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The Emerging Role of Extracellular Vesicle-Derived miRNAs: Implication in Cancer Progression and Stem Cell Related Diseases

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Abstract

Cells release into the extracellular environment, diverse types of membrane vesicles of endosomal and plasma membrane origin called exosomes and microvesicles. A number of studies indicate that these extracellular vehicles (EVs) mediate the interaction between cancer cells and their microenvironment; and thereby, play a critical role in the development of cancers. EVs contain cargo which consist of proteins, lipids, mRNAs, and miRNAs that can be delivered to different types of cells in nascent as well as distal locations. Discovery of this latter cargo has drawn an increasing amount of attention, due to their altering effects on the transcriptome, proteins, and subsequent cellular characteristics in recipient cells. Cancer cell derived exosomes (CCEs) have been identified in body fluids of cancer patients including urine, plasma and saliva. Because CCE content largely depends on tumor type and stage, they invariably lend great potential in serving as prognostic and diagnostic markers. Notably, accumulating evidence demonstrates that EV-derived miRNAs have key roles in regulating various aspects of cellular homeostasis, including proliferation, survival, migration, metastasis, and the immune system etc. More recently, diagnostic and therapeutic exploitation of stem cells derived EVs are under investigation. This review aims to summarize recent advances in EV-derived miRNAs in a variety of tumor types, and suggests that these cancer-derived exosomal miRNAs play a critical role in regulating cellular functions in surrounding and distant locations. It also discusses the role of adverse environmental exposure in altering stem cell exosomal miRNA profiling, which we believe leads to changes in the extracellular environment as well as a diverse range of biological processes.

Keywords: Extracellular vesicles; Exosomes; miRNA, Cancer cells; Stem cells

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Introduction

miRNA and their functions

MicroRNAs (miRNAs) are small, non-coding RNA molecules (21-23 nt) found in various species that play an important role in transcriptional and post-transcriptional regulation of gene expression [1, 2]. For most miRNAs, primary miRNA transcripts are generated by RNA polymerase II, either as separate transcriptional units or embedded within the introns of protein coding genes [3]. MiRNAs are generated from a long primary transcript (pri-miRNA) which is cleaved in the nucleus by a complex formed by the protein DGCR8/Pasha and the RNAse III Drosha to a shorter hair-pin structure (50–120 nt) which constitutes the miRNA precursor (pre-miRNA) [4]. The pre-miRNA is exported to the cytoplasm, where it is processed by the RNAse III enzyme Dicer to a 22-nucleotide duplex and then loaded onto the miRNA-containing RNA-induced silencing complex (miRISC) where miRNA duplex is unwound and the single stranded 22 nt mature miRNA, which originates from one arm of the pre-miRNA hairpin, is bound to an Argonaute protein [5]. Only one strand of the duplex remains in the RISC-Loading complex as a mature miRNA (guide strand or miRNA), whereas the other (passenger strand) is rapidly degraded. Mature miRNAs function by binding to complementary sequences within mRNA molecules, usually resulting in gene silencing via translational repression or target degradation [5, 6]. The presence in the target mRNA 3'-UTR of multiple sites for the same or different miRNAs generally confers

repression, more effectively. To date, approximately 2,000 miRNAs have been identified in the human genome [7-9] which target over 60% of mammalian genes [10, 11]. Sharing of seed sequences and the presence of overlapping targets indicate that a single miRNA can target many different mRNAs and that different miRNAs can target the same mRNA as well. This suggests that miRNAs are involved in integrating gene regulatory networks, in both physiological and pathological conditions. miRNA was first discovered in the early 1990s [12]. Subsequently, many short regulatory RNAs were identified in almost all multicellular organisms [13-17], in single cellular algae, and DNA viruses [18, 19]. miRNAs have been found to be involved in many biological events including normal development, differentiation, growth control, and numerous diseases including cancer [20-25]. Importantly, microRNAs are already considered diagnostic and prognostic biomarkers for patient stratification as well as therapeutic targets and agents.

miRNA in cancers/tumors

miRNA negatively regulates gene expression by binding to the 3' untranslated region (3'UTR) of mRNA, leading to degradation or translation blockade. Deregulation of miRNA is tightly linked to cancer initiation and progression, and circulating miRNAs have emerged as potential biomarkers for cancer diagnosis and prognosis. In 2002, the first study to demonstrate the link between deregulation of mir-15 and 16 and the pathogenesis of chronic lymphocytic leukemia was reported [26]. Subsequently, miRNAs have been suggested as biomarkers in cancer, because miRNA-expression arrays are found to be more efficient in classifying cancer than mRNA-expression arrays [27]. miRNAs have been found not only in tissues, but also in body fluids including serum, plasma, and urine in a stable form that is protected from RNase activity in association with RISC, either free in blood or in membrane-enclosed vesicles (such as exosomes) [28-31]. In other words, miRNAs can be secreted into the extracellular environment through exosomes or in complexes with protein or lipid-based carriers. Accumulating evidence demonstrates that miRNAs as well as proteins can be transferred to neighboring or distant cells in these secretory forms to modulate cell function. Extracellular miRNAs are therefore emerging as a new group of messengers and effectors in intercellular communication.

The major causes of altered miRNA expression in cancer include genetic and epigenetic alterations, aberrant transcription factor activity, mutation in miRNA biogenesis pathways, and other mechanisms which seek to elude miRNA repression [3, 32].

miRNAs in extracellular vesicles

Exosomes: Exosomes are cell-derived extracellular vesicles (EVs) present in many and perhaps all biological fluids, including blood, urine, and cultured media of cell cultures [33, 34]. Exosomes range in diameter, between 40 and 150 nm. In mammals, exosomes are either released from the cells when multivesicular bodies fuse with plasma membrane or they are released directly from the plasma membrane [35]. It is becoming increasingly clear that exosomes have specialized functions and play a key role in many biological events including tumorigenesis, metabolism, coagulation, intercellular signaling, and the immune system. Consequently, there is growing interest in the clinical applications of exosomes. Exosomes could potentially be utilized in the prognosis, therapy, and as biomarkers for health and disease.

Exosomes contain various molecular constituents, including proteins, nucleic acids, and lipids [36]. According to ExoCarta (http://www.exocarta.org), an exosome database, 4563 proteins, 194 lipids, 1639 mRNAs, and 764 miRNAs have been identified in exosomes from multiple organisms [37, 38]. Although the exosomal protein composition varies with cell and tissue of origin, most exosomes contain an evolutionary-conserved, common set of protein molecules such as chaperones, subunits of the trimeric G proteins, cytoskeletal proteins, tetraspanin proteins, and other proteins [39, 40]. The 2007 hallmark study, which demonstrated that exosomes contain RNA cargo [41] has garnered great interest in understanding the role of exosomes in cell-to-cell interaction leading to an altered expression pattern in recipient cells. That study also revealed the differences in cellular and exosomal mRNA – while for the first time, miRNA content was described, as well as the functionality of the exosomal mRNA cargo [41]. Soon after this discovery, exosomal miRNA, and exosome derived miRNAs were evidenced to be surrogate diagnostic markers for biopsy profiling [42, 43].

Many advances have been made in the field of exosome isolation with improving knowledge and emerging novel technologies. To date, several strategies including ultracentrifugation, size-based isolation, precipitation, and affinity-based capture have been utilized to isolate exosomes [44].

Several mechanisms have been hypothesized in describing the interactions of exosomes and recipient cells. Exosomes can bind to cells through receptor–ligand interactions. Alternatively, exosomes can putatively attach or fuse with the target-cell membrane, delivering exosomal surface proteins and perhaps cytoplasm to the recipient cell. Finally, exosomes may also be internalized by the recipient cells via endocytosis [41]. Therefore, more research studies are focused on investigating the role that exosomes may play in cell-to-cell signaling; often hypothesizing that delivery of their RNA cargo molecules will explain biological effects. Accumulating evidence demonstrates that these cell-to-cell communications influence both physiological and pathological processes [45-47].

Role of miRNAs from cancer cell-derived exosomes: Evidence indicates that both normal and cancer cells communicate via the release and delivery of molecules. These molecules include miRNAs which are packed into exosomes and significantly influence the physiological and pathological conditions of cells [48]. Tumor-derived exosomes can modify normal, healthy cells by altering their translational profile to promote tumor progression. Exosomes are quickly emerging as powerful sources for molecule transfer between cells in the mediation of both beneficial and pathological processes. The oncogenic profiling of miRNA in exosomes varies with cancer type and exhibits a variety of functions depending on the recipient cell [49-51] **(Table1).**

Diagnostic signatures: An ever-increasing body of literature demonstrates that molecular constituents of exosomes, especially exosomal miRNAs hold great promise as novel biomarkers for

Table 11 Extracellular vesicle-derived miRNAs in cancers and stem cells .

miRNAs	Tissues/cells/diseases	Functions	References	Year
miR-146b, miR-222	indolent papillary thyroid cancer	Proliferation	Lee JC, et al.	2015
miR-24-3p, miR-891a, miR106a-5p, miR-20a-5p, miR-1908	nasopharyngeal carcinoma	proliferation	Ye SB, et al.	2014
miR-30a, miR-138, miR-146, miR-203	chronic myeloid leukemia	chemoresistance	Taganov; Duncan; Yu et al.	2006, 2008, 2012
miR-21, miR-155	neuroblastoma	chemoresistance	Chal.lagundla et al.	2015
miR-122	breast cancer	metabolism	Fong et al.	2015
miR-135	multiple myeloma	angiogenesis	Umezu et al.	2014
miR-19a	astrocyte	metastasis	Zhang et al.	2015
miR-21, miR-141, miR-200, miR-203, miR-205, miR-214	ovarian cancer	diagnostic signatures	Taylor et al.	2008
miR-17, miR-19a, mir-21, miR-126, miR-149	melanoma	diagnostic signatures	Pfeffer et al.	2015
miR-21, miR-34a	MSCs	tumor supportive miRNAs	Vallabhaneni et al.	2015
miR-221	GC-MSCs	proliferation, migration	Wang et al.	2015
miR-23b	BM-MSCs	metastasis	Ono et al.	2014
miR-200	breast cancer stem cells	metastasis	Shimono et al.	2009
miR-19b, miR-26b, miR-203	colonic stem cells	dietary response miRNAs	Shah MS, et al.	2016

clinical diagnosis. Among ovarian cancer patients, cancer-derived exosomal miRNAs included miR-21, miR-141, miR-200a, miR-200c, miR-200b, miR-203, miR-205, and miR-214 which exhibited significantly elevated levels as compared to benign disease. These results suggest that microRNA profiling of circulating tumor exosomes could potentially be used as surrogate diagnostic markers for ovarian cancer [42]. In melanoma, exosomal miRNAs included miR-17, miR-19a, miR-21, miR-126, and miR-149 and expressed higher levels in patients with metastatic sporadic melanoma as compared with familial melanoma patients or unaffected control subjects This indicates that distinct exosomal miRNAs may play important roles in tumor progression and metastasis, and may be used as predictive biomarkers to monitor remission as well as relapse following therapeutic intervention [52]. In addition, based on high-throughput next generation sequencing data, the exosomal miRNA signature is potentially well-suited to serve as a peripheral screening tool for Alzheimer's disease [37, 53].

Cell proliferation

Increasing evidence shows that cancer-derived exosomes contain miRNAs, which regulate the proliferation of recipient cells. In indolent papillary thyroid cancer (PTC), PTC-derived exosomes contain miR-146b and miR-222, which alter proliferation of other cells in a complex manner [54]. In nasopharyngeal carcinoma (NPC), NPC sera and NPC-cell derived exosomes commonly contain miR-24-3p, miR-891a, miR106a-5p, miR-20a-5p, and miR-1908. These over-expressed miRNA clusters down-regulate the MARK1 signaling pathway to alter cell proliferation and differentiation [55]. In pediatric cancer neuroblastoma (NB), using ultracentrifugation and exosome precipitation procedure, isolated exosomes from MYCN-amplified NB exhibit highly expressed miRNAs which are associated with a range of cellular and molecular functions related to cell growth and cell death [56].

Resistance to chemotherapy

Development of chemoresistance is a persistent problem during the treatment of local and disseminated disease. In chronic myeloid leukemia (CML), miRNAs can act as oncogenes or tumor suppressor genes which then contribute to the pathogenesis, disease progression, and resistance to therapy [57]. For example, several miRNAs including miR-146, miR-138 and mir-30a, miR-203 are involved in imatinib treatment related resistance, [58-61] suggesting that the potential use of these small RNAs as therapeutic targets holds new opportunities in the treatment of CML . Another recent experiment showed that cross-talk between NB cells and human monocytes by exosomal miR-21 and miR-155 plays a crucial role in chemotherapy resistance [61]. This study demonstrated that NB cells secrete exosomic miR-21 transferred to human monocytes. The miR-21 is able to bind to the Toll-Like Receptor 8 in the recipient cells of human monocytes leading to increased expression of miR-155. Then, the monocytes secrete exosomal miR-155 transferred to NB cells leading to decreased Telomeric Repeat Binding Factor (NIMA-Interacting) 1 (TERF1, an inhibitor of telomerase) which is associated with chemoresistance in NB [62]. Exosomic miR-155-mediated cross-talk between human monocytes and NB cells increases the telomerase activity and confers the drug resistance to NB cells in response to cisplatin treatment. These data demonstrate that the miR-21/Mir-155/TERF1 circuitry contributes to chemoresistance in NB [61].

Metabolism

In breast cancer, miRNA from exosomes have been implicated in metabolism and metabolic disorders [63]. Breast cancer-secreted miR-122 via exosomes reprograms glucose metabolism in the pre-metastatic niche to promote metastasis. *In vivo* inhibition of miR-122 restores glucose uptake in distant organs such as brain and lungs, and decreases the incidence of metastasis. These results demonstrate that miR-122 from CCEs are able to reprogram

systemic metabolism in the facilitation of disease progression [63].

Angiogenesis

Exosomal miRNA transfer is believed to be involved in angiogenesis. In blood vessels, EV transfer of miRNAs modulates atherosclerosis and angiogenesis [64]. Several studies demonstrate the roles of miRNAs in activating cellular changes and modulating angiogenesis via the shuttling of miRNAs from other cells into endothelial cells (ECs). The human monocytic cell line, THP-1 is known to have abundant levels of miR-150, whereas miR-150 is low to absent in ECs. miR-150 transfers from THP-1 monocytes via EVs into ECs resulting in significantly elevated miR-150 levels in ECs. Subsequently, protein levels of miR-150 target c-Myb are decreased in ECs resulting in enhanced cell migration [65, 66]. One of the major hallmarks of cancerous cells lies in their ability to grow tumors and generate their own vasculature; an essential element in disease progression. It becomes clear that cancer derived EV can exert complex effects on ECs, their progenitors and on supporting cells; thereby, contributing to vessel formation within tumors. For example, Tspan8 is expressed in pancreatic cancer cells, and exhibits characteristics of promoting angiogenesis [67]. Tspan8 is involved in ECs and cancer cell EV interaction [68]. Subsequently, EV uptake by ECs elevated expression levels of pro-angiogenesis related factors to enhance angiogenesis [68]. In multiple myeloma (MM), the massive proliferation of plasma cells causes hypoxia. The hypoxia-resistant MM cells (HR-MM) produced more exosomes than the parental cells under normoxia or acute hypoxia conditions. Furthermore, HR-MM derived exosomes exhibit high levels of miR-135, which directly suppressed its target factor-inhibiting hypoxia-inducible factor 1 (FIH-1) in ECs, leading to enhanced endothelial tube formation under hypoxia via the HIF-FIH signaling pathway [69]. These experiments indicate that exosome-derived miRNAs from various tumors/cancers target surrounding or distant cells, ultimately changing the recipient cell's function.

Invasion/Metastasis

Cancer biology is tightly regulated by cell-to-cell interaction. It is believed that initiation and progression of cancer is tightly regulated by tumor-associated stroma, which consists of extracellular matrix components and several cell types, including cancer-associated fibroblasts (CAF), immune cells, vascular cells, and bone marrow–derived cells [70]. It has been shown that fibroblasts secrete exosomes that promote breast cancer cells (BCCs) protrusive activity, motility, and metastasis by activating autocrine Wnt-PCP signaling in BCCs [71].

Epithelial-to-mesenchymal transition (EMT) is a process by which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties. EMT has been implicated in the initiation of metastasis for cancer progression. In bladder cancer, the cancer-derived exosomes are capable of decreasing expression of epithelial markers E-cadherin and β-catenin, and increasing the migration and invasion of urothelial cells [72]. These studies suggest the important role of exosomes in the invasiveness and metastasis of disease.

The dynamic and reciprocal cross-talk between metastatic cells and their microenvironment during the adaptive metastatic outgrowth has recently been demonstrated via EV-derived miRNA [73]. In brain, astrocyte-derived exosomes mediate an intercellular transfer of PTEN-targeting microRNAs (miR-19a in miR-17~92 cluster play a major role in the down regulation of PTEN) to metastatic tumor cells, while astrocyte-specific depletion of PTEN-targeting microRNAs or blockade of astrocyte exosome secretion rescues PTEN loss and suppresses brain metastasis. In addition, this adaptive PTEN loss in brain metastatic tumor cells leads to an increased secretion of the chemokine CCL2, which recruits IBA1-expressing myeloid cells that reciprocally enhance the outgrowth of brain metastatic tumor cells via increased proliferation and decreased apoptosis [73]. These data suggest that exosomal miRNAs may play an essential role in the dynamic interaction between metastatic tumor cells and extrinsic signals at individual metastatic organ sites which significantly contribute to subsequent outgrowth.

Role of miRNAs in stem cell derived exosomes

Stem cells in cancer/tumor: Stem cells are undifferentiated biological cells that can differentiate into specialized cells, and divide to produce more stem cells. Normal stem cells (NSCs) have two main defining properties. First, they can renew themselves, which allows self-perpetuation and maintenance of a pool of totipotent stem cells. Self-renewal can occur by means of symmetric mitosis in which a stem cell produces two daughter stem cells, or asymmetric division in which a stem cell produces a daughter stem cell and another cell that is committed to a certain line of differentiation [74]. Second, NSCs can differentiate into multiple lineages, thus replacing and maintaining major functional elements that characterize surrounding tissue [75] **(Table1).**

Cancer stem cells (CSCs) are cancerous cells (found within tumors or hematological cancers) that possess characteristics associated with normal stem cells, specifically the ability to give rise to all cell types found in a particular cancer sample. CSCs are tumorigenic and may generate tumors through stem cell processes of self-renewal and differentiation into multiple cell types. CSCs are small cancerous cell populations with stem-like properties including cell proliferation, multiple differentiation and tumor initiation capacities. CSCs are therapy-resistant and cause cancer metastasis and recurrence. One recurrent key issue in cancer therapy lies in the targeting and elimination of CSCs, in order to cure cancer completely without relapse and metastasis [76]. To target CSCs, miRNAs are considered CSC markers.

Conclusive evidence for CSCs was first discovered by isolating a subpopulation of leukaemic cells that expressed the specific surface marker CD34, but lacked the CD38 marker [77]. The $CD34^{+}/CD38^{-}$ subpopulation is capable of initiating tumors in NOD/SCID mice that are histologically similar to the donor. Later on, CSCs and tumor stem cells (TSCs) from various types of cancer/tumor were identified including breast cancer, ovarian cancer, prostate adenocarcinomas, brain gliomas, lung cancer, colorectal carcinomas, melanoma, and fibroids [78-86]. Recently, our group isolated and characterized myometrial and fibroid stem cells using dual Stro-1/CD44 surface markers and demonstrated the role of these stem cells in the pathogenesis of uterine fibroids [87].

Exosomal miRNAs in cell proliferation, migration and apoptosis: Tumor-stroma interaction is critical for carcinogenesis and cancer progression. Recently published studies have demonstrated that exosomes from stem cells induce proliferation and migration in several types of cells [88, 89]. Cancer-derived MSCs exhibit aberrant miRNA expression patterns. For instance, gastric cancer-associated MSCs (GC-MSCs) and their non-cancerous tissue MSCs (GCN-MSCs) exhibit a different miRNA profile. Isolated exosomes from GC-MSCs exhibited a higher content of miR-221, were instantly internalized by gastric cancer cells, and significantly promoted cell proliferation and migration [90].

Human mesenchymal stem cells (hMSCs) can act as stromal cells for solid tumors. For example, when hMSCs were localized, and integrated into tumor associated stroma, they were shown to promote tumor growth and angiogenesis through juxtacrine, paracrine and endocrine mechanisms [91-93]. A recent study on hMSCs [94] demonstrated that exosomes/EVs from serum deprived hMSCs act as carriers that transport tumor supportive miRNAs. Through a series of ultracentrifugation steps, isolated exosomes containing anti-apoptotic miR–21 and miR-34a [94] have been demonstrated to be involved in cell survival and proliferation [95, 96]. In addition, these hMSC-derived EVs increase the overall survival of cancer cells under serum-deprived conditions.

Exosomal miRNAs in invasion/metastasis: MicroRNAs (miRNAs) have recently been recognized as targets for anti-metastatic therapy against cancer malignancy [97]. Mesenchymal-epithelial transitions (MET), are integral steps of cell fate specification during gastrulation and organogenesis. MET occur during normal development, cancer metastasis, and induced pluripotent stem cell reprogramming. In cancer progression, reactivation of the MET program promotes tumor metastasis by driving tumor cell invasion and enhancing tumor cell survival during the metastatic cascade. MicroRNAs have emerged as key regulators of CSCs and MET. Among them, the miR-200 family plays a particularly important role in integrating the MET program and core stem cell pathways [98]. The overexpression of miR-200 suppresses the clonogenicity of breast cancer stem cells (BCSCs) and the ability of multipotent mammary stem cells (MaSCs) to regenerate mammary ductal trees. On the other hand, inhibition of miR-200 increases the number of CSCs in breast cancers.

Another study focused on the role of miRNAs in metastasis used the co-culturing system of bone marrow-metastatic human breast cancer cell line (BM2) with human bone marrow mesenchymal stem cells (BM-MSCs). This study revealed that BM-MSCs suppressed the proliferation and invasion of BM2 cells [99]. Acquisition of these dormant phenotypes in BM2 cells was also observed by culturing the cells in BM-MSC-conditioned media or with exosomes from BM-MSC cultures. Although various miRNAs exhibited increased expression in BM-MSC-derived exosomes as compared with those from adult fibroblasts, overexpression of miR-23b in BM2 cells induced dormant phenotypes through the suppression of the target gene, MARCKS. This latter target gene encodes a protein that promotes cell cycling and motility [99]. These studies suggest that exosomal miRNAs from BM-MSCs may promote breast cancer cell dormancy in a metastatic niche, and EV-mediated communication has a major influence on key aspects of cancer progression. Future directions could include studies which focus on developing a thorough understanding as to whether EV-derived miRNA profiling from metastatic CSCs differs from those of CSCs in a primary site.

miRNAs from stem cells in response to environmental exposure

Exosome production and content may be influenced by molecular signals, depending on the origin of the cells. The origin of CSCs is still an area of ongoing investigation. In brief, CSCs can be generated as mutants of developing stem cells, adult stem cells, or differentiated cells that require stem-like attributes.

Alternately, exosome production and content may be altered in response to adverse environmental exposure. For example, tumor cells exposed to hypoxia secrete exosomes with enhanced angiogenic and metastatic potential, suggesting that tumor cells adapt to a hypoxic microenvironment by secreting exosomes to stimulate angiogenesis or facilitate metastasis in a more favorable environment [100, 101].

Abnormal environmental exposures have been shown to be involved in many diseases [102, 103]. Since the involvement of miRNAs and tumor-initiating cells/progenitor cells play a crucial role in the development of tumors and many other diseases, the environmental exposure can impact stem cell regulatory networks by modulating the steady-state levels of miRNAs. Therefore, miRNA changes may be sensitive indicators of the effects of acute and chronic environmental exposure. To understand how colonic stem cell populations respond to environmental factors such as diet and carcinogens, Shah et al, determined the effects of the chemoprotective fish oil/pectin diet on miRNAs and mRNAs in colonic stem cells obtained from Lgr5-EGFP-IRES-creER knock-in mice [104]. Following global miRNA profiling, 26 miRNAs ($P<0.05$) were differentially expressed in Lgr5 (high) stem cells as compared to Lgr5 (negative) differentiated cells. Fish oil/pectin treatment up-regulated miR-19b, miR-26b and miR-203 expression as compared to corn oil plus cellulose (CCA) specifically in Lgr5 (high) cells. They further demonstrated that only miR-19b and its indirect target PTK2B were modulated by the fish oil/pectin diet in Lgr5 (negative) cells [104].

In a separate observation from another animal diet model, 8-week-old mothers of 1-day old rat pups were fed diets containing deficient or enriched amounts of n-3 polyunsaturated fatty acids (n-3 PUFAs) from two weeks before breeding up until delivery. The results indicated that rat neural stem cells/neural progenitors (NSC) proliferation and differentiation were dually altered by the *in utero* polyunsaturated fatty acid supply, along with marked alterations in mRNA and miRNA expression [105]. Therefore, fetal exposure to n-3 PUFA deficient diet altered NSC characteristics, and reprogramed expression patterns of mRNAs and miRNAs. However the link between miRNA and exosomes in stem cells in response to adverse environmental exposure requires further investigation.

Conclusion

Exosomes naturally carry miRNAs; therefore, they are used as carriers to deliver miRNAs in several therapeutic applications. A number of studies have shown that exosomes can deliver

tissue-targeted siRNA and miRNAs to modulate gene expression pattern within target cells [106-108]. Moreover, several clinical trials on exosome-based therapies for cancer treatment are being conducted [109-111].

Although great strides have been made in understanding the role of miRNAs, and we now know that their associated networks play in a variety of biological progress and diseases, there still remains limited information regarding how the interaction between stem cells and their surrounding differentiated cells happens through EV derived miRNAs, therefore alters the transcriptome in recipient cells leading to cancer/tumor progression. Further studies focused on the interplay between gene expression reprogramming and stem cell features via exosomes will augment our understanding and identification of critical exosomal miRNA targets and related events. Considering the reversibility of epigenetic alterations as well as the pivotal role they play in early carcinogenesis and other diseases, reversion of these alterations could be a promising approach for providing novel therapeutic targets for treatment of a variety of diseases.

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Conflict of Interest

None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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