer metastasis [47]. It was demonstrated that exosomes from metastatic rat adenocarcinoma BSp73ASML transfer such exosomal miRNAs (miR-494 and miR-542-3p) that can target cadherin-17 and MMP expression to dictate the formation of de novo pre-metastatic sites [44]. As well, it was clear that exosomes bearing MMP13 protein can promote nasopharyngeal carcinoma cell metastasis via the degradation of ECM [48]. Increasing data demonstrate that prostate cancer exosomes have an inevitable role in ECM degradation and tumor expansion. These exosomes carry various miRNAs, for instance, miR-21-5p, miR-139-5p, and miR-100-5p, which control the MMP translation process (e.g., MMP2, MMP9, and MMP13) [49]. It is worth considering the fact that exosomal miRNA probably alters adhesion molecules, cell cycle genes, chemokine ligands, pro-angiogenesis genes, proteases, and genes participating in oxidative stress response [44]. Under hypoxia condition, tumor cells release a large number of exosomes through the activation of the HIF-1α pathway which may result in promoting survival, invasion, and angiogenesis [50]. Scrutiny of the molecular mechanism showed that exosome recipient endothelial cells release many effective growth factors and cytokines to stimulate pericyte migration via PI3K/AKT signaling pathway, thus promote angiogenesis [51]. Of note, oncogenic exosomes from malignant mesothelioma cells are enriched with angiogenic proteins which promoted the motility of angiogenic cells, augmenting the tumor progression via vascular remodeling and angiogenesis [52]. Glioma-released exosomes could transfer tissue factors which promote angiogenesis by the up-regulation of protease-activated receptor 2 in epithelial cells [53]. Thus, in a hypoxic condition which frequently observed in the tumor environment, tumor cells potentially produce exosomes with pro-angiogenic and pro-metastatic properties, suggesting the compensatory activity of these cells against hypoxic condition [54]. The tumorigenic effects of exosomes were mainly ascribed to the hypothesis that TDEs are involved in EMT process [55] (Fig. 3). It was considered that EMT is the sign of aggressive tumors and cells shifted to EMT have the potential to acquire plasticity and tendency to indwell far from the site of origin. Cho and co-worker showed that exosomes released from ovarian cancer cell lines can contribute to induce EMT

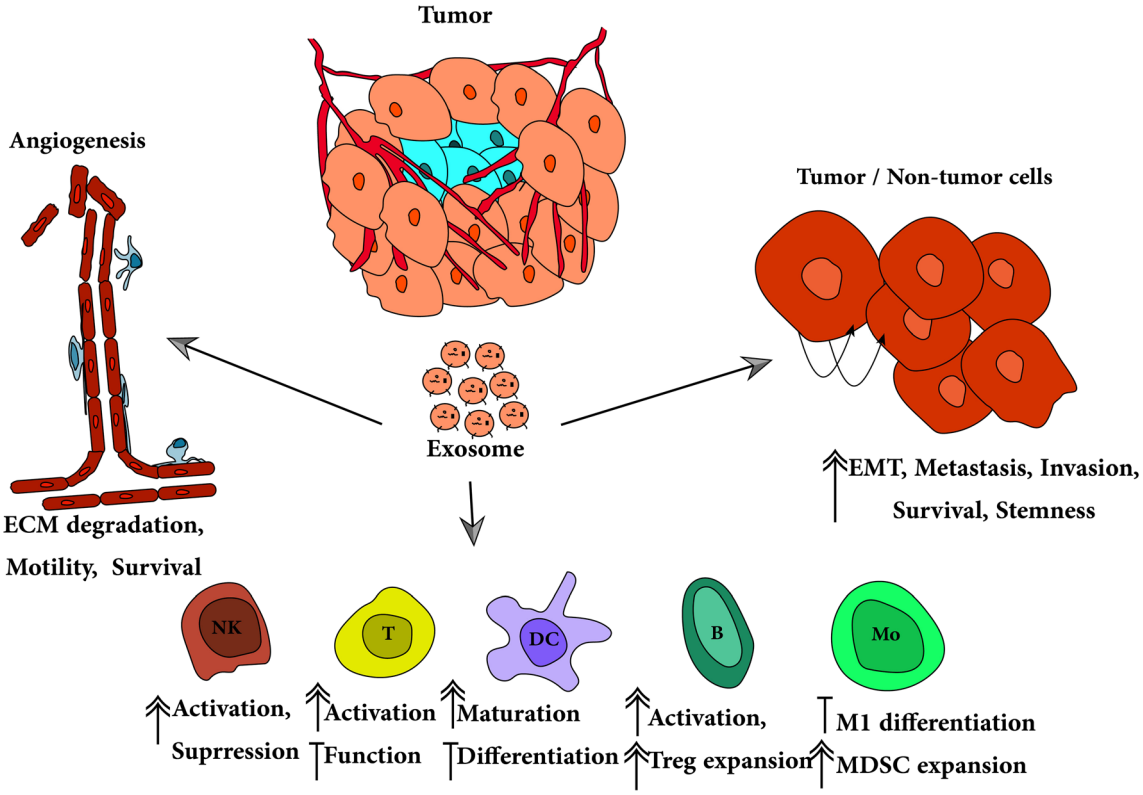

Fig. 3 Tumor-derived exosomes in the tumor microenvironment. Exosomes from tumor cells abundantly release exosome to send signals to neighboring cells. Recipient cells such as surrounding cells, tumor cells, stromal cells, endothelial cells, and immune cells are functionally affected by tumor-derived exosomes. It is believed that angiogenesis was increased through promotion in ECM degradation, migration, and also the survival of endothelial cells. In response to exosomes, EMT induction, metastasis, invasion, expansion, and cancer stemness were enhanced in a neighboring cell. Additionally, the effect of tumor-derived exosomes on immune cells is contradictory determined; this arises from the simultaneous presence of different stimulatory and inhibitory molecules on the exosome membrane. Tumor-derived exosomes contribute to activation of B, T, and NK cell. Reversely, function, differentiation, of T, DC, and Mo were inhibited, respectively. B: B lymphocyte; DC: dendritic cell; EMT: epithelial-mesenchymal transition; MDSC: myeloid-derived suppressor cell; Mo: macrophage; NK: natural killer; T: T lymphocyte. ↑ means increase; T means inhibition

phenotype in adipose tissue-derived MSCs [55]. In this context, exosome-based treatment results in tumor characteristics of adipose tissue-derived MSCs as the tumor-associated myofibroblasts, with enhanced expression of α-SMA, SDF-1, and TGF-β. Based on data from experiments, ovarian cancer-derived exosomes induced the myofibroblastic phenotype through activating an intracellular TGF-β signaling pathway [55]. Besides, proteins from TDEs such as Rab3D and LMP1 were reported to promote EMT phenomenon which helps to tumor oncogenicity and metastasis of cancer cells [56]. Garnier et al. confirmed that cancer cells induced mesenchymal phenotypes to secrete EVs containing tissue factors [57]. In addition, exosomes released from bone marrow EMT can encourage multiple myeloma development in an animal model system [58]. Thus, evidence indicated that the EMT cells are capable of producing paracrine factors that influence neighboring cells and cancer resistance. The most important part of TDE functions is to transfer the inhibitory/stimulatory signaling to almost immune cells. Exosomes isolated from breast cancer cell culture medium can effectively mediate the suppression of immune cells through destruction of T cell proliferation and NK cell cytotoxicity in in vitro and in vivo experiments. Moreover, ovarian cancer exosomes mediated down-regulation of the TCRζ chain and T cell apoptosis [59]. Wieckowski et al. determined that TDEs suppressed proliferation of $CD8^+$ T helper cells coincided with the activity of $CD4^+$ T helpers. In contrast, exosomes originated from healthy cells supported the proliferation of both T cells [60]. Evidence showed that TDEs are implicated in the modulation of other immune cells. For example, exosomes bearing MICA/B (MHC class I chain-related protein A and B) isolated from acute myeloid leukemia patients can suppress the function of NK cells through down-regulating NKG2D receptors [61]. TDEs were shown to disrupt monocyte differentiation and to switch monocytes into TGF-β-expressing dendritic cells (DCs). TGF-β-expressing DCs produce prostaglandin E_2 which inhibits the proliferation of $CD8^+$ T cells [62]. Notably, TDEs contain a broad spectrum of stimulatory factors on their membrane and lumen such as MHC I, II, and intraluminal growth cytokines [63]. This feature indicates

the multidimensional function of cancer exosomes in immune-oncology and may serve a profound opportunity in developing exosome-based cancer therapies [64]. Commensurate with these comments, it seems that TDEs could act as stimulatory and inhibitory agents on immune cells at the same time. In spite of this property, the underlying mechanisms involved in the dual activity of cancer exosomes on immune cell responses remain elusive. As an example, when TDEs co-cultured with antigen presenting cells contributed to the colonization of the tumor-draining lymph nodes that might encourage the generation of pro-inflammatory cytokines thereby changing the lymph node cytokine pool. Under these conditions, lymph node tissues are abundant in IL-6, IL-12, and IFN-γ while low contents of IL-10 produced which can induce the Th1 immune responses. In this regard, the immuno-stimulatory property of TDE treatment may be mediated directly by exosome-induced M1 macrophages. M1 cells secrete exosomes in response to exosomal treatment, shifting the lymph nodes cytokine profile to encourage Th1 immune response [65]. However, the current agreement in this area is that different tumor cells release exosomes that promote cancer maintenance, metastasis, niche formation, angiogenesis, and immunomodulation [6] (Fig. 3). It is noteworthy to mention that despite the deep focus on understanding key signaling mechanisms and targeting these exosomes as potential strategies for therapeutic intervention, detailed molecular mechanisms driving cancer exosome biogenesis, uptake, and transcriptional or translational responses are still vague.

Tumor-derived exosome cargoes

It is well-established that TDEs bear cargoes that are different from normal cell exosome content [52]. For example, Pfeffer et al. showed an increased level of specific exosomal miRNAs in esophageal squamous cell carcinoma which related to tumorigenesis and aggressiveness. It was shown that the expression of exosomal miR-126, miR-149, miR-17, miR-19a, and miR-21 was elevated; thus, these miRNAs contribute to the promotion of melanoma metastasis [66]. Data support the enhanced expression of a distinct cluster of miRNAs including let-7i, miR-16, miR-21, and miR-214 in circulating exosomes (Table 1) [67]. The existence of such miRNAs (Let7a and miRNA-409) enables us to discriminate exosomes from specific cancer cell types such as glioblastoma, colorectal, and prostate [68]. Considering the fact that miRNA pattern of exosomes is similar to the parent tumor mass makes exosomal miRNA pattern eligible to be considered as a non-invasive prognostic and diagnostic tool in cancer biology [69] (Table 1). In addition, exosomal proteins have recently received much attention regarding their potential role in the expansion of tumor cells to surrounding tissue cells. Meanwhile, these factors could be used as prognostic and diagnostic biomarkers [56]. In a recent experiment, it was revealed that exosomal oncogenic proteins such myoferlin is related to pancreatic and breast cancer progression and metastases [70]. Lung cancer-derived exosomes carry an abundant volume of CD317 and EGFR molecules on their surface. It is suggested that these molecules are considered as a biomarker for diagnosing of non-small cell lung cancer [71]. In line with these data, a compressive proteomic experiment showed a distinct panel of proteins in urinary exosomes from bladder cancer patients. Other oncoproteins were identified in exosomes released from many cancer types including colorectal, nasopharyngeal, pancreatic, renal, stomach, etc. that may serve diagnostic biomarkers (Table 1) [72]. Even, comparative proteomic analyses of the exosomal contents from the different stage of cancer showed typical and unique compositions (e.g., integrins and tetraspanins) that can be exploited as a tool to discriminate cancer development, progression, and metastasis [73]. Moreover, onco-exosomes exhibit exclusive lipid profile compared to normal exosomes. For example, Roberg-Larsen and co-workers confirmed the existence of 27-hydroxycholesterol (27-OHC) in breast cancer exosomes [74]. Other lipidomic analysis showed a specific lipid profile of exosomes driven from urinary of prostate cancer patients [75]. Calling attention, early-stage cancer detection is a pivotal state to inhibit cancer progression and reduce cancer-related mortality. In conclusion, the study of TDEs biogenesis and their miRNA/protein cargoes may lead to a deep understanding of exosome pathological role of in cancer development and thereby support the notion that miRNAs/proteins could be used as diagnostic and prognostic biomarkers for various cancers.

Biological responses of irradiated stem cells

In the field of cancer therapy, radiotherapy was traditionally used as an effective approach to eliminate abnormal cells and shrink tumor mass [76]. It is well established that IR induces failure to DNA and other bio-molecules of target cells [77]. In this context, ionizing stress radiation is a critical mediator that induces the generation of oxidative molecules in cells [78]. In fact, free radicals generated by the energy of low linear radiation such as X-rays and γ-irradiation is mainly responsible for IR-induced cell injury that can be enhanced in the presence of oxygen and participate to the formation of various reactive oxygen species (ROS) [79]. Free radicals/ROS actively disrupt the structure and function of DNA, lipids, and proteins, contributing to metabolic and structural alterations [7]. It was shown that DNA is the real target of the biological effect of IR [80]. Intracellular ROS generation has also been reported in both direct irradiated and bystander cells [81]. Data indicated that IR affects the structure and function of cell organelles including PM, cytosol, endoplasmic reticulum, Golgi

Table 1 Exosomal miRNAs and proteins as a biomarker

Cancer type	Exosomal miRNAs as biomarker	Reference	Exosomal proteins as biomarker	Reference
Bladder	miR-15b, miR-24, miR-135b, miR-1224-3p	(Huang et al. 2013)	α6-integrin, Basigin, TACSTD2, Mucin4, EDIL-3, EPS8L2, MUC-1,	(Chen et al. 2012)
Breast	miR-10, miR-21, miR-182, miR-373, miR-1246	(He Y et al. 2018)	Survivin, Survivin-2B, CEA, Tumor antigen 15-3	(Khan et al. 2014)
Cervical	miR-21, miR-146a	(Wang X et al. 2008)	ATF1, RAS	(Shi et al. 2017)
Colorectal	let7a, miR-21, miR-192, miR-221	(Chiba et al. 2012)	CEA	(Silva et al. 2012)
GBM	miR-320, miR-574-3p	(Manterola et al. 2014)	EGFRvIII	(Skog et al. 2008)
HNC	BART-miRNAs	(Principe et al. 2013)	NR*	
Liver	miR-221, miR-18a, miR-122, miR-222, miR-101, miR-224, miR-106b, miR-195	(Sohn et al. 2015)	NR	
Melanoma	miR-31, miR-19b-2, miR-20b, let-7a, miR-182, miR-221, miR-30b, miR-30d, miR-222, miR-92a-2, miR-21, miR-15b, miR-210miR-532-5p	(Gajos-Michniewicz et al. 2014)	CD63, Caveolin1, TYRP2, VLA-4, HSP70	(Peinado et al. 2012)
Lung	miR-155, miR-17-3p, miR-205, miR-21, miR-106a, miR-146, miR-191, miR-192, miR-212, miR-214 miR-203, miR-210,	(Molina-Pinelo et al. 2012)	EpCAM, EGFR, CEA, LRG-1	(Jakobsen et al. 2015)
Ovarian	miR-21, miR-205, miR-206, miR-103, miR-141, miR-200b, miR-200a, miR-557, miR-200c, miR-203, miR-214	(Taylor and Gercel-Taylor 2008)	MAGE3/6, Claudin-4, L1CAM, TGFβ1, CD24, ADAM10, EMMPRIN,	(Szajnik et al. 2013)
Renal	miR-15a, miR-378, miR-451	(Redova et al. 2012)	NR	
Pancreatic	miR-21,miR-1246, miR-17-5P,miR-4644	(Madhavan et al. 2015)	GPC1, MIF	(Melo et al. 2015)
Prostate	miR-107, miR-574-3p, miR-375, miR-141	(Hessvik et al. 2013)	Survivin, PTEN, Transmembranes, Protease, ITGB1, Serine2-ETS, β-catenin, PSA, PCA3, PSMA, ITGA3,	(Gámez-Valero et al. 2015)

NR not reported

apparatus, lysosomes, mitochondria, nucleus, and ribosomes [7]. The paracrine activity of tumor cells was shown to be influenced by post-IR [82]. Destructive effects of IR on non-cancerous cells such as stem cells have been previously examined. For example, human embryonic stem cell gene expression was altered following exposure to IR in vitro. It was documented that these cells entered apoptosis. It seems that cell response is depended on X-ray dose and intensity [83]. IR potentially affects HSCs via different signaling ways. Significant progress in IR-induced HSCs damage indicated that HSC apoptosis can occur via the p53 pathway while HSC differentiation increased by the activation of the G-CSF/Stat3/BATF-signaling. Synchronically, the close relationship between ROS-p38 pathways was implicated in HSC senescence (Table 2) [84]. Concurrently, IR has the potential to increase cell stress in each stem cell type such as neural, mesenchymal, and muscle stem cells outlined in Table 2 [85]. IR-induced effects can be easily exported by directly irradiated cells to surrounding cells such as non-irradiated cells, which are known as NTEs of IR [8]. NTEs have been shown to participate in initiating different signaling in target cells through a gap junction or paracrine soluble factors [86]. These paracrine factors secreted by radiated cells are transported and delivered in the form of cargoes and are transported within an enclosed membrane termed exosome, which facilitates targeted cell IR-induced alternations [87]. Precise insight into molecular mechanisms involved in radiation injury enables us to understand their origin at a more basic level and to design more effective radiotherapy with fewer side effects.

Classification of radiation NTEs

Growing studies indicated that cells receiving IR are promising sources to send signals to non-irradiated neighboring cells, which are well-known as NTEs of IR. In this context, radiation NTEs is classified into three main phenomena including cohort effect, abscopal effects, and Bes (Table 3) [88]. Cohort effects explain the communication between heterogeneously irradiated cells within an irradiated field [89]. Noticeably, it has been proposed that following miscellaneous irradiation, high-dose irradiated cells could launch signals to influence low-dose irradiated cells and vice versa [89]. To confirm this

Table 2 Effect of IR on stem cells biology

Stem cell type	IR-induced biological effects	Reference
Embryonic	Impaired apoptosis, proliferation, cancer induction	(Wilson et al. 2010)
	Increased apoptosis and DNA damage,	(Saha et al. 2014)
Hemaiotoeic	Increased oxidative stress, apoptosis, and radiosensivity; decreased stemness	(Kato et al. 2011; Rodrigues-Moreira et al. 2017)
Mesenchymal	Dose-dependently Increased apoptosis, metabolism, stress, and DNA repair	(Jin et al. 2008)
	Downregulated cyclin B1 and cyclin E2	(Kurpinski et al. 2009)
	impaired Autophagy, and cell cycle; declined proliferation and stemness	(Alessio et al. 2015)
Neural	Augmented oxidative stress and radiosensivity	(Tseng et al. 2013)
	Increased and proliferation, differentiation; decreased apoptosis, DNA damage, and radiosensivity	(Wei et al. 2012)
	Increased apoptosis and oxidative stress	(Tseng et al. 2013)
	Impaired apoptosis, cell cycle, development, proliferation, and stress responses	(Bajinskis et al. 2010)
Muscle	Decreased proliferation	(Masuda et al. 2015)

hypothesis, Zhang et al. designed a uniform and gradient irradiation experiment on MCF-7 cells indicating that ROS production, survival, and cell death correlated with gradient irradiation. Even, these effects were higher in cell-exposed gradient irradiation compared to the group exposed to uniform irradiation. Thus, these effects provide new insight into radiation therapy in which irradiated cells additionally were affected by signals from neighboring irradiated cells, contributing to amplifying direct irradiation effects [8]. Growing evidence has suggested the involvement of abscopal effects following radiotherapy (Table 3). Abscopal effects have been detected in metastatic patients receiving radiotherapy [88]. Indeed, irradiated cells may produce messengers to regulate non-irradiated cell function out of an irradiated area [88]. Association of this phenomenon has been shown in the treatment of various malignancies such as lymphoma, hepatocellular carcinoma, and melanoma [88]. This knowledge received additional support following activation of RNS, ROS, p53, and cytokines including IL-1α, IL-6, and TNF-α in the cells outside of the irradiated area [88]. IR can diminish tumor progression out of the radiation area. For instance, Demaria et al. demonstrated that the abscopal effects contributed to immune responses and T cells are recruited to inhibit distant tumor generation. In a mice model of carcinoma, the growth of tumor cells outside of the irradiated field was significantly inhibited following radiotherapy due to abscopal effects [90]. The well-known NTEs are the radiation-induced BEs (Table 3). BEs are identified as biological effects ignited after irradiation in cells that have not been directly radiated [8]. This mechanism can be directed via the gap junction between the target and non-target cells and/or in a paracrine manner. In the case of paracrine way, secreted soluble factors (ROS, nitrogen species, or cytokines) and extracellular vesicles can transmit messengers from irritated cells to non-irradiated cell [9]. In a better word, this phenomenon explains the signaling effects of direct-irradiated cells to non-irradiated cell through a mixture of cytokines such as TGF-β, TNF-α, and IL-8 [91]. The Bes that were launched in bystander cells may consist of DNA destruction, chromosomal inconsistency, transformation, survival, proliferation, and apoptosis [15]. Further experiments are needed to discover underlying mechanisms involved in cancer biology induced by NTEs. It seems that in vitro and in vivo studies on NTEs are recommended for validating its clinical application to cancer radiotherapy.

Table 3 Classification of non-targeting effects of IR

Non-targeting effects	Bystander	Cohort	Abscopal
Affected volume	Non-irradiated	Irradiated	Non-irradiated
Cell interval	Neighboring	Neighboring	Distant
Biological effects	Genomic instability, ROS generation, oncogenic transformation	Genomic instability, ROS generation, cell death	Genomic instability, tumor growth inhibition

Furthermore, understanding the possible benefits of combination therapies is critical to introduce optimized preserve strategies following radiotherapy.

Exosome kinetic in irradiated cells

Numerous cancer cells release EVs to the ECM for communication with surrounding cells [37]. The effects of IR on exosome biology have been reported in some of the in vitro experiments (Fig. 4). Previous studies showed that cells produce EVs under pathological and stress conditions with distinct property in molecular composition [30]. Under the pathological condition, the molecular dynamic of exosome biogenesis differs from normal condition [30]. IR potentially makes changes in the proteins and miRNAs cargo of EVs [19]. Previous studies indicated that IR significantly impacts intercellular communication through different mechanisms [7]. IR exposure to human epithelial lung H460 cell line causes DNA break and the activation of the p53 signaling. In turn, p53 protein recruits transmembrane protein tumor suppressor-activated pathway 6 to induce exosome formation and secretion [92] (Fig. 4). This protein is associated with the endosomal compartments, Golgi apparatus, and cytoplasmic membrane involved in exosome formation and abscission [30]. The pivotal role of the p53 in the secretion of exosomes was previously confirmed. p53 overexpression in cancer cells led to an extraordinary level of radiation-induced exosome secretion [19]. Similarly, the involvement of IR-activated p53 pathway in the exosome secretion pathway has been shown in other tumor cells [9]. Additionally, alteration of the exosome cargoes has been reported in response to stress conditions such as radiotherapy [82]. A work conducted by Lehmman et al. indicated that X-rayed prostate cancer cells releasing exosomes burdened with B7-H3 (CD276) protein, which was recently introduced as a diagnostic biomarker of prostate cancer [82]. Furthermore, their team observed that changes in exosome cargoes and secretion ratio were associated with the initiation of senescence in tumor cells. Hurwitz and colleague found that serum level of Hsp72 protein in patients with prostate cancer was elevated following radiotherapy in comparison to healthy subjects [93]. In this context, investigation on the composition of IR-treated glioma-derived exosomes showed an abnormally elevated insulin-like growth factor binding protein 2 and connective tissue growth factor mRNA levels, which contribute to migration and invasion of various tumors [19]. Mutschelknaus et al. showed that head and neck squamous cell carcinoma release exosomes with

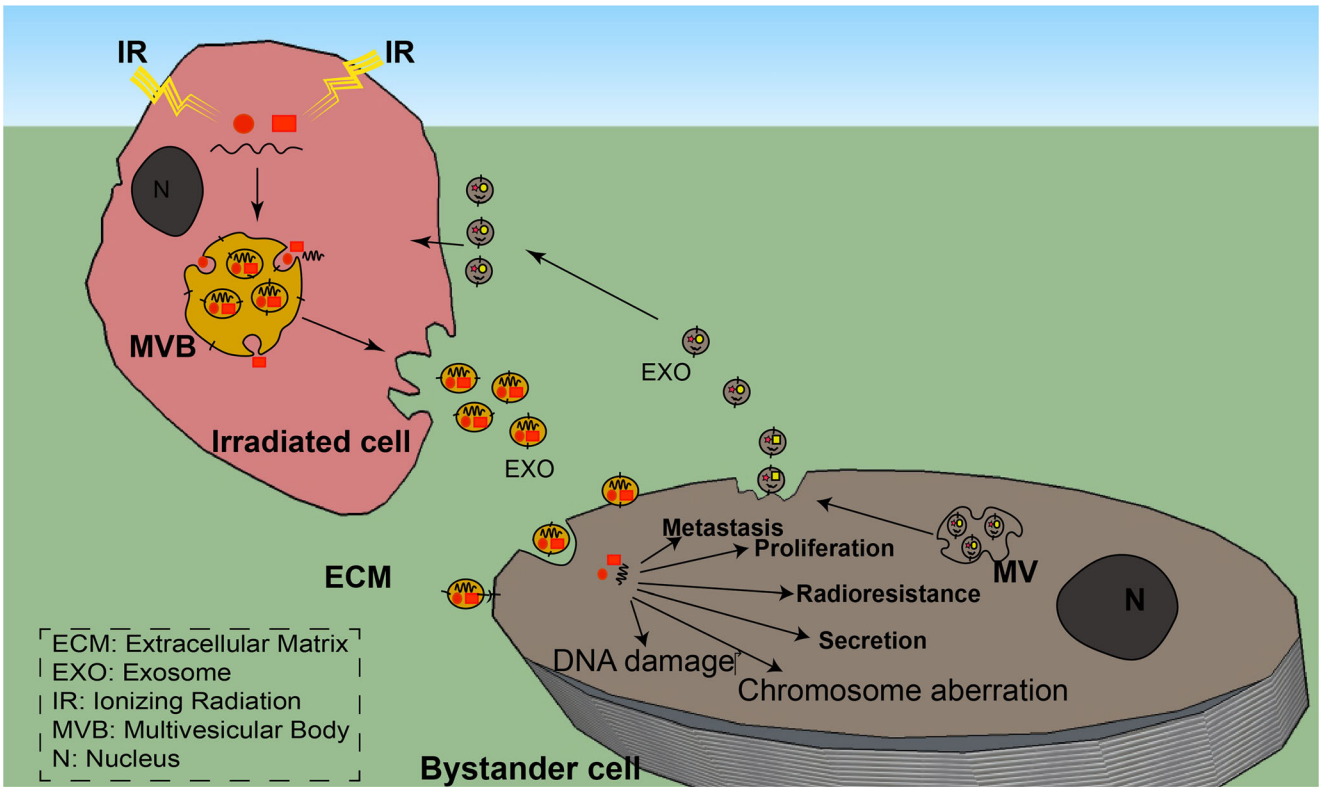

Fig. 4 Exosome-mediated bystander effects. IR directly induces damage and alternation to the directly irradiated cell components. In response to IR, directly irradiated cells release a variety of soluble factors and exosomes. IR-induced molecular profile of irradiated cells can be sorted to the exosomal secretory pathway. Unirradiated cells (or BCs) can uptake exosomes received from irradiated cells result in switch cell signaling pathways including metastasis, proliferation, radioresistance, secretion, and genetic damage in BCs. Additionally, in an intercellular volume, BCs could send signals to irradiated cells through exosomes

exclusive compositions that influence non-irradiated cell migration and motility in vitro after exposure gamma-ray radiation [82]. Recently, a comprehensive analysis on the composition of exosomes purified from irradiated head and neck squamous cell carcinoma showed that level of proteins involved in gene expression including EIFs, PSMs, RPLs and RPSs, cell proliferation, and cell signaling (RAB-GTPase, ARFs, and RASs proteins) was significantly elevated. Future investigations on same exosomes discovered a reduced level of apolipoproteins and immunoglobulins. Authors discussed that this data may sign a cellular adaptation mechanism to IR stress by expelling intracellular garbage components in the way of exosomes. Of note, increased level of proteins in radiated cells is related to cell cycle arrest, inhibition of transcription, translation, and cell division [94]. Besides the impact on the intracellular communication through exosomes, IR affects the dynamic of the exosome capture in irradiated recipient cells with the support of CD9/CD81 molecules. Using irradiated stem cells, Hazawa et al. showed that at the PM level, these molecules join together to interact with exosomal membrane receptors [95]. More recently, the potency of laser irradiation has been studied in the kinetic of exosomes by our group. Based on our data, we found that low-level laser irradiation of endothelial cells increased exosome secretion through Wnt signaling pathway and the transcription of genes involved in exosome biogenesis and secretion like, Alix, CD63, Rab27a, and Rab27b was significantly induced. We also showed the close relation of autophagy with exosome secretion [31]. The role of IR as a key factor of exosomal secretory pathway is ineffectively understood yet. Hence, further investigations are necessary to disclose the exact effect of IR on exosome signaling pathways in tumor cells.

Exosome-mediated bystander effects

The possibility of exosomal-mediated BEs was examined in various tumor cell models (Fig. 4). For instance, exosome-mediated BEs were examined in breast cancer carcinoma cells in an in vitro irradiation model. Exosomes harvested from irradiated cell media contain specific RNAs that induce early and late chromosomal break in BCs [96]. Arscott et al. showed that exosomes purified from condition media of irradiated glioblastoma cells potentially augment the migration of recipient cells through the recruiting of molecules involved in cell motility such as neurotrophic tyrosine kinase receptor type 1, focal adhesion kinase, proto-oncogene tyrosine-protein kinase Src, and Paxillin in target cells. Using molecular profiling, authors discovered a large number of molecules related to cell migration signaling pathways [91]. The ability of exosomes to transmit bystander information confirmed in squamous head and neck cancer irradiation in the in vitro model where exosomes derived from irradiated cells contribute to enhance the growth rate of non-irradiated cells. In addition, these exosomes are capable of rescue directly irradiated cells from death, thus induce radio-resistance of tumor cells through repairing damaged DNA content [97]. Activation of DNA repair machinery was considered as the promising mechanism for the augmented survival after IR. Interestingly, exosomes released from irradiated MCF7 cells are capable of inducing adverse effects in the recipient BCs via broadcasting of information vertically in time and horizontally [98]. For example, Al-Mayah showed that exosomes from conditioned media of irradiated and bystander progeny cells, when co-cultured with non-irradiated cells, caused BEs-related DNA damage. This effect was heretically present in cells that were not X-rayed [98]. Treatment of non-irradiated keratinocyte cells with exosomes from irradiated cells resulted in BEs such as the decrease in viability, calcium influx and generation of ROS [99]. Consistent with findings that exosome-mediated BEs, exosomes produced by irradiated mouse breast cancer cells transfer DNA strands to DCs and therefore induce DCs up-regulation of co-stimulatory molecules and activation of IFN-γ. In An in vivo experiment, it was shown that the induction of tumor-specific $CD8^+$ T cell response and inhibition of tumor development occurred in the mice model after IR [9]. Recently, the significance of exosomes and exosomal miRNAs in several areas has been documented. MiR-21 was involved in producing BEs through a condition media exposing experiments [100]. X-rayed MRC-5 cell model revealed that exosomes actively transfer miRNA-21 to target cells and contributed to DNA damage and chromosome aberration [101]. In another experiment, it was found that irradiated BEP2D cells potentially enclosed miRNA-7-5p to exosomes in order to participate in bystander autophagy progression. miRNA-7-5p could induce autophagy through modulating the EGFR and downstream signaling molecules such as Akt-mTOR [102]. In this regard, recent evidence has lightened the modulator function of ultraviolet (UV) radiation in exosomes kinetic. For example, Cicero et al showed that UV-irradiated keratinocyte cells release exosomes that promote melanin production by melanocytes [103]. The work of Le et al. confirmed that exosomes extracted from UV-irritated human colon carcinoma, HCT116 $p53^{+/+}$, caused a significant mitochondrial membrane depolarization and a decrease in cell survival rate exposed to exosomes. Authors showed that exosomal RNAs are responsible for eliciting bystander responses. Further investigations are needed to uncover the key roles of exosomal RNA profile in the generation of BEs. In the future, we need more understanding of signaling that deals with exosomal mediated BEs. It might be possible to interfere in exosome biogenesis and functions by design additional treatment approaches following radiotherapy.

Conclusion

In the field of radiotherapy, although directly irradiated cells are killed, there is the idea those NTEs play a pivotal role in the tumoricidal effect of IR. Irradiated cells release exosomes to cross-talk with neighboring cells and exert alternations in bystander cells. Because of variations in cell type and IR dose in cancer experimental models, there is the controversy that NTEs could promote radio-sensitivity or radio-resistance and metastasis. Unfortunately, our knowledge about IR-induced exosome biogenesis and BEs of exosomes is not complete. Preliminary data suggest the importance of IR in modulating exosome kinetic. Further investigations are essential to discovering molecular mechanisms involved in IR-induced alternation in dynamic of the exosomal secretion and composition. Exosomal-mediated BEs is another issue that will be considered in cancer therapy. In this regard, the novel strategy of exosomal targeting maybe has potential to inhibit relapse and improve the outcome of IR in patients. Furthermore, studies in exosome field may open a new hopeful avenue to fight tumor especially in case of cancer prognosis, diagnosis, and also exosomal-based delivery systems.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Abbreviations ALIX, ALG2-interacting protein X; BCs, Bystander cells; ESCRT, Endosomal sorting complex required for transport; EMT, Epithelial-to-mesenchymal transition; ECM, Extracellular matrix; EVs, Extracellular vesicles; HSCs, Hematopoietic stem cells; HRS, Hepatocyte growth factor-regulated tyrosine kinase substrate; ILVs, Intraluminal vesicles; IR, Ionizing radiation; MHC I, II, Major histocompatibility complex class I and class II molecules; MMPs, Matrix metalloproteinase; MSCs, Mesenchymal stem cells; miRNAs, Micro-RNAs; MVs, Microvesicles; ABs, Apoptotic bodies; MVBs, Multivesicular bodies; NTEs, Non-targeting effects; PM, Plasma membrane; ROS, Reactive oxygen species; SNARs, Soluble NSF attachment protein receptor; TEM, Transmission electron microscopy; TDEs, Tumor-derived exosomes

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