Chapter

Exosomes in Cancer Diagnosis and Radiation Therapy

Ai Nakaoka, Kana Kobayashi, Mennaallah Hassan and Ryohei Sasaki

Abstract

Exosomes are a subgroup of extracellular vesicles that are released by all types of cells, including tumor cells, and mediate intercellular communication via the transport of various intracellular components, including microRNAs, messenger RNAs, and proteins. Radiation produces reactive oxygen species and induces DNA double-strand break in cancer cells and normal cells. Cancer cells have severe damage and die by irradiation, but normal cells can keep proliferation with their high DNA repair ability. Irradiated cells generate communication signals and cause biological changes in neighboring or distant non-irradiated cells. This review outlines the role of exosomes in radiation therapy. In the tumor microenvironment, exosomes are considered to regulate cell survival, migration, and resistance to therapy by interacting with vascular endothelial cells and various types of immune cells. Nowadays, radiation therapy is typically combined with immunotherapy. Regulation of the activity of exosomes may overcome the problem of resistance to immunotherapy. Furthermore, exosomes can attenuate resistance to chemotherapy by transporting certain types of microRNA. The current evidence suggests that exosomes may be useful in the diagnosis and treatment of cancer in the future.

Keywords: exosomes, cancer, microRNAs, liquid biopsy, radiation therapy

1. Introduction

Approximately half of all patients with cancer receive radiation therapy as part of their treatment making it critical in the treatment of cancer [1]. Radiation therapy is used in curative and palliative regimens to achieve locoregional control [2]. Furthermore, radiation therapy is often combined with surgery, chemotherapy, and more recently, immunotherapy [3]. Despite the progress made in approaches to deliver radiation, precision medicine and combined therapies, resistance to treatment, and recurrence continue to occur in the clinical setting. According to recent studies, components of exosomes such as miRNAs and lncRNAs perpetuate drug resistance. In gastric cancer, it is reported that exosomes from M2-macrophage-induced miR-21 mediated upregulation of PI3K/Akt signaling and reduced apoptosis and cisplatin resistance [4]. In breast cancer, exosomal miR-221/222 modulated p27 and estrogen receptor (ER) for tamoxifen resistance, and exosomal cargo-lncRNA UCA1 mediated tamoxifen resistance [5, 6]. In prostate cancer, cancer-associated fibroblast-derived exosomes conferred gemcitabine resistance via Snail and miR-146a [7].

Biologically, radiation induces damage to DNA and other host cell structures by oxidative stress [8]. Following exposure to X-rays, free radical production caused by the interaction of ionizing radiation with water molecules and redox-mediated biological pathways are responsible for oxidative DNA damage and cell death. Interaction of free radicals with DNA causes the formation of different types of DNA oxidation in both nucleus and mitochondria. Oxidation of DNA and also cell death through necrosis or apoptosis can stimulate inflammatory responses and oxidative stress, leading to further DNA damage. Oxidized cell-free DNA is elevated in cancer patients and also patients undergoing radiation therapy for their malignancies [9, 10].

Irradiated cells generate communication signals and cause biological changes in neighboring or distant non-irradiated cells. This phenomenon is known as the radiation-induced bystander effect (RIBE) [11, 12]. Although, the exact mechanism of RIBE remains unclear, there is increasing evidence to suggest that exosomal microRNAs (miRNAs) support various cellular regulatory roles in the response to radiation [13–15].

Radiation therapy has recently been shown to have systemic immune-modulating effects in patients with cancer, including an abscopal effect whereby local radiation elicits a systemic immune response and alleviates the tumor burden in untreated areas [16]. Although, the mechanism of the abscopal effect remains to be fully elucidated, there is accumulating evidence indicating a strong association between this effect and tumor-derived exosomes.

Exosomes are membrane vesicles with a diameter of 30–150 nm that are released by the fusion of an organelle in the endocytic pathway, the multivesicular body, and the plasma membrane [17]. Exosomes are released in a four-stage process, namely, initiation, endocytosis, formation of multivesicular bodies, and release of exosomes. The exosome with its cargo, consisting of messenger RNA (mRNA), miRNA, mitochondrial DNA (mtDNA), single-stranded DNA, double-stranded DNA, retrotransposons, and various proteins (MHC class I and II molecules, cytokines, adhesion

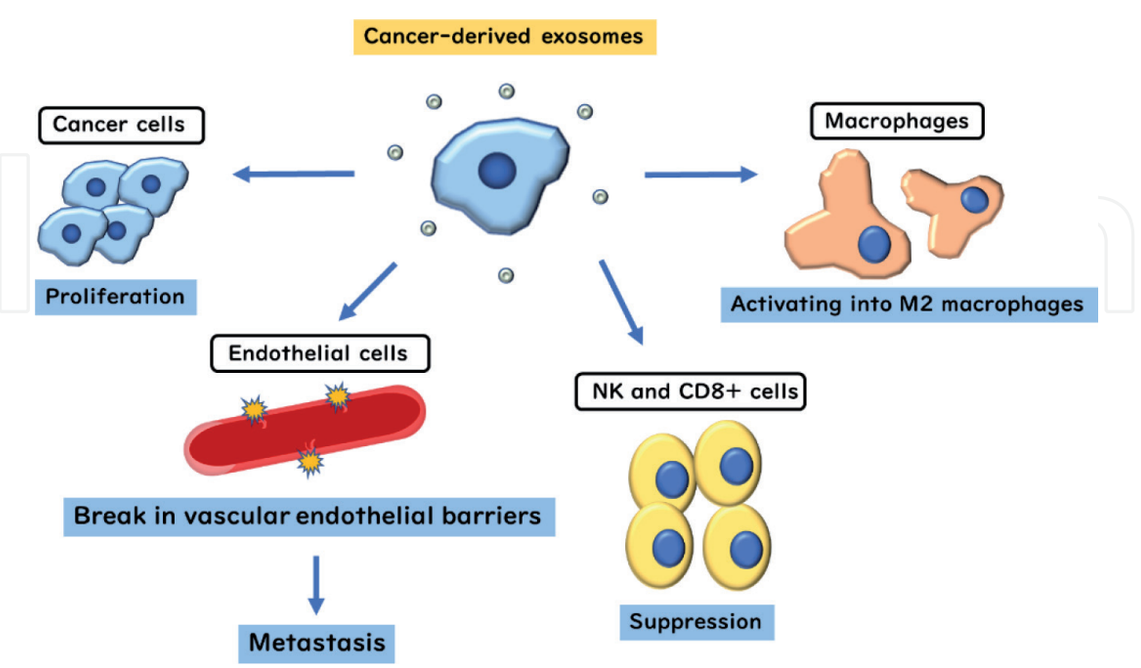

Figure 1.
Cancer-derived exosomes mediate various intercellular communications and induce the proliferation of cancer cells. Through communication with endothelial cells, vascular endothelial barriers are destroyed and metastasis is induced by cancer-derived exosomes. NK and CD8-positive cells are suppressed by cancer-derived exosomes. Macrophages are activated into M2 macrophages by cancer-derived exosomes. NK, natural killer.

molecules, transmembrane molecules, integrins, and tetraspanin) are released by the host cells and transferred to the recipient cells [18–20]. Exosomes are a subgroup of extracellular vesicles that are released by all types of cells, including tumor cells. Exosomes mediate intercellular communication by transporting various intracellular components, including cargos [21] and induce a variety of phenomena, including the proliferation of cancer cells [22], metastasis [23], and resistance to treatment [24]. Tumor-derived exosomes affect not only cancer cells themselves but also vascular endothelial cells and various types of immune cells (**Figure 1**). Exosomes released from tumor cells may affect proximal tumor cells and stromal cells in the local microenvironment and can also have systemic effects via their functional components, such as microRNAs and proteins, via the circulation. Emerging evidence suggests that tumor-derived exosomes have both immunostimulatory and immunosuppressive activity that depends on the molecules inside these structures and the status of immune cells in the tumor microenvironment [24, 25].

2. Diagnosis of exosome-mediated cancer using liquid biopsies

Liquid biopsy is a term generally used to describe the collection of body fluid and has been explored as a non-invasive complementary tool for the diagnosis of cancer. Non-invasive measurement of cancer biomarkers using liquid biopsy allows for patient stratification, screening, monitoring of response to treatment, and detecting minimal residual disease following recurrence. Extracellular vesicles are considered to be significant biomarkers in the liquid biopsy-based diagnosis of cancer, and profiling of these vesicles has the potential to improve the early detection of cancer.

Liquid biopsy can provide more comprehensive information on the genetic landscape and track genomic evolution during disease progression in patients. The main biological biomarkers used in liquid biopsy include circulating tumor cells, circulating tumor DNA, and exosomes originating from healthy tissue or tumor tissue [26]. Given that many tissues are difficult or impossible to biopsy or resect, conventional biopsies cannot provide information on the efficacy of treatment. Exosomes can be easily acquired from most types of body fluid; therefore, they are attracting attention as biomarkers in liquid biopsy for diagnosis (**Figure 2**).

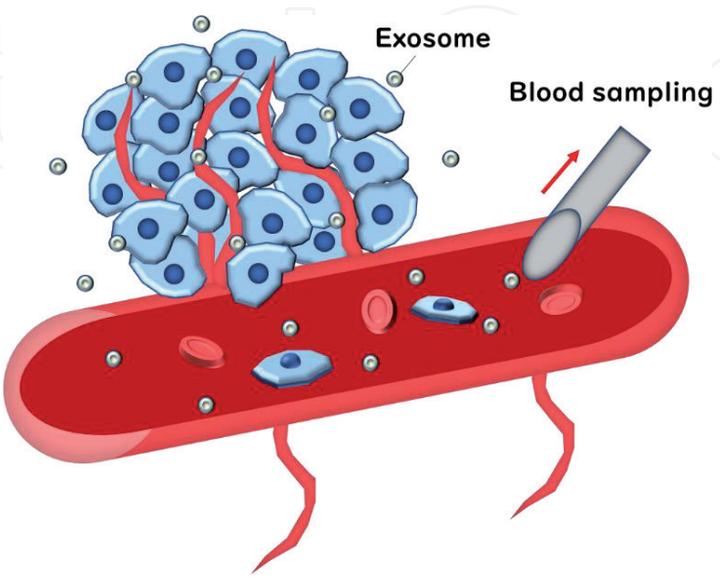

Figure 2.
Schema showing the liquid biopsy technique. Exosomes are released from cancer cells and enter blood vessels. Early diagnosis can be assisted by minimally invasive analysis of exosomes in blood vessels.

3. Roles of exosomes in common malignant diseases

3.1 Breast cancer

Early diagnosis of breast cancer increases the chances of cure and survival. Although, mammography is widely used, it is difficult to detect cancers in women with dense mammary gland tissue when using this method [27], and unnecessary radiated exposure should be avoided. Blood, nipple suction fluid, sweat, urine, and tears are being investigated as alternative diagnostic methods; among these, urine and tear tests have relatively high sensitivity and specificity. For example, the urinary miRNA profile in patients with primary breast cancer is different from that in healthy controls [28–30]. Cala et al. identified cancer-specific patterns and constructed models for the early diagnosis of breast cancer. They found that a combination of succinic acid and dimethylheptanoyl carnitine was able to separate patients with breast cancer (n = 31) from healthy controls (n = 29) with a sensitivity of 93.5% and a specificity of 86.2% [29].

Currently, reported urinary biomarkers of breast cancer are still in the discovery stage, and their specificity and sensitivity need to be verified in cohort studies. There are also multiple reports of biomarkers in tears [31–35], which are relatively easy to obtain using non-invasive methods. Inubushi et al. compared the exosomes in tears from five women with metastatic breast cancer with those from eight healthy volunteers and found higher amounts of exosome markers in tears than in serum and higher expression of breast cancer-specific miR-21 and miR-200c in tears from the patients with metastatic breast cancer [35]. Therefore, exosomes in tears could also be a useful diagnostic and prognostic biomarker.

Drug resistance is also a factor that affects prognosis. The role of exosomes in drug resistance has received a great deal of attention. There are numerous reports on the association between exosomes and drug resistance in breast cancer [36–43]. Biomarkers can be used to predict the response of a tumor to a particular treatment and may reflect tumor susceptibility and drug resistance. Although, few methods are currently recommended for routine clinical application, blood-based monitoring of the therapeutic response is a minimally invasive and promising technique. Based on those methods, liquid biopsy analysis may bring novel information as biomarkers of breast cancer [44, 45].

3.2 Prostate cancer

Prostate cancer is a tumor with a high mortality rate, and early diagnosis has a significant effect on the prognosis. Serum and urinary exosomes are promising non-invasive biomarkers that could aid in early diagnosis [46–48]. Prostate cancer has a good prognosis when hormone therapy is effective, but castrate-resistant prostate cancer has a markedly worse prognosis. Hessvik et al. identified 36 exosomal miRNAs as candidate biomarkers for prostate cancer in clinical studies [49]. Moreover, plasma miR-1290 and miR-375 levels correlate with reduced overall survival and have been identified as potential prognostic biomarkers of castrate-resistant prostate cancer [50, 51]. Expression of AR-V7 RNA in circulating tumor cells was identified as a predictor of the response to enzalutamide (an anti-androgen) and abiraterone (an anti-androgen and CYP17 inhibitor) in prostate cancer [52]. Del Re et al. detected the AR-V7 transcript preferentially in patients resistant to treatment with enzalutamide or abiraterone and proposed that the level of the AR-V7 transcript measured in extracellular vesicles in plasma could serve as a biomarker [53].

3.3 Lung cancer

Screening with low-dose CT is currently used for early diagnosis of lung cancer but is associated with radiation exposure. miRNAs derived from exosomes in body fluids are stable and relatively easy to obtain. Therefore, they are expected to be useful biomarkers for the early diagnosis of lung cancer [54–61]. Asakura et al. reported that serum miR-1268b and miR-6075 expression levels showed 99% sensitivity and 99% specificity for the diagnosis of lung cancer regardless of histological type or TNM stage [61]. This finding may lead to significant improvements in the results of screening for lung cancer and selection of more effective treatment for non-small cell lung cancer (NSCLC). Lebanony et al. reported that expression of exosomal miR-205 can distinguish between squamous and non-flat epithelial lung cancer even in poorly differentiated tumors [60]. Biomarkers that can predict the progression of lung cancer are also being investigated. Downregulation of miR-503 relative to non-malignant lung tissue has been observed in NSCLC tissue. For example, Liu et al. reported a link between the miR-503 level and advanced tumor stage and a poor prognosis [62]. These findings indicate that miR-503 may be a useful biomarker of survival in patients with NSCLC.

4. Exosome in radio-resistance

Radio-resistance is induced by certain conditions or pathways, and eventually limits the efficacies of radiation therapy. Molecular oxygen status is recognized as one of the most influential factors regulating radio-resistance. In general, cancer cells may be radio-resistant under hypoxic conditions in solid tumors [63–65]. Adapting to the hypoxic conditions, tumor cells acquire a hypoxia-resistant phenotype with the characteristic alterations in signaling, gene expression and metabolism. It is widely known that tumor cells in the hypoxia microenvironment induced radio-resistance by hypoxia-inducible factor 1 (HIF-1) and several pathways such as phosphatidylinositol 3-kinase Akt/mammalian target of rapamycin (mTOR), nuclear factor-κB (NF-κB) [66–68]. Tumor-derived exosomes promote angiogenesis by suppressing the expression of factor-inhibiting hypoxia-inducible factor 1 (HIF-1) and by transporting numerous pro-angiogenic biomolecules like vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), and microRNAs. The hypoxic condition induces angiogenesis. Uptake of the tumor-derived exosomes by normal endothelial cells activates angiogenic signaling pathways in endothelial cells and stimulates new vessel formation [69]. Exosomes can affect T cells through direct receptor-ligand interactions, but in ECs, exosomes usually use the internalization pathway [70].

The NF-κB is known as the most important transcription factors regulating the expression of a large number of genes involved in cellular processes, such as inflammatory responses, cellular growth, developmental processes and apoptosis. The inducers of NF-κB activity are highly variable, including ROS, TNFα, IL-1β, LPS, isoproterenol, ionizing radiation, viruses, and chemotherapeutic reagents [71]. Active NF-κB enters the nucleus and up-regulates the transcription of Bcl-2, Bcl-xL, XIAP, survivin and Akt leading to chemo- and radio-resistance. Yoshida and coworkers demonstrated that NF-κB is the strongest indicator of radio-resistance in laryngeal squamous cell carcinoma cases treated with radiation therapy alone without surgery nor combined chemotherapy [72]. As for the relationship between exosomes and NF-κB, Zeng et al. elaborated that exosomal miR-183-5p shuttled by M2-TAM mediated Akt/NF-κB pathway to accelerate colon cancer progression through targeting thioesterase superfamily member 4 (THEM4) [73]. Li J et al.

demonstrated that hypoxic colorectal cancer-derived extracellular vesicles deliver microRNA-361-3p to facilitate cell proliferation by targeting TRAF3 via the noncanonical NF-κB pathways [74].

Farias et al. reported that exosomes derived from irradiated MSCs (mesenchymal stem cells) delay tumor growth and reduce metastasis after treatment with MSC plus radiation therapy [75]. In this report, the authors indicate that the effect of MSCs on the tumor is associated with ANXA1; recognized as an anti-inflammatory mediator which regulates migration and cellular responses. They also showed MSCs plus radiation therapy administered on an experimental murine model had a positive effect on the tumor-volume reduction of the contralateral, untreated tumor [75]. According to these reports, exosomes derived from MSC are expected to be able to improve the efficacy of irradiation for radiation-resistant cancers.

5. Perspectives on the future use of exosomes in radiation therapy

Radiation induces oxidative stress in host cells [8], and the RIBE induced by exosomes has been investigated in several studies. It has been reported that irradiated cells release more exosomes than non-irradiated cells [12, 76, 77]. Arscott et al. identified a relationship between increased secretion of exosomes and overexpression or mutation of p53 in glioblastoma cells [78]. Furthermore, it has been found that exosomes derived from irradiated cells are taken up by neighboring cells in greater numbers than those derived from non-irradiated cells [79]. Hazawa et al. suggested that one reason for this difference could be the enhancement of cellular attachment to exosomes via augmented formation of the CD29/CD81 complex induced by radiation [80]. Thus, the influence of irradiation is transferred to neighboring cells via interaction with exosomal cargo. Although, the mechanism via which cargo is included in exosomes is not clearly understood, exosomal expression of miRNA influences the effect of irradiation on cells [79, 81]. Nakaoka et al. also investigated this effect and found that exosomes derived from irradiated MIAPaCa-2 human pancreatic cancer cells induced a radiosensitive effect on neighboring cells through an increase in levels of reactive oxygen species in cells [79], which they attributed to a reduction in expression of antioxidant enzymes via changes in the miRNA profile in exosomes. A summary of the molecules involved in RIBE is presented in **Table 1**.

Radiation therapy can have profound immune-stimulatory effects and is increasingly viewed as a promising partner in combination therapy for patients receiving immunotherapy. Intrinsic events in cancer cells induced by DNA damage are central to the immune-modifying effects of radiation therapy [82]. These events have been investigated in studies of the DNA damage response, which focuses on the tumor microenvironment. In addition to its ability to destroy cancer cells by damaging DNA, radiation therapy can modulate both the immunotherapy and adjuvant therapy of tumors by triggering the release of pro-inflammatory mediators, increasing tumor-infiltrating immune-stimulatory cells. and enhancing the expression of neoantigens [82–84] and immune-stimulatory signaling by cyclic dinucleotide cyclic GMP-AMP. Understanding the mechanistic basis of radiation therapy as an anticancer treatment has been transformed by the recent discovery that DNA damage in cycling cancer cells can activate cytoplasmic nucleic acid sensors [82, 83]. The cGAS (cytoplasmic DNA-sensing cyclic GMP–AMP synthase)-STING (stimulator of interferon genes) pathway is involved in this process [85, 86]. Using this pathway, tumor-derived exosomes can shuttle TREX1-sensitive type I interferon-stimulatory double-stranded DNA from irradiated cancer cells to dendritic cells (**Figure 3**) [87].

Cargo	Molecule	Cell line	Interactions	Ref.
miRNAs	miR-7-5p	BEP2D	Induction of autophagy	13
	miR-208a	Non-small cell lung cancer cells	Promotion of cell proliferation Induction of radioresistance	14
	miR-301a	Glioblastoma cells	Promotion of radiation resistance	15
	miR-6823-5p	MIAPaCa-2	Enhancement of radiosensitive effect	79
Proteins	Connective tissue growth factor Insulin-like growth factor-binding protein 2	LN18, U87MG and U251	Enhancement of tumorigenesis and migration	78

Table 1.
Radiation-induced bystander effect induced by exosomes.

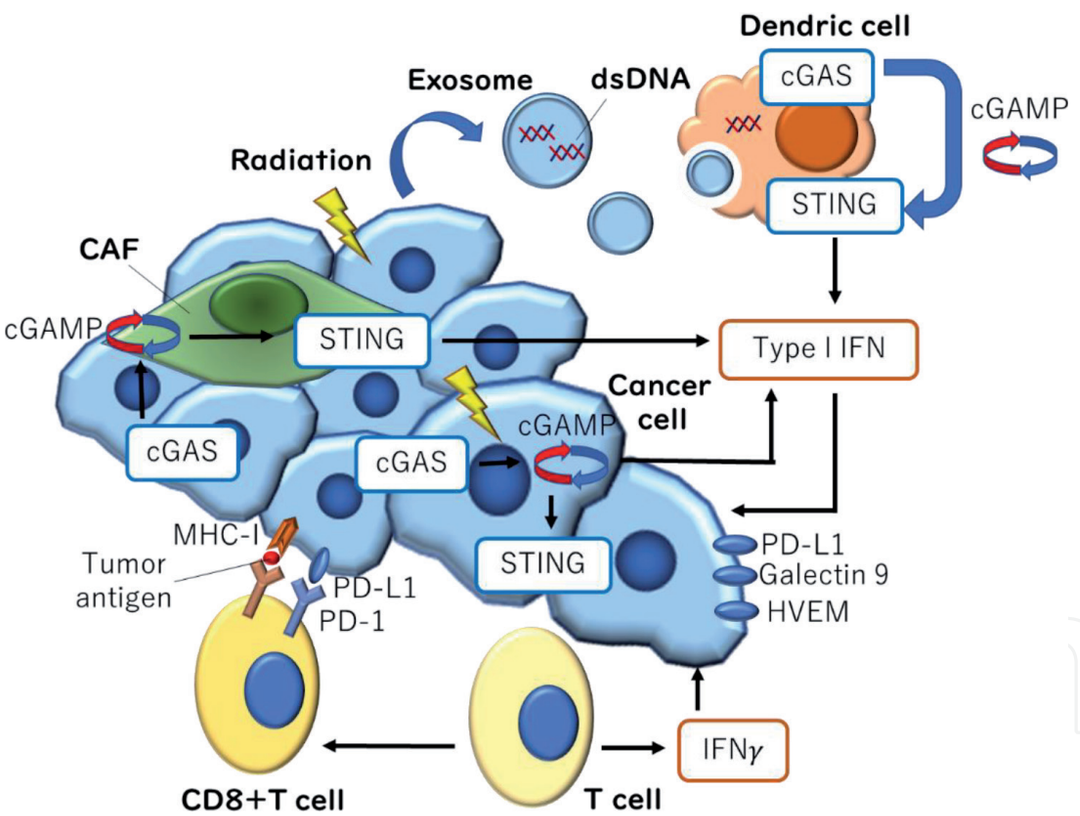

Figure 3.
Radiation and immune response via cancer-derived exosomes in the tumor microenvironment. The pathway related to activation of cytoplasmic nucleic acid sensors. Tumor-derived exosomes can transport type I IFN-stimulatory dsDNA from irradiated cancer cells to dendritic cells via the STING pathway. T-cells produce IFN-γ. Inhibition of activation of CD8-positive T-cells by PD-1/L1 binding. cGAS cytoplasmic DNA-sensing cyclic GMP-AMP synthase; dsDNA, double-stranded DNA; IFN, interferon; STING, stimulator of interferon genes.

6. Exosomes in chemotherapy and other therapy

Chemo-resistance should be solved for effective cancer therapy. Several studies indicated that signal transducer and activator of transcription 3 (STAT3), focal adhesion kinase (FAK) and epithelial-mesenchymal transition (EMT) contribute

to the development of chemotherapeutic resistance [88–91]. The anti-apoptotic signal which is activated by the STAT3 pathway is an important factor in causing initial drug resistance [88]. FAK is reported to promote invasive tumor growth with β-catenin [89]. EMT is popular with the induction of cancer metastasis [90], but it is also deeply related to drug resistance. Mani et al. reported that EMT may cause cancer cells to acquire epithelial stem cell properties [91]. EMT formation induces chemo-resistance [92] and it is induced by various signaling pathways, such as TGFβ, Wnt signal, etc. [93, 94].

Several studies have demonstrated that exosomal activity might be involved in chemo-resistance. In EMT, Shan et al. indicated that downregulation of exosomal miR-148b-3p may offer opportunities in the treatment of bladder cancer by increasing chemosensitivity via inhibition of the Wnt/β-catenin pathway and promoting expression of PTEN [95]. In addition to it, Liu et al. reported that exosome-transmitted miR-128-3p increased chemosensitivity of oxaliplatin-resistant colorectal cancer by suppressing epithelial-mesenchymal transition and inducing drug accumulation in cancer cells [96]. Furthermore, exosomes derived from cancer-associated fibroblasts and cancer cells are often reported to contribute to chemo-resistance [97, 98]. According to these reports, exosomes are also important to the regulation of chemotherapy.

7. Conclusions

Exosomes play a critical role in cancer progression, including cell–cell communication, tumor-stromal interactions, activation of signaling pathways, and immunomodulation. Emerging data indicate that radiation-derived exosomes increase tumor burden, decrease survival, cause radiation-induced bystander effects and promote radio-resistance. Exosomes show abilities as diagnostic and predictive biomarkers in various malignancies. Exosomes play a role in radio- and chemo-resistance through multiple pathways. The mechanism of the intercellular communication by exosomes, the transport of exosomal cargo, the secretion mechanism, cell dependence, immune response and radiation response have been intensively explored. Specifically, research on exosomes about radiation therapy has been expanding. Radiation-exposed cells release altered exosomes, and those exosomes play a role as cargo to bring multiple messages to their recipient cells leading to various radiation responses. Further understanding of the mechanisms of exosome-mediated radio-resistance might ultimately lead to the development of novel treatment strategies.

Acknowledgements

This research was supported by Grants-in-Aid for Exploratory Research (21 J21801 to AN, and 21 K07642 to KK) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Conflict of interest

The authors declare no conflict of interest.

DOI: http://dx.doi.org/10.5772/intechopen.101684

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