Aptamer-based therapy for targeting key mediators of cancer metastasis (Review)

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Abstract. Cancer-related deaths remain a challenging and devastating obstacle to defeat despite the tremendous advances in cancer treatment. Cancer metastasis is the major cause of these cancer-related deaths. Metastasis involves sequential steps during cancer cells' journey to a new site. These steps are coordinately regulated by specific intracellular regulators and cellular interactions between the cancer cells and the supporting microenvironment of the different organs. The development of aptamer-based therapeutics is a promising strategy to fight cancer metastasis as it holds potential advantages. Oligonucleotide and peptide aptamers are short sequences of single-stranded nucleic acids or amino acids, respectively, that target proteins, genetic materials, and cells. Antimetastatic aptamer-based therapeutics exert their pharmacological effect by direct interaction with the signaling pathways inside the cancer cells or the communications between cancer cells and the tumor microenvironment. In addition, aptamers have been utilized as a guiding ligand to deliver a therapeutic moiety to cancer cells or the supporting microenvironment. The selected aptamer possesses high specificity since it is designed to recognize and interact with its target. This review summarizes recent advances in the development of aptamer-based therapeutics targeting mediators of cancer metastasis. In addition, potential opportunities are discussed to inspire researchers in the field to develop novel aptamer-based antimetastatic treatments.

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Abbreviations: Angptl4, angiopoietin-like-4; CEA, carcinoembryonic antigen; circRNA, circular RNA; COX-2, cyclooxygenase-2; CSCs, cancer stem cells; CTCs, circulating tumor cells; CTLA4, cytotoxic T lymphocyte-associated antigen 4; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; EREG, epiregulin; EVs, extracellular vesicles; FAK, focal adhesion kinase; HepG2, hepatocellular carcinoma cells; HER2, human epidermal growth factor receptor 2; HGF, hepatocyte growth factor; IL, interleukin; IL4R-α, Interleukin 4 receptor-α; LAC, lung adenocarcinoma; lncRNAs, long non-coding RNAs; LOX, lysyl oxidase; MDSCs, myeloid-derived suppressor cells; MED1, mediator complex subunit1; miRNAs/miRs, microRNAs; MMPs, matrix metalloproteinases; MUC1, mucin 1 antigen; MVs, microvesicles; OPN, osteopontin; Psap, prosaposin; PTDs, protein transduction domains; RANKL, receptor activator of nuclear factor-B ligand; RNA-seq, RNA sequencing; sasRNA, small antisense RNA; SDF-1, stromal cell-derived factor 1; SELEX, Systemic Evolution of Ligands by Exponential Enrichment; sLex, sialyl Lewis X; ssDNA/RNA, sequences of single-stranded nucleic acids; TGF-β, transforming growth factor-β; TICs, tumor-initiating cells; Tregs, regulatory T cells; VEGFR1$^+$, VEGF receptor 1-positive; VEGFs, vascular endothelial growth factors

Key words: cancer metastasis, antimetastatic treatment, aptamer-based therapeutics, targeted treatment, drug delivery

Contents

1. Introduction

Cancer consists of more than 200 types with incomplete knowledge of the origin, tumorigenesis, and progression (1-3). According to the most recent statistics, approximately 19.3 million cases of new patients have been diagnosed with cancer, and a fatality of approximately 10 million (4). The majority of these massive numbers of deaths are caused by cancer metastasis (5). Cancer metastasis involves the process of tumor cells spreading from a primary tumor mass to different sites through blood and lymphatic vessels. It involves a series of events known as the cancer metastasis cascade (Fig. 1).

First, the epithelial cancer cells in primary tumors invade the extracellular matrix (ECM) and stromal cell layers within the site and enter into the lumina of the blood vessels. After that, cancer cells deal with several unfavorable conditions during their transport in the circulation until they arrive at specific organs. Next, cancer cells extravasate into the parenchyma of these organs, survive in these new microenvironments and initiate micrometastases. Finally, cancer cells restart their proliferative ability at the metastatic sites, thereby generating neoplastic growths known as 'metastatic colonization' (6).

During the last few decades, cumulative scientific discoveries that have been made in the field of molecular and cellular oncology have transformed clinical practice. For instance, the precise detection of cancer at an earlier stage and tailoring a therapeutic approach toward tumor type-specific intervention have been improved significantly (7). Molecular phenotypes associated with cancer metastasis have been extensively studied which has led to identifying potential metastasis-associated targets. The design of molecular-targeted therapies that specifically interact with those targets, whether they are components of a specific intracellular pathway or cell-cell communication signaling, have demonstrated potential benefits by overcoming systemic toxicities associated with traditional treatments such as chemotherapy and radiation therapy as well as improving the pharmacokinetics and pharmacodynamics of these traditional treatments (8,9).

One of the most recently developed molecular-targeted therapy is short sequences of single-stranded nucleic acids (ssDNA or RNA) or amino acids which are known as oligonucleotide or peptide aptamers, respectively (Fig. 2). Aptamers mimic antibodies in which they possess high selectivity when they bind with the selected targets. The first observation of the binding capability of aptamers was reported when researchers found that a subpopulation of isolated RNA molecules was able to bind specific ligands. An *in vitro* technique called SELEX (Systemic Evolution of Ligands by Exponential Enrichment) was introduced as a procedure to generate these oligonucleotide aptamers (10,11). The three-dimensional structure of a short sequence (20-100 bases long) RNA or ssDNA gives aptamers the capability to interact specifically with particular ligands with selectivity and affinity similar to those of antibodies. However, aptamers are more favorable compared to antibodies due to their cheap and rapid synthesis (12,13). A few years after oligonucleotide aptamers had been introduced, a research study demonstrated that designing a short peptide (5 to 20 amino acids long) then embedding it in a protein scaffold generated a protein with high specificity to a selected target. Therefore, a new concept launched for this type of aptamer is called peptide aptamer (14-16). To date, peptide and oligonucleotide aptamers have been developed and exploited for different diagnostic and therapeutic purposes. Aptamers have been employed for diagnostic applications in cancer detection and imaging (17) and infectious disease (18). For therapeutic purposes, aptamers have been utilized to treat different diseases such as cancer (17,19), infectious diseases (18,20), coagulation disorders (21), diabetic nephropathy (22) and ocular vascular diseases (23). This review aims to summarize recent advances in utilizing aptamers as a treatment strategy against cancer metastasis specifically and highlight key mediators and molecular factors involved during different metastasis stages. Moreover, potential opportunities to improve aptamer-based antimetastatic therapeutics are discussed. We used Web of Science and PubMed databases to retrieve the most recent information in this review.

2. Cancer metastasis cascade

Local invasion. Local invasion is the access of the cancer cells that were contained within the primary tumor into the surrounding stroma, and then into the adjoining normal parenchymal tissue. To enter the stroma, the carcinoma cells modify the ECM, which has a significant function in arranging epithelial tissues. The integrins (transmembrane proteins) bind to the ECM and form the integrin-mediated cell-matrix adhesions (24). This binding initiates several pathways leading to signal transduction events within the carcinoma cells that leads to disturbances in cell polarity, proliferation, invasiveness, and survival (25).

A majority of the carcinomas have the ability to invade as cohesive multicellular units known as a collective invasion. However, a single cancer cell may attack through two different mechanisms: integrin-dependent (mesenchymal invasion) and integrin-independent, Rho/ROCK-dependent (amoeboid invasion) pathways (26). It may be noted that the cancer cells may interconvert between these mechanisms due to the change in the microenvironment (27). While the patterns of cancer cell invasion are classified as collective and individual cell migration (28), the individual cell invasion mechanism is not compatible with an important element of epithelial tissue organization, specifically the E-cadherin-mediated intercellular junctions that lead to the development of the epithelial cell sheets and remain associated with the surrounding epithelial cells. To attenuate tight junctions and cellular polarity, carcinoma cells may undergo epithelial-mesenchymal transition (EMT). EMT is vital for different facets of normal embryonic morphogenesis which ultimately help to liberate cancer cells from epithelial cell sheets (29). Several transcription factors, namely Slug, Snail, Twist, zinc finger E-box-binding homeobox 1 (ZEB1), and ZEB2 play significant roles in EMT. They trigger mesenchymal entry by downregulating the expression of E-cadherin and other epithelial markers such as cytokeratin, zonula occludens-1 (ZO-1), laminin-1, and α1(IV) collagen (29). In addition, other regulatory non-coding genes, such as microRNAs (miRNAs/miRs), govern EMT. The miR-200 family regulates EMT programs by post-transcriptionally suppressing the expression of ZEB1 and ZEB2, while on the other hand, ZEB1 and ZEB2 inhibit the transcription of the miR-200 family. Such a relationship establishes a double-negative-feedback loop that operates as a bistable switch controlling the fate of cancer cells to go in either the mesenchymal or epithelial state (29). The debilitation of the ECM is further aggravated by active proteolysis activated by the matrix metalloproteinases (MMPs), which promotes the invasion of carcinoma cells to the stromal compartment. As the stroma becomes more chronically inflamed upon tumor progression, cancer cells are challenged by fibroblasts, endothelial cells, adipocytes, mesenchymal stem cells from the bone marrow, macrophages, and other immune cells (30). These stromal cells further influence the aggressiveness of

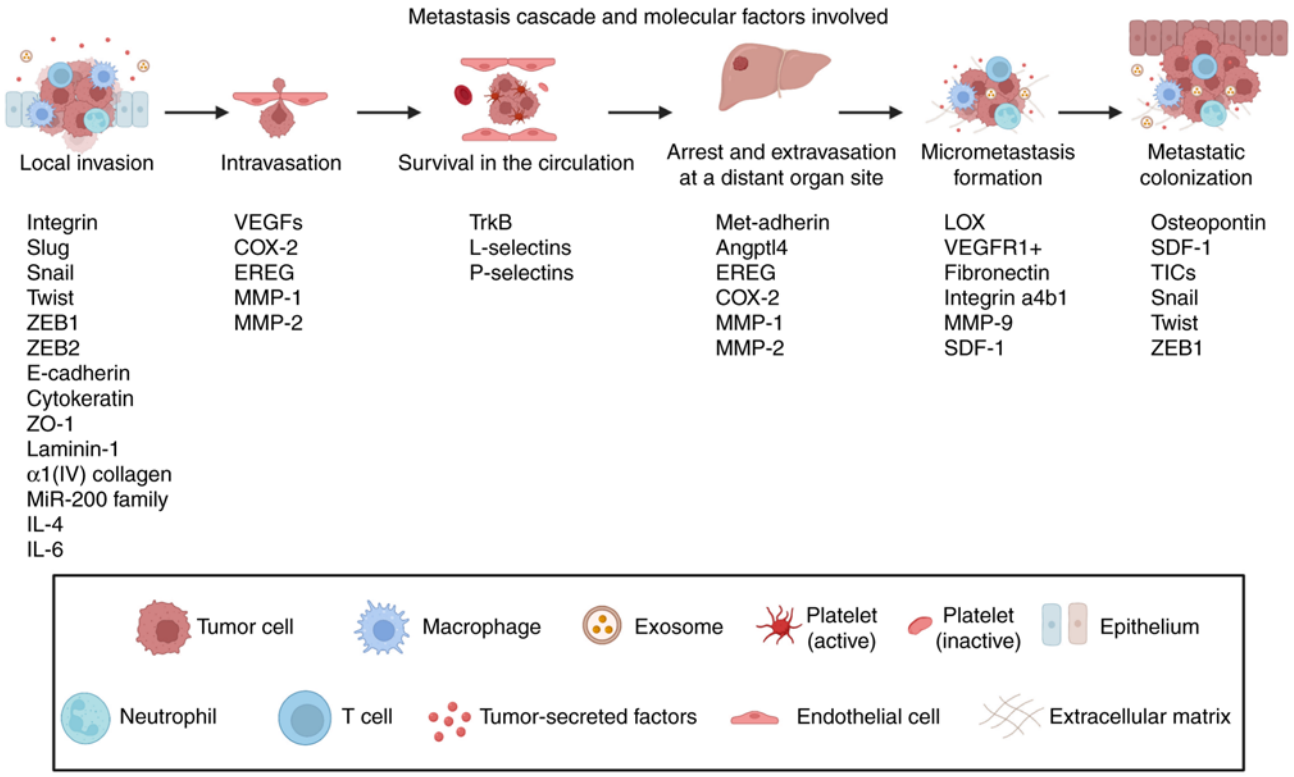

Figure 1. Metastasis cascade and molecular factors involved. Six stages of metastasis and molecular factors involved in the metastasis as discussed in the text. These stages are local invasion, intravasation, survival in the circulation, arrest and extravasation at a distant organ site, micrometastasis formation, and metastatic colonization. In addition, molecular factors that are involved during cancer metastasis cascade can be transcriptional factors, secreted proteins and cellular pathways mediators as discussed in the text. The figure was created with BioRender.com. ZEB1, zinc finger E-box-binding homeobox 1; ZEB2, zinc finger E-box-binding homeobox 2; ZO-1, zonula occludens-1; IL, interleukin; VEGFs, vascular endothelial growth factors; COX-2, cyclooxygenase-2; EREG, epiregulin; MMP, matrix metalloproteinase; TrkB, tropomyosin receptor kinase B; Angptl4, angiopoietin-like-4; LOX, lysyl oxidase; SDF-1, stromal cell-derived factor 1; TICs, tumor-initiating cells.

carcinoma cells via different types of heterotypic signaling. For example, the invasiveness of breast cancer is stimulated by IL-6 secreted by the adipocytes (31). Similarly, it has also been demonstrated that the cathepsin protease activity in tumor-associated macrophages is activated by the secretion of IL-4, which fuels the invasiveness of carcinoma cells (32).

Intravasation. Intravasation occurs when the carcinoma cells enter into the lumina of lymphatic or blood vessels. This process is normally observed in human tumors and signifies a vital prognostic marker for its progression; dissemination through blood vessels is the key mechanism for the spread of metastatic carcinoma cells (33). Intravasation is enhanced by molecular variations that increase the capacity of carcinoma cells to penetrate the microvessels that are composed of pericyte and endothelial cells. Intravasation is influenced by the vascular endothelial growth factors (VEGFs) secreted by the tumor cells which enhance the generation of new blood vessels within their local microenvironment through neoangiogenesis. In comparison to the normal blood vessels, the neovasculature developed by carcinoma cells is prone to leakiness and is subjected to continuous reconfiguration (34). Furthermore, it has been reported earlier that cyclooxygenase-2 (COX-2), epiregulin (EREG), MMP-1, and MMP-2 synergistically promote breast carcinoma intravasation due to their capacity to stimulate neoangiogenesis (35).

Survival in the circulation. After the intravasation has been achieved, the carcinoma cells are widely transported through the systemic circulation, known as circulating tumor cells (CTCs). Before reaching other organs, the CTCs deal with different types of stresses for its survival, such as the absence of the integrin-dependent adhesion to ECM components that is required for cell survival. Consequently, the epithelial cells undergo anoikis, which is a form of apoptosis that is activated by the loss of attachment to the substratum (36). However, the tyrosine kinase TrkB has been observed to suppress anoikis (37). Additionally, the tumor cells also face potential damage due to the hemodynamic shear forces and the innate immune system, specifically natural killer cells. These challenges have been observed to be evaded simultaneously through the formation of large emboli through the interactions with blood platelets (regulated by the tissue factor and/or L- and P-selectins by the carcinoma cells) (30). In this way, the platelet-coated tumor cells escape immune detection until they are arrested at distant organ sites.

Arrest and extravasation at a distant organ site. Despite the capacity of the CTCs to spread to a wide range of distant organ sites, it has been previously observed that specific carcinoma types metastasize to specific organs which leads to the proposal of the 'seed and soil hypothesis' (6). Seed and soil hypothesis suggests that cancer cells (seed) disseminated from the primary tumor spread to all organs, but only specific

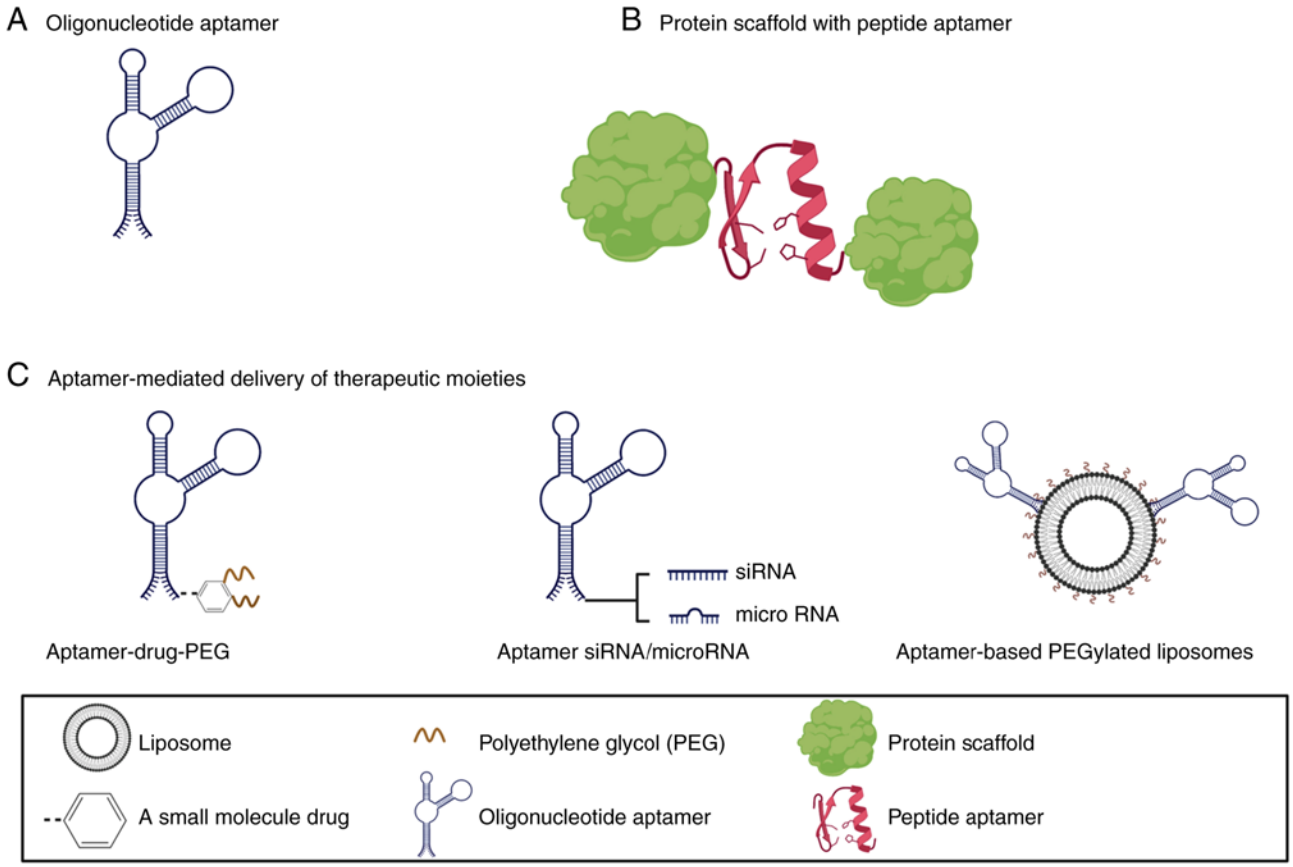

Figure 2. An overview of aptamer-based therapeutics. (A) Oligonucleotide aptamer. (B) Peptide aptamer. (C) Aptamer-mediated delivery of therapeutic moieties in which the aptamer is conjugated with a small-molecule drug and polyethylene glycol (PEG) (left), or conjugated with siRNA/microRNA (middle), or conjugated with PEGylated liposomes or any nanoparticle (right). The figure was created with BioRender.com.

microenvironments (fertile soil) that support metastatic tumor formation in specific organs. Once the cancer cells reach the suitable organs, the organ microenvironment supports the attachment and seeding of cancer cells in the specific organ. This is because some carcinoma cells depend on specific adhesive interactions in particular tissues that assist their trapping. For example, the generation of metadherin in breast cancer cells initiates the spread of carcinoma cells to the lungs through increased binding to the pulmonary vasculature (38). In addition, it has been observed that a proinflammatory environment in the liver causes Kupffer cells to secrete chemokines which upregulate vascular adhesion receptors and ultimately enable adhesion of the circulating colorectal and lung carcinoma cells to the liver microvasculature (39). Then, the trapped cancer cells grow inside the vasculature and develop a microcolony that ultimately penetrates through the surrounding vessels, resulting in the straight contact of the tumor cells with the tissue parenchyma (40). In another way, the carcinoma cells might rupture from vessel lumina into the tissue parenchyma by going through the endothelial cell and pericyte layers that separate the vessel lumina from the stromal microenvironment. This process is known as extravasation. Further, the physical barriers to extravasation may be breached due to the ability of the primary tumors to secrete factors that cause an imbalance in the microenvironments and induce vascular hyperpermeability. For example, the factors protein angiopoietin-like-4 (Angptl4), epiregulin (EREG), COX-2, MMP-1, and MMP-2, disrupt pulmonary vascular endothelial cell-cell junctions in order to facilitate the extravasation of breast carcinoma cells in the lungs (35,41).

Micrometastasis formation. The formation of micrometastases is initiated following the survival of the extravasated cancer cells in the parenchyma of distant tissues. It is worth mentioning that several factors contribute to this stage of cancer metastasis including the type of stromal cells, ECM constituents, growth factors, and cytokines. In the beginning, cancer cells establish a 'premetastatic niche' to ascertain the compatibility in the foreign microenvironment (42). For this to happen, the primary tumors liberate systemic signals consisting of lysyl oxidase (LOX) (43), that stimulates organ-specific upregulation of fibronectin from fibroblasts, which in turn, activates VEGF receptor 1-positive ($VEGFR1^+$) hematopoietic progenitor cells from the bone marrow to these prospective sites of metastasis through homing interactions between the deposited fibronectin and its cognate receptor, integrin a4b1that is expressed by the hematopoietic progenitor cells. The hematopoietic progenitor cells secrete MMP-9 that changes the immediate microenvironments at these loci. The MMP-9 activation triggers the release of various integrins and discharge of molecules from the ECM, such as the carcinoma cell chemoattractant stromal cell-derived factor 1 (SDF-1) (42). All of these changes convert the distant microenvironments into growth sites for the disseminated tumor cells.

Metastatic colonization. Even after the successful survival of the tumor cells in the new microenvironment, it is still not ensured that they can grow and form metastases, the process known as metastatic colonization. Instead, it has been observed that a large number of tumor cells either slowly perish over a period of time or are sustained as microcolonies during long-term dormancy, retaining the overall cell number (44). These dormant microcolonies may continue to remain dormant because of incompatibilities with the foreign microenvironments that surround them (44), such as in mammary carcinoma cells, where the focal adhesion kinase (FAK), integrin b1, and Src pathways are unable to engage within distant tissues (45-47). However, they may escape dormancy to initiate active proliferation cell-nonautonomous mechanisms that are stimulated by osteopontin (OPN) or SDF-1 (48,49). Secondly, the dormant microcolonies may proliferate continuously but the overall number may remain the same due to the high apoptotic rate. For example, the prostate tumor cell secretes prosaposin (Psap) that inhibits metastatic colonization by upregulating the anti-angiogenic factor thrombospondin-1 in stromal cells (50). Metastatic colonization is also dependent on another attribute known as 'tumor-initiating cells' (TICs), which possess such an extensive self-renewal capacity to achieve malignant growth. The entry into the TIC state is promoted by miRNAs and the EMT-promoting transcription factors, such as Snail, Twist, and ZEB1, as already discussed (51).

3. Aptamers as targeted therapeutics

Approaches of targeted therapy. Exploiting aptamers as a new generation of therapeutics has attracted the attention of the scientific community due to various advantages that are offered by aptamers. An individual aptamer possesses high specificity since it depends on its three-dimensional conformation to bind to its specific target. The aptamer molecule binds to the target through a hydrogen bond, electrostatic interaction, van der Waals, hydrophobic interactions, or stacking interactions (14,52). Moreover, aptamers can be chemically synthesized with flexible customization providing an opportunity to improve pharmacokinetics and meet a wide range of applications needed (53,54). They can bind diverse targets, ranging from small molecules, proteins to viruses and cells (55-58). From a therapeutic perspective, aptamers can be used to exert pharmacological action by themselves as agonists (target activation), antagonists (target inhibition), or to act as ligands for targeted delivery of therapeutics (12) (Fig. 2). Most of the developed aptamers fall into the former category. Pegaptanib (Macugen®) is the first FDA-approved aptamer and works by antagonizing the action of vascular endothelial growth factor (VEGF) (23). For targeted delivery purposes, aptamers can be constructed to deliver a wide range of diagnostic or therapeutic moieties such as fluorescent materials, radioisotopes, cytotoxic drugs, RNA oligonucleotides, and nanoparticles (59-63). In addition, a multifunctional, aptamer-based theranostic conjugate can be designed by coupling a diagnostic marker and a therapeutic moiety with the aptamer for simultaneous diagnosis and treatment (64-66).

Pharmacokinetic considerations when designing aptamers. Once an aptamer enters the body, it is susceptible to various factors that minimize or prevent its therapeutic action. It is vital to implement strategies to overcome obstacles and produce aptamers suitable for clinical settings. Stability in the circulation and tumor microenvironment, rapid excretion by the kidney, and delivery to intracellular targets affect the therapeutic actions of the aptamers. First, oligonucleotide aptamers can undergo enzymatic degradation by nucleases present in the circulation and tumor microenvironment. Strategies to produce nuclease-resistant aptamers include chemical modification of the structure by attaching a functional group to one of the following positions: 2'position of monosaccharide and 3' or 5'termini of the aptamer (67,68). Pegaptanib aptamer, an approved therapeutic aptamer against age-related macular degeneration, is an example of such a modification. Another strategy is synthesizing an RNA or DNA backbone composed of *L*-ribose or *L*-deoxyribose for RNA and ssDNA aptamers, respectively. Spiegelmer is the name used to refer to these types of aptamers. This strategy relies on the fact that nucleases degrade *D*-oligonucleotide while the *L*-oligonucleotide is resistant to degradation (68,69). A second challenge facing an aptamer inside the body is the rapid excretion of the aptamer by the kidneys. The low molecular weight of the aptamer is responsible for the short-time presence of an aptamer in the circulation. An effective approach to overcome this obstacle is by conjugating the aptamer with cholesterol or high molecular weight moieties, such as proteins and polyethylene glycol (70-72). Aptamer-PEG conjugate possesses an enhanced half-life in the circulation compared to the unconjugated form of the aptamer (72). Crossing the cell membrane is another obstacle that can prevent an aptamer from exerting its pharmacological action. While the majority of targeted aptamers can easily interact with targets that are present in the circulation or on the surface of the cancer cells, some aptamers face difficulties crossing the cell membrane to reach their intercellular target. To overcome this obstacle, several strategies have been explored. The first strategy is the use of cell-internalization SELEX to develop a therapeutic cell-internalizing aptamer which has the capability to bind its target on the cell membrane and subsequently internalize to exert its action (73-75). The second strategy relies on coupling the therapeutic aptamer to protein transduction domains (PTDs), also known as cell-penetrating peptide. PTDs can cause the internalization of the therapeutic aptamer (76-78). The third strategy depends on the cellular uptake of a vector containing the DNA sequence of the therapeutic aptamer which is subsequently expressed intracellularly. The expressed aptamer is called an intramer. After cellular uptake of the vector by the targeted cell, intramer expression takes place inside the cells which makes the intramer available to exert its action (79,80).

4. Targeting key mediators of metastasis by aptamer-based therapeutics

Targeting cancer stem cells (CSCs). A small population of cancer cells inside the tumor mass, known as cancer stem cells (CSCs), have been found to possess the capability of self-renewal and generation of cancer progeny cells (81). These cells defeat the process of anoikis, a type of programmed cell death that is triggered when the cells are removed from the surrounding ECM. In addition, research has found that

these CSCs are resistant to therapeutic drugs since there is an upregulation in the expression of the ATP binding cassette transporter which results in drug efflux. *In vitro* and *in vivo* studies show that CSCs play a role in the formation of metastatic nodules (81-83). For example, it has been reported that the pluripotent genes octamer-binding transcription factor 4 (*OCT4*) and *NANOG* force-expressed in lung adenocarcinoma (LAC) increase the tumorigenic and metastatic capability of the cells since they induce EMT through the Slug protein. In addition to the increase in the number of $CD133^+$ cells, the new cells have increased sphere-forming ability, drug resistance, and migration (84). In addition, $CD24^{-/low}$ breast cancer stem cells were found to have an increased capability for the formation of the tumor as compared to $CD24^+$ breast cancer cells. Several important pathways are engaged in the CSC self-renewal process, such as the Wnt/β-catenin and NOTCH pathways. Further, the stemness of CSCs is maintained through the hepatocyte growth factor (HGF), and the transcription factors, OCT4, NANOG, SOX2, and BMI-1 (85-89). OCT4 expression has been reported to induce dysplasia and expansion of progenitor cells in the intestines (90). Furthermore, SRY-box transcription factor 2 (SOX2) maintains the vital signaling cascades for tumorigenesis. BMI1 proto-oncogene, polycomb ring finger (BMI-1) has a vital role in the self-renewal of normal stem cells. It has been observed that the knockdown of BMI-1 expression in $CD133^+$ laryngeal cancer cells led to the restriction of cell growth, colonization, cell invasion *in vitro*, and tumorigenesis *in vivo* (91). It has been earlier demonstrated that the Y-box binding protein 1 could augment the stemness characteristics of human hepatoma cell lines (92,93). Moreover, the KRAS signal c-MYC axis supports the advancement of stem cell characteristics in pancreatic cancer (94).

The selective targeting capability of aptamers has been exploited to deliver cytotoxic drugs or nanoparticles carrying different types of gene therapy or small-molecule drugs to CSCs (Fig. 3). These therapeutic aptamers selectively target proteins overexpressed on the surface of the cell membrane of CSCs such as, but not limited to, human epidermal growth factor receptor 2 (HER2), CD133, CD44, CD20, and EpCAM receptors. In addition, CSC-targeted aptamers were developed against different types of cancer (95-100). A multifunctional nanoparticle decorated with RNA aptamer that can specifically target the HER2 receptor overexpressed on the surface of human breast cancer cells was examined *in vitro* and *in vivo*. The HER2 aptamer was able to deliver the nanoparticle carrying two different siRNAs against mediator complex subunit 1 (MED1) to HER2-overexpressing breast cancer cells and inhibit metastatic behavior of the cells *in vitro*. An *in vivo* study demonstrated that the targeted nanoparticle eliminated breast cancer metastatic nodules in the lung and significantly downregulated the expression levels of proteins involved in cancer metastasis such as c-Myc, MMP-9, Trefoil factor 1 (TFF-1), and cyclin D1. Most importantly, the examination of the stem cell population showed a significant decline and complete depletion of $CD44^+/CD24^{-/low}$ stem cells after monotherapy and combination therapy with tamoxifen, respectively (98). Moreover, RNA aptamers targeting CD133 receptors that present on the surface of breast cancer cell stem cells were used to deliver nanoparticles carrying anti-microRNA to inhibit microRNA-21. An *in vitro* study demonstrated selective nanoparticle uptake by breast cancer stem cells compared to a control cell line with a loss of metastatic capability of the stem cells (96). In addition, functional nanoparticles decorated with two different aptamers against CD44 and transmembrane glycoprotein mucin 1 antigen (MUC1) were able to simultaneously deliver doxorubicin to CSCs and cancer cells, respectively. Dual-aptamer targeting nanoparticles inhibited breast cancer metastasis to the lung in an animal model of breast cancer metastasis (100).

Targeting circulating tumor cells (CTCs). Following the progression of the disease in the primary organ, the cancer cells leave the organ, survive in the blood circulation, and metastasize in an organ-specific manner. This process was first reported by Stephen Paget (101). Research evidence has demonstrated the vital role of CTCs in the development of metastasis. Poor prognosis is associated with the increased numbers of CTCs in the circulation. This makes targeting CTCs a novel strategy to disrupt cancer metastasis cascade (102). Comprehensive knowledge established concerning molecular changes such as overexpression of adhesion molecules that support the attachment of CTCs to the tissues of the distant organs led scientists to develop therapeutics that block or prevent CTCs from attaching to the new organ. A clinical study examining the impact of using anti-EpCAM monoclonal antibodies, which target EpCAM, which is overexpressed in CTCs, on patients with metastatic colorectal carcinoma was conducted. The study demonstrated a significant prolongation of patient survival (103). Another treatment strategy that showed prolonged survival of an animal model of metastatic ovarian cancer involved utilizing nanotechnology and a magnetic field to isolate CTCs (104).

The development of aptamer-based therapeutics that target receptors that are involved in CTC attachment has shown effective prevention of cancer metastasis. DNA aptamer that displayed the capability to selectively target carcinoembryonic antigen (CEA), which plays a critical role in CTC adhesion and implantation, has been developed. Researchers mixed the anti-adhesive DNA aptamer with CEA-overexpressing colonic carcinoma cells before they injected these pretreated cells intraperitoneally in a mouse model. The *in vivo* study showed a significant decrease in the numbers and volumes of metastatic nodules compared to the control group (105,106). Another study developed a DNA aptamer against sialyl Lewis X (sLe^x), which helps cancer cells to bind endothelial-selectin (E-selectin) and metastasize to distant organs. *In vitro* study demonstrated that treating metastatic hepatocellular carcinoma cells (HepG2) with the DNA aptamer against sLe^x significantly inhibited adhesion and migration of HepG2 cells (107,108).

Targeting tumor-secreted extracellular vesicles. Extracellular vesicles (EVs) are cell-derived, cytosol-containing vesicles that are involved in cell communications with the surrounding environment. They have been categorized based on the size into three types: exosomes (30-100 nm diameter), microvesicles (MVs) (100-1000 nm diameter), and larger vesicles known as oncosomes (1-10 μm diameter) (109-112). It has been found that EVs play a critical role in supporting metastasis. Tumor-secreted EVs contain thousands of active

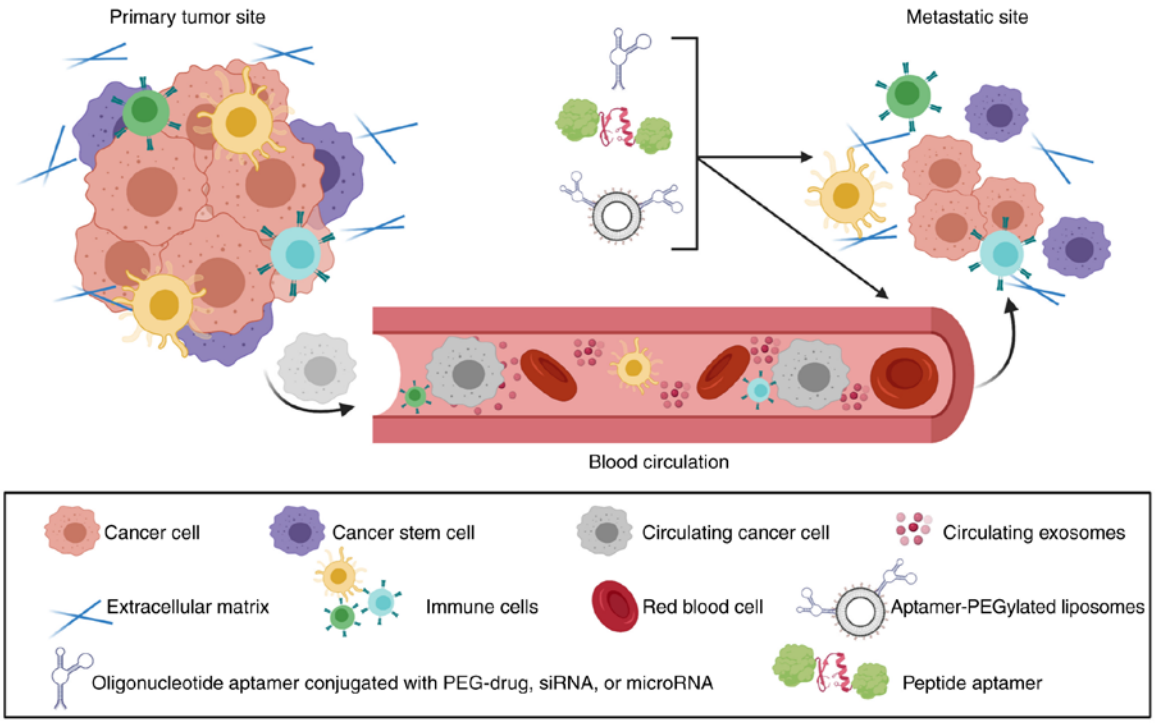

Figure 3. Aptamer-based antimetastatic therapeutics targeting key mediators of cancer metastasis. Antimetastatic aptamer-based therapeutics target key mediators of cancer metastasis such as cancer stem cells (CSCs), circulating tumor cells (CTCs), tumor-secreted extracellular vesicles and tumor-associated immune cells as discussed in the text. The figure was created with BioRender.com.

constituents such as lipids, proteins, and genetic materials for intercellular communication (113-116), which act as messengers in local and distant microenvironments (117-119). RNAs function as the chief bioactive factor of tumor cell-derived EVs, together with other non-coding RNAs namely microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (120-122). These non-coding RNAs delivered by exosomes to recipient cells can control the expression of many genes to support oncogenic reprogramming of malignant cells, tumor growth, local invasion, and premetastatic or metastatic niches formation (123-126). For example, it has also been reported that exosomes can destroy the blood-brain barrier by the action of miR-181c that increases central nervous system (CNS) metastasis (127). Furthermore, the breast cancer cell-derived exosomes were found to utilize miR-122 to create a metastatic niche in the brain and enhance disease progression by downregulating glucose uptake in non-tumor cells and rerouting the available nutrients for cancer cells themselves (128). Moreover, the exosomes also adversely affect the immune function leading to an immunosuppressed phenotype and facilitating tumor progression (129). It has been observed that the *c-Myc* mRNA in the recipient microglia and macrophages was decreased due to the uptake of glioblastoma-derived exosomes by microglia/macrophages that led to an immunosuppressive phenotype mediated by the transport of miR-21 and miR-451 (130). It is worth mentioning that EVs can be released from stroma cells and support tumor metastasis. For example, exosomes released by astrocytes were found to downregulate phosphatase and tensin homolog (*PTEN*), a tumor-suppressor gene, in brain tumor cells leading to elevated oncogenicity (131).

The growing body of evidence regarding the crucial role of EVs in the development of cancer metastasis has attracted some researchers to identify aptamers that target EVs and seek to develop aptamer-based therapeutics to treat cancer metastasis. Recently, a novel modification of SELEX technology, which has been called Exo-SELEX, has been established to identify aptamers that specifically bind cell-derived exosomes. Using the most aggressive subtypes of breast cancer, triple-negative and HER2$^+$, Exo-SELEX helped researchers to identify novel Ex-50.T aptamer. In addition, *in vitro* assessment of the therapeutic activity of EX-50.T was carried out using MDA-MB-231 and MCF-7 cell lines. The study relied on the fact that treating MCF-7 cells with exosomes derived from the highly metastatic MDA-MB-231 cells stimulates MCF-7 migration. The results demonstrated that MCF-7 cell migration was significantly inhibited when treating MCF-7 cells with exosomes that were pre-incubated with Ex-50.T (132). Another approach for targeting cancer cell-derived EVs is with the help of nanotechnology. Researchers have exploited the well-known fact regarding the liver uptake of mesoporous silica nanoparticles and the subsequent elimination of the nanoparticles into the small intestine to develop nanoparticles that specifically bind and eliminate circulating EVs from the blood. Mesoporous silica nanoparticles decorated with an aptamer that specifically target epidermal growth factor receptor (EGFR-targeting aptamer) that presents on the surface of EVs were developed. *In vivo* study to monitor the biodistribution of injected, labeled exosomes derived from metastatic lung cancer showed that the aptamer-targeted nanoparticles were able to significantly increase the accumulation of labeled exosomes to the liver and consecutively to the small intestine. In addition, aptamer-targeted nanoparticles inhibited the

pulmonary metastasis formation in a subcutaneous murine tumor model (133).

Targeting tumor-associated immune cells. Immune cells can play dual opposite roles in cancer. Immune cells can recognize and kill immunogenic cancer cells and inhibit tumor growth. Conversely, they can promote tumor growth by inducing the formation of new blood vessels, known as angiogenesis, and facilitating cancer metastasis (134-136). Intercellular communication between cancer cells, immune cells, and other stroma cells in the tumor microenvironment is responsible for this observed plasticity of immune cell function. Myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) recruited to the primary and secondary tumor sites contribute to the immunosuppressive environment. They are recruited in response to numerous growth factors, chemokines, and cytokines that are secreted by the cancer cells and other stromal cells (137-141). Through different mechanisms, MDSCs and Tregs negatively affect the antitumor activity of NK cells and prevent tumor infiltration of antitumor cytotoxic $CD8^+$ T cells (139,142). In addition, the types and levels of bioactive molecules and growth factors secreted in the tumor microenvironment and involved in signaling pathways can modulate the function of immune cells. For example, pro-inflammatory cytokines stimulate the antitumor activity of the immune cells while anti-inflammatory cytokines create an immunosuppressive environment that shifts the function of immune cells toward tissue repair and regeneration. Interleukin-4 (IL-4) and interleukin-13 (IL-13) can shift tumor-associated macrophages toward the alternatively activated M2 phenotype which promotes tumor growth and stimulates the metastatic behavior of cancer cells. Similarly, transforming growth factor-β (TGF-β) shifts tumor-associated neutrophils toward a protumor phenotype (141,143,144). The communication between cancer cells and the immune cells continues during different stages of the cancer metastasis cascade. During cancer cell invasion and intravasation, immune cells secrete enzymes, such as MMPs, that contribute to ECM remodeling. In addition, the newly developed blood vessels facilitate cancer cell spread to different organs. Receptor activator of nuclear factor-B ligand (RANKL) which is secreted by T regulator cells improves CTC survival during the circulation and contributes to the development of the pre-metastatic niche in the secondary site (145,146). More details regarding the role of immune cells in cancer metastasis are reviewed elsewhere (147,148).

The use of aptamer-based therapeutics to inhibit the recruitment and function of immunosuppressive cells and modulate the immune cell phenotype from a protumor to an antitumor phenotype are promising strategies by which to inhibit cancer metastasis. The development of aptamer-based therapeutics that prevent immunosuppressive cells from exerting their action has been exploited. The interleukin 4 receptor-α (IL4R-α) antagonist aptamer that targets IL4R-α-expressing M2 tumor-associated macrophages and myeloid-derived suppressor cells led to the elimination of immunosuppressive cells *in vivo*. Subsequently, the numbers of antitumor $CD8^+$ T cells were increased which suppressed the formation of pulmonary metastasis of 4T1 metastatic breast cancer cells (149). The utilization of aptamers to deliver therapeutics to tumor metastases also has been developed. Doxorubicin-loaded liposomes decorated with a T1 aptamer that target MDSCs showed significant depletion of MDSCs and increased intratumoral accumulation of cytotoxic T cells in animals with bone metastasis of MDA-MB-231 breast cancer cells (150). In addition, the 4-1BB aptamer was used to deliver a small non-coding antisense RNA (sasRNA) to Treg cells for transcriptional gene silencing of a key regulator of the immunosuppressive phenotype of Treg cells. Aptamer-sasRNA conjugate inhibited the Treg immunosuppressive phenotype *in vitro* and significantly improved coadministered antitumor vaccine against B16F10 metastatic melanoma cells that were grown subcutaneously *in vivo* (142,151). Similar findings have been reported after using an aptamer that targets cytotoxic T lymphocyte-associated antigen 4 (CTLA4) to deliver STAT3 siRNA to Treg cells. The CTLA4-STAT3 siRNA conjugate depleted Treg cells in the primary and metastatic sites and prevented pulmonary metastasis of B16 melanoma cells *in vivo* (19). In addition, aptamers that target and prevent the functions of anti-inflammatory cytokines or their receptors can inhibit immune cell polarization toward a protumor phenotype. Aptamers that target IL-6, IL-6R, IL-10R have been developed (152-155), yet their therapeutic activities against metastasis need to be examined.

5. Limitations and future perspectives

The development of aptamer-based anticancer therapeutics has been increasingly growing in the last few years. Encouraging findings introduce aptamer-based therapeutics as a potential class of agents in the treatment of primary tumors. However, cancer metastasis is the main cause for cancer-related deaths, thus preventing or treating cancer metastasis is pivotal to improving survival rates. Recently, extensive efforts have been directed toward developing and evaluating aptamer-based therapeutics against cancer metastasis. Table I documents examples of aptamer-based therapeutics that have been examined against cancer metastasis in preclinical and clinical settings and reported between 2019 and 2022 (133,156-161). Unfortunately, no aptamer-based therapeutic has been approved by the FDA to treat cancer metastasis thus far limiting the use of aptamer-based therapeutics in cancer metastasis treatment. It has been mentioned earlier in this review that some strategies had been introduced to overcome the challenges concerning aptamer pharmacokinetics such as enzymatic degradation, rapid excretion by the kidney, and poor delivery to intracellular targets. Nevertheless, other challenges still exist, and overcoming these challenges is crucial before aptamer-based technology can be approved for use against cancer metastasis. For example, immunological reactions resulting from aptamer administration have been observed. A randomized clinical trial evaluating the effect of REG1 anticoagulation system, which is an RNA aptamer-based inhibitor of the coagulation factor IXa, was terminated due to a severe allergic reaction (162). Research has demonstrated that allergic reactions observed with aptamer-based therapeutics result from factors such as the CpG-containing sequence and PEGylation (163-165). Understanding the underlying mechanisms by which the immunological reactions are triggered as well as finding the design elements contributing to

Table I. Examples of aptamer-based therapeutics that have been examined against cancer metastasis in preclinical and clinical settings and reported between 2019 and 2022.

A, Aptamer-based therapeutics with confirmed efficacy in animal models of cancer metastasis

Aptamer name	Aptamer type	Target	Function	Cancer type	Animal model	Cell line used	(Refs.)
TR4	RNA	Human transferrin receptor 1 (hTfR1)	Targeted delivery and internalization of C/EBPα-saRNA (C/EBPα-saRNA-TR4 or C/EBPα-saRNA-P19 conjugates)	Pancreatic ductal adenocarcinoma (PDAC)	Direct intrahepatic implantation of pancreatic cancer (liver-metastatic model of pancreatic cancer)	PANC-1	(156)
P19	RNA	PDAC cell specific					
Gint4.T	RNA	Platelet-derived growth factor receptor β (PDGFRβ)	Inhibition of ligand-dependent receptor activation and its downstream signaling	Triple-negative breast cancer	Spontaneous lung metastasis in orthotopic mouse model of TNBC	4T1	(157)
GL21.T	RNA	AXL, an oncogenic tyrosine kinase receptor	Inhibition of AXL downstream signaling and as targeted delivery of miR-148b (axl-miR-148b conjugate)	Breast cancer Melanoma	Spontaneous lung and liver metastases in orthotopic breast cancer or melanoma subcutaneous mouse models	4175-TGL/MA-2	(158)
AIA1	RNA	Metastasis phenotype	Invasion inhibition	Prostate cancer Osteosarcoma	Widespread metastases of prostate cancer using intracardiac injection/Spontaneous lung metastasis in orthotopic primary model of osteosarcoma	PC-3/82L	(159)
EpCAM aptamer	RNA	Epithelial cell adhesion molecule (EpCAM)	Targeted delivery of miR-203b-3p (EpCAM-Apt/miR-203b-3p)	Ovarian cancer	Ovarian peritoneal metastasis model	SK-OV-3 ES2	(160)
Anti-EGFR	DNA	Epidermal growth factor receptor (EGFR)	Helping nanoparticles for specific recognition of oncogenic exosomes and decreasing exosomes circulation	Lung cancer	Spontaneous lung metastases in subcutaneous mouse model of human lung cancer	A549	(133)
P42	Peptide	SRY (sex determining region Y)-box 2 (SOX2)	Inhibition of SOX2 function and its downstream targets	Esophageal squamous cell carcinoma (ESCC)	Zebrafish model of human esophageal squamous cell carcinoma metastasis	KYSE-450	(161)

Table I. Continued.

B, Aptamer-based therapeutics evaluated against cancer metastasis in clinical trials

Aptamer name	Aptamer type	Target	Phase	Status	Cancer type	ClinicalTrials.gov Identifier
Olaptesed (NOX-A12)	RNA	C-X-C motif chemokine 12 (CXCL12)	Phase I/II	Completed March 2020	Metastatic colorectal cancer Metastatic pancreatic cancer	NCT03168139

immunogenicity is important for the successful development of safe aptamers. Furthermore, it is extremely important to identify unwanted immunological reactions when using aptamers that target and modulate immune cells involved in cancer metastasis. In addition, information concerning the long-term consequences of targeting tumor-associated immune cells, and whether the impact can be extended to affect other cells in the immune system network are lacking. In addition, insufficient toxicity data and lack of appropriate regulatory guidelines for preclinical toxicity assessment studies are challenges that could restrain aptamers from being approved for treatment against cancer metastasis. Toxicity observed with other oligonucleotide-based therapeutics such as antisense oligonucleotides have raised a red flag about possible toxicity that could not be detected with current study designs when assessing the toxicity of oligonucleotide aptamers (166,167). Pegaptanib is the only FDA-approved aptamer-based therapeutic for the treatment of neovascular age-related macular degeneration. However, postmarketing surveillance of the adverse drug reactions of pegaptanib suggests a critical need for a long-term assessment for possible severe adverse drug reactions and toxicity (168). Moreover, a survey conducted by the Oligonucleotide Working Group of the European Federation of Pharmaceutical Industries and Associations (EFPIA) demonstrated a discrepancy in preclinical safety assessment studies performed for oligonucleotide-based therapeutics (169). Thus, establishing optimal toxicity assessment guidelines is critical for fully characterizing aptamer toxicity. Another limitation is the lengthy and complex production process which is an obstacle for large-scale production for clinical use. The SELEX process is carried out in multiple rounds in which each round consists of multiple steps: incubation of the target with a random library, removing the unbound sequences from the bound sequence, elution of the desirable bound sequence, and amplification of the desirable bound sequence (11). Moreover, a successful selection of an aptamer necessitates optimizing the experimental conditions such as temperature, pH, ionic strength, and ratio of the target to the random library which creates a complex process. Therefore, identification of strategies to improve the aptamer selection process will help facilitate the large-scale production of aptamer-based therapeutics.

Targeting key mediators of cancer metastasis using aptamer-based therapeutics is a promising strategy against metastasis; yet, researchers should solve concerns with the current research. First, exploring new targets and assessing the efficacy of aptamer-based therapy in relevant *in vivo* models are still limited. Enormous molecular targets and factors that regulate cancer metastasis have not been explored as potential targets for aptamer-based antimetastatic therapy. For example, miRNAs are RNAs with short, highly conserved, non-coding sequences. By binding to the 3'untranslated region of target mRNAs, miRNAs control gene expression at the posttranscriptional level. Recent findings demonstrate that miRNAs control metastasis by regulating metastasis-related genes in cancer stem cells and during the processes of EMT and metastatic colonization (170,171). Using aptamers to bind oncogenic precursor microRNAs to inhibit functional miRNA formation or deliver tumor suppressor miRNAs in the form of aptamer-miRNA conjugate

may show antitumor activity (172-175). Furthermore, circular RNAs (circRNAs), which range in length from a few hundred to thousands of nucleotides, is a type of RNA that has been recently re-recognized (176). Contrary to linear RNAs that have 5'caps and 3'tails, circRNAs are single-stranded with covalently closed circular transcripts (177). They were earlier known to be the products of mis-splicing or by-products of pre-mRNA processing with poor abundance, but now they have been recognized as a class of non-coding RNAs following the use of high-throughput RNA sequencing (RNA-seq) technologies. circRNAs are observed to be involved in the pathogenesis of cancer tumorigenesis, metastasis, and therapy resistance (178,179). These potential strategies have been examined *in vitro* or clinically irrelevant *in vivo* models (142,173,175). Thus, assessment of the efficacy of developed aptamer technology against newly identified targets in appropriate animal models of cancer metastasis is crucial to develop effective aptamer-based therapeutics.

6. Conclusion

In conclusion, this review summarizes recent advances in the development of aptamer-based antimetastatic therapeutics. Studies discussed here demonstrate that targeted aptamers possess a promising future in fighting cancer metastasis. Despite the potential findings in the field of molecular and cellular oncology, there are still opportunities to explore aptamer technology against potential targets. This could result in the development of aptamer-based antimetastatic therapy to target cancer metastasis. In addition, more research is needed to examine the existing antimetastatic aptamers in situations that mimic cancer metastasis inside the body.

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Competing interests

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