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REVIEW

Development of transferrin receptor aptamers as drug delivery vehicles for the treatment of brain metastases

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ABSTRACT

Affecting approximately up to 10–40% of all cancer patients, the prognosis for patients suffering from metastatic brain tumours is poor. Treatment of these metastatic tumours is greatly hindered by the presence of the blood brain barrier which restricts the overwhelming majority of small molecules from entering the brain. A novel approach to overcome this barrier is to target receptor mediated transport mechanisms present on the endothelial cell membranes, in particular the transferrin receptor. Given their specificity, safety profile and stability, nucleic acid-based therapeutics are ideal for this purpose. This review explores the development of bifunctional aptamers for the treatment of brain metastases.

KEYWORDS: Blood-brain-barrier, transferrin receptor, brain metastases, aptamers, bifunctional aptamer, drug delivery

BRAIN METASTASES: PREVALENCE, PROGNOSIS AND TREATMENT

Classified as the most frequent cancer in the central nervous system, brain metastases (BMs) are ten more common than primary brain tumours and are associated with an extremely poor prognosis. Studies investigating the incidence of brain metastases are lacking, with reported percentages of patients diagnosed varying from 10–40% and therefore an exact incidence in not known (Walbert et al, 2009; Fortin, 2012; Singh et al, 2014). This is further complicated by the fact that some patients remain undiagnosed due to being asymptomatic or the disease has progressed too far and diagnosis would not alter their care (Murrell et al, 2017). This is demonstrated by the frequency of brain metastases in autopsy studies being higher than those reported in population studies (Nayak et al, 2012). While advances in cancer treatment have led to an increase in progression free survival of primary malignancies, the battle for many cancer patients is not over following the release of recent epidemiological data which highlighted an increase in the incidence and prevalence of brain metastasis (Langley et al, 2013; Singh et al, 2014). While increasing the length of the patients' lives, these treatments in turn allow greater time for metastasis to occur. This increase in incidence may also be attributed to advances in imaging and diagnostic techniques (Nayak et al, 2012). Of all the primary tumour types, lung, breast, melanoma, renal and colorectal have the highest tendency to metastasize to the brain (Table 1) (Soffietti et al, 2012; Singh et al, 2014).

The burden of this disease is further exemplified by the fact that median survival time is merely measured in months (Singh et al, 2014). The main objective when treating BMs is to maximise the patient's survival and functional state, without compromising neurological status. To achieve this, treatment protocols are based around multiple factors, including age, health status, systemic disease burden, number of lesions and their location (Hardesty et al, 2016). Tak-

Table 1. Common primary sources of brain metastasis.

Primary source	Incidence of brain metastasis
Lung	40%–50%
Breast	15%–25%
Skin (melanoma)	6%–11%
Colorectal	3%

ing these factors into account, treatment generally revolves around a multi-modality strategy utilising a combination of treatment options, including surgical resection, whole brain radiation therapy, stereotactic radiotherapy and traditional chemotherapy. While each of these treatments possess limitations, systemic treatments such as chemotherapy have the poorest response rate, partly explained by the presence of the blood brain barrier (BBB), a highly restrictive barrier which limits the movement of 98% of small molecules from entering the brain and disrupting homeostasis (Gabathuler, 2010).

INFLUENCE OF THE BLOOD-BRAIN-BARRIER ON THE TREATMENT OF BRAIN METASTASES

The human brain requires a precise microenvironment to function optimally. This environment is provided and maintained through the defined function of the BBB. This highly impermeable barrier plays a key role in brain homeostasis by segregating the brain from the blood impeding the influx of blood-borne molecules (Ballabh et al, 2004; Abbott et al, 2010). Because of this restrictive barrier, the availability of systemic treatments for BMs is greatly reduced compared with the available treatments for extracranial cancers. However, it is important to consider that majority of brain macro-metastases (metastases greater than 1mm in diameter) show signs of BBB disruption, suggesting it may not be the factor hindering successful treatment with chemotherapy (Grossi et al, 2001; Preusser et al, 2018). This is evident from the fact that in some studies, BMs have demonstrated similar responses to systemic chemotherapy as other metastatic sites (Grossi et al, 2001; Edelman et al, 2010; Barlesi et al, 2011). However, the question then arises as to whether this barrier breakdown allows sufficient chemotherapeutic concentrations to enter the brain. Furthermore, this breakdown is only observed for macro-metastases, with barrier integrity still observed in the presence of micro-metastases (Grossi et al, 2001).

Structure and the function of the blood-brain-barrier

Present at all levels of the vascular tree, the BBB is composed of endothelial cells lining the brain vasculature coupled with surrounding astrocytes and pericytes (Figure 1). Forming the morphological basis of the BBB, brain endothelial cells are phenotypically dissimilar to those of the peripheral circulation (Bernacki et al, 2008). Brain endothelial cells are characterised by their highly polarised tight junctions connecting them to adjacent cells. This junction, in combination with minimal pinocytic vacuoles, limits

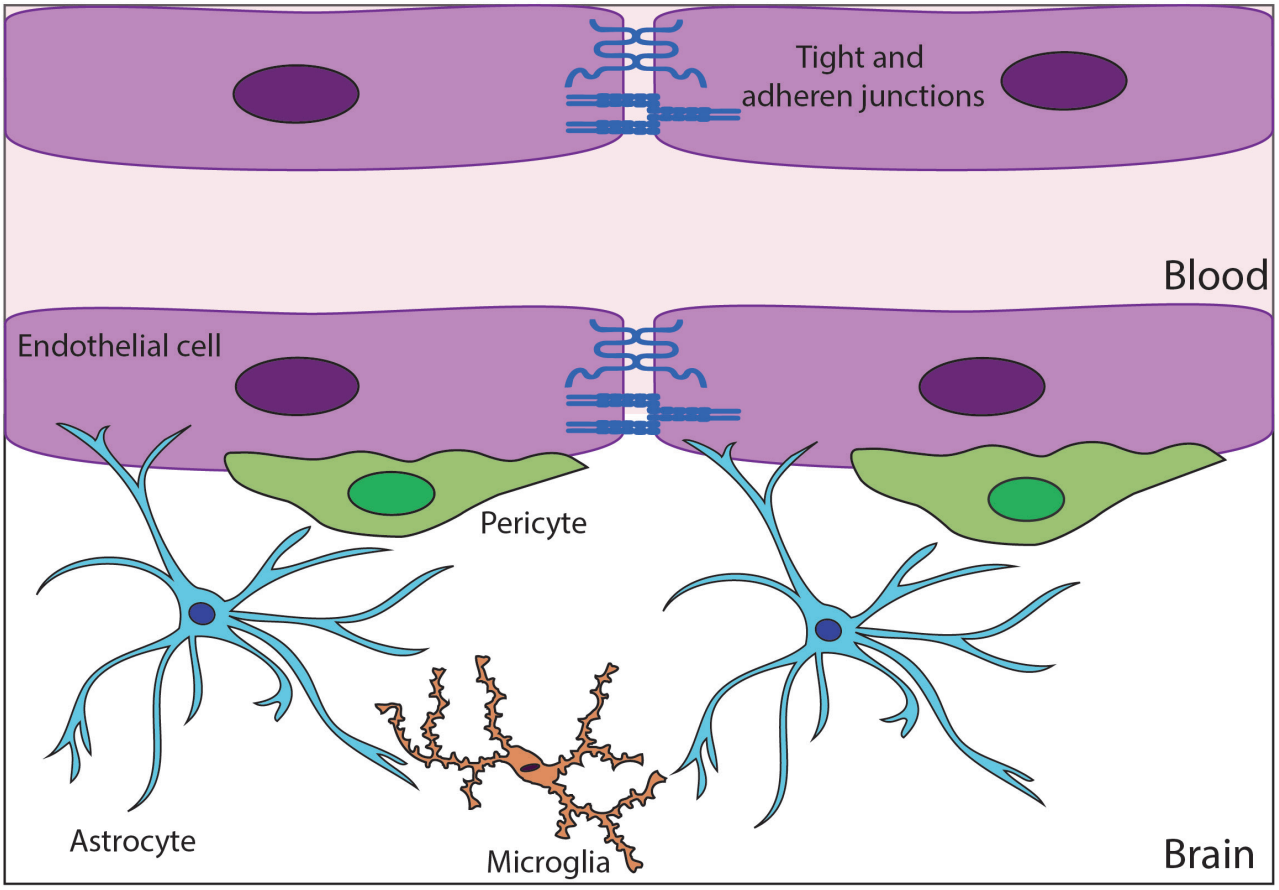

Figure 1. Cellular components of the BBB. Blood vessels of the brain are lines with polarised endothelial cells connected via tight and adheren junctions. On the abluminal side of the barrier these cells are lined by surrounding astrocytes and pericytes.

the free transport of molecules into the brain (Deeken et al, 2007; Daneman, 2012).

The junctional complexes present between adjacent endothelial cells of the brain microvessels are the most important factors responsible for the impermeable nature of the BBB (Bernacki et al, 2008). Two main types of junctional complexes are present between the endothelial cells, tight (TJ) and adheren junctions (AJ) (Figure 2), that together determine the overall paracellular permeability of the BBB. Composed of an intricate balance of cytoplasmic and transmembrane proteins linked to an actin cytoskeleton, the TJs of the BBB create a rate-limiting barrier to paracellular diffusion of solutes (Huber et al, 2001). Structurally, these junctional complexes form a series of multiple barriers being formed of a continuous network of intramembrane strands of parallel proteins (Figure 2) (Huber et al, 2001; Luissint et al, 2012). Three key integral proteins constitute the transmembrane component of these TJs, occludin, claudin and junctional adhesion molecules. Together, these proteins create the primary seal of TJs through the formation of dimers and connection to their counterpart on adjacent endothelial cells (Huber et al, 2001; Luissint et al, 2012). While these transmembrane proteins are essential for TJ composition and function, several additional cytoplasmic proteins are required for the full function of the complexes. Numerous accessory proteins are crucial in providing structural support for TJs. Of these, zonula occluden (ZO) proteins are of the most importance. Linked to the cytoplasmic C-terminal of both claudin and occludin, ZO proteins link these transmembrane proteins to an actin skeleton, providing structural and functional integrity to the endothelium (Abbott et al, 2010). While the TJs of the BBB limit the diffusion of solutes, AJs provide structural support, holding adjacent endothelial cells together (Figure 2). Spanning the intercellular cleft between endothelial cells, cadherin proteins bridge neighbouring endothelial plasma membranes via their homophilic interactions (Huber et al, 2001; Meng et al, 2009). Similar to TJs, cytoplasmic catenin proteins play an analogous role to ZO proteins, intracellularly linking cadherins to the cytoskeleton (Gloor et al, 2001; Huber et al, 2001).

TRANSPORT SYSTEMS OF THE BLOOD-BRAIN-BARRIER

The highly polarised nature of the BBB endothelial cells and the overall tightness of the structure, restricts the transport of molecules greater than 500 daltons into the brain (Daneman, 2012). This results in the prevention of an overwhelming majority of small molecules from crossing the BBB (Gabathuler, 2010). In addition to this, the few drugs capable of crossing the barriers are actively pumped back out by protein efflux transporters present on the endothelial cells membranes. The overall restrictive nature of this barrier tightly regulates the delivery of vital nutrients, minerals and molecules essential for the maintenance of brain homeostasis. Though the restrictive architecture of the

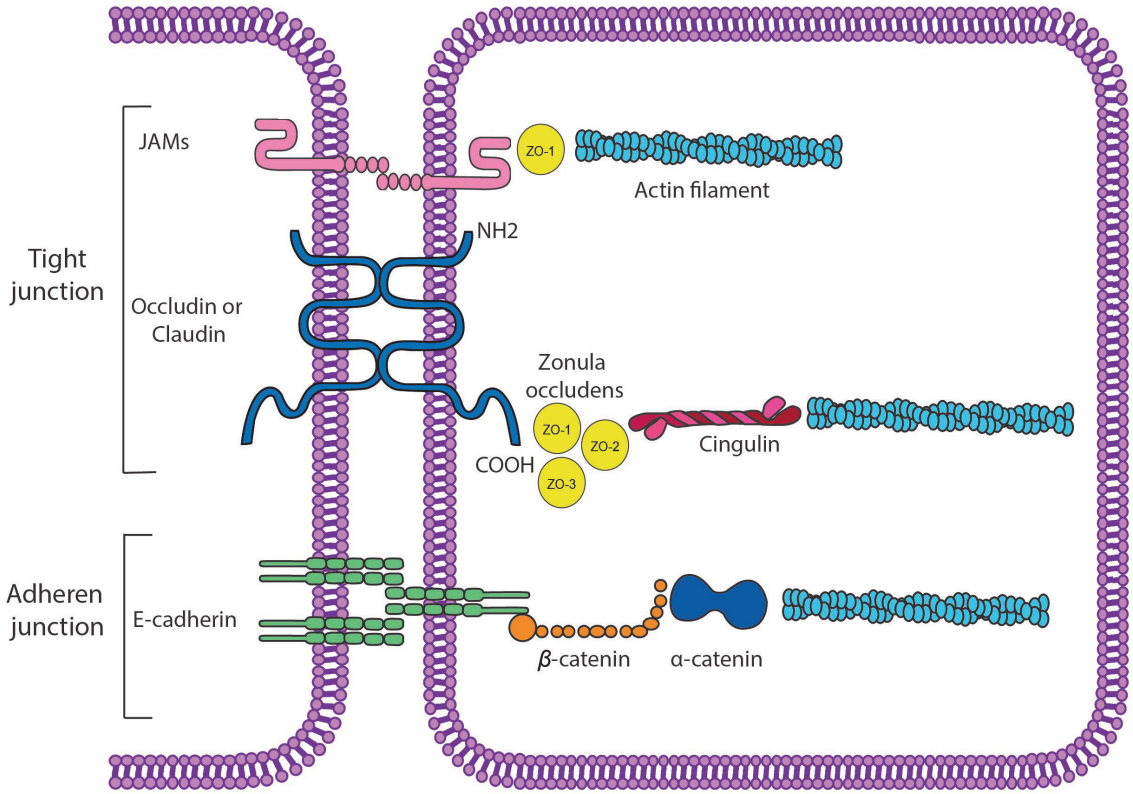

Figure 2. Junctional complexes of the BBB tight junctions. The BBB consists of a physical and physiological barrier whereby the physical component is comprised of TJs and AJs, which together restrict the movement of molecules from the blood to the brain. Generated through three integral transmembrane proteins, occludin, claudin and junctional adhesion molecules, interconnected with accessory proteins, TJs limit the paracellular diffusion of solutes. AJs provide structural support for the BBB through holding adjacent endothelial cells together.

BBB tightly regulates the influx of hydrophilic substances essential for the maintenance of brain homeostasis from the blood to the brain, the presence of carrier and receptor mediated transport systems (Figure 3) promotes the transportation of molecules essential for cerebral function (Svetlana et al, 2011).

ATP- binding cassette transporter efflux

The protective role of the BBB is further enhanced by ATP-binding cassette transporter efflux proteins. Present on the abluminal and luminal sides of the endothelial cells, these transporters have a dual role, firstly to protect the brain microenvironment from the influx of neurotoxins and secondly, restrict the access of therapeutics (Figure 3B) (Miller, 2010). Identified as playing a key role in the chemo-resistance of cancer cells, these efflux pumps have a similar effect in the endothelial cells of the BBB. Their presence has significant implications on the systemic treatment options available for patients suffering from BMs, as they efficiently remove drugs before a therapeutically relevant dose can be achieved. This is evident in the case of lipid-soluble drugs which would be expected to easily diffuse across the BBB but due to these transporters, have lower permeability than that predicted by their lipid solubility (Deeken et al, 2007). Numerous efflux transporters have been identified on the BBB endothelium, including MDR1, multidrug resistance proteins and OATP/OCTP organic anion and cation transporters (Deeken et al, 2007; Cioni et al, 2012).

Transport proteins

The highly restrictive nature of the BBB results in the majority of small polar molecules being incapable of diffusing into the brain (Figure 3C). These molecules include glucose, amino acids, nucleosides and many other molecules. Given the essential roles these molecules play in cell growth and metabolism, it is essential for them to be transported across the BBB. This is achieved through the presence of carrier mediated transport proteins present on both the luminal and abluminal membranes of the endothelial cells of the BBB. These proteins mediate the bi-directional movement of these molecules between the blood and the brain. Examples of these transporters, include the GLUT1 glucose transporters, LAT1 large neutral amino-acid transporters, CNT2 concentrative nucleotide adenosine transporters and OATP/OCTP organic cation and anion transporters.

Receptor mediated transcytosis

Transcytosis across the BBB via endocytic mechanisms is the main route by which large macromolecules enter the brain microenvironment (Figure 3D). In this mechanism, following ligand binding to the receptor on the apical membrane, the membrane invaginates the complex,

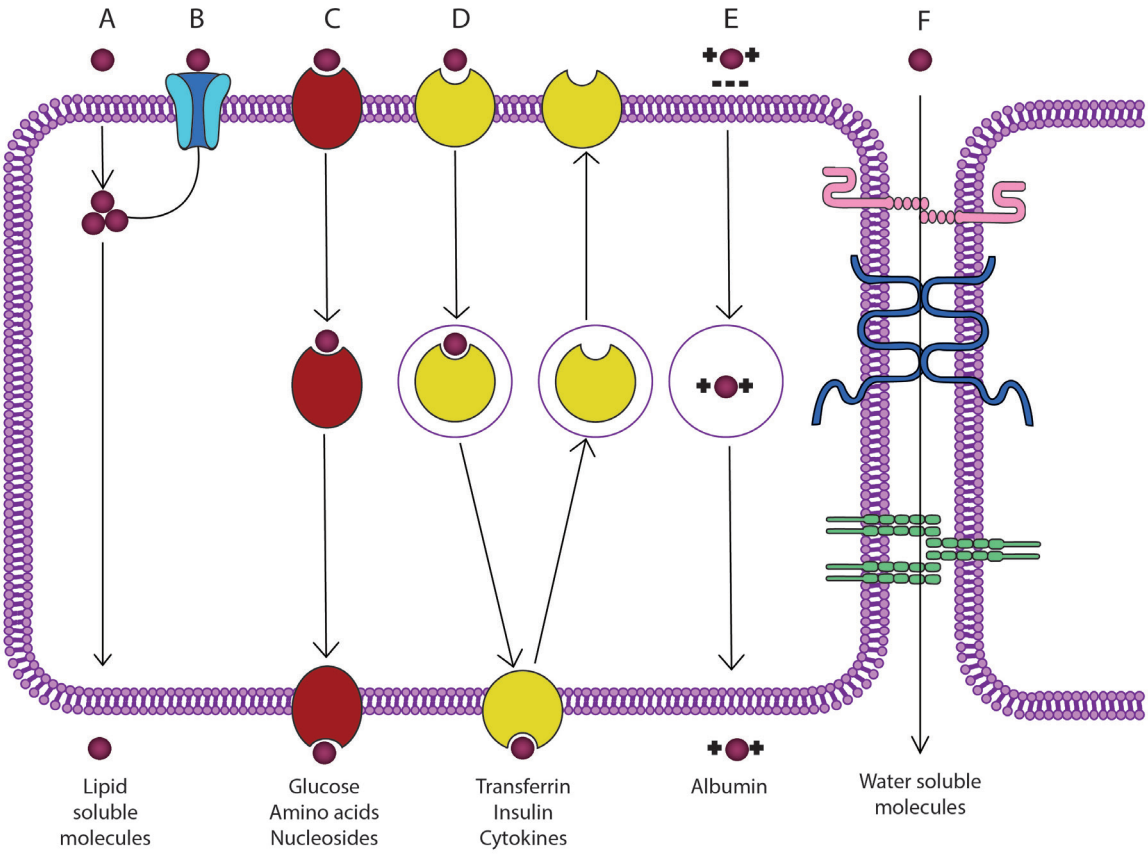

Figure 3. Mechanisms of transport across the BBB. A number of transport processes can be distinguished: Passive diffusion, a mechanism driven by a concentration gradient mainly involving small hydrophobic molecules. ATP- binding cassette Transporter efflux, transporters which play a critical role in preventing neurotoxic substances from entering the brain. Carrier mediated transport, transport mechanisms of many essential polar molecules. Receptor mediated transcytosis, requires receptor binding of ligand and can transport a variety of macromolecules Adsorptive transcytosis, a non-specific transport mechanism dependant on charge. Paracellular aqueous pathway, transport limited to water soluble molecules.

forming an intracellular vesicle, where it is shuttled to the basolateral membrane and the contents of the vesicle are released (Lajoie et al, 2015). An example of receptor-mediated transcytosis (RMT) operating in the BBB is the transport of iron loaded transferrin (Tf). Playing an essential role in mitochondrial energy generation, neurotransmission, oxygen transport, and cellular division, the transport of iron into the brain microenvironment is critical for normal brain function (Ponka et al, 1999; McCarthy et al, 2015). Upon association with the transferrin receptor (TfR) at the apical endothelial cell surface, the iron-bound Tf is internalised and following a drop in pH (pH 7.4 to 6.0), iron is released into the early endosome, where it is then released into the brain following the fusion of the vesicle with the basolateral membrane (Ponka et al, 1999; Georgieva et al, 2014).

Adsorptive mediated transcytosis

Adsorptive mediated transcytosis is the main route by which large positively charged macromolecules non-specifically enter the brain microenvironment (Figure 3E). This process is initiated by the adsorption of cationic molecules on the negatively charged domains on the apical surface of the endothelial cell membranes (Lajoie et al, 2015). This adsorption triggers the endocytosis process and similar to RMT, the membrane invaginates this complex forming an intracellular vesicle, where it is shuttled to the basolateral membrane and the contents of the vesicle are released (Lajoie et al, 2015).

METHODS TO OVERCOME THE BLOOD-BRAIN-BARRIER

Given the significant need to increase cytotoxic therapy accumulation in metastatic brain tumours, there are numerous strategies to enhance delivery which could be investigated. These approaches include hijacking receptor mediated transport mechanisms present on the BBB endothelium, bypassing the BBB through local delivery of therapeutic agents, the delivery of chemotherapeutics simultaneously with drug transport inhibitors and disruption of the barrier (Table 2).

Table 2. Summary of advantages and disadvantages of the methods to overcome the BBB

Method	Description	Advantages	Disadvantages
Direct Administration	Delivery in this manner is achieved through intrathecal delivery.	▪ Lower required dose ▪ Only the brain is exposed to indiscriminate cytotoxic agent	▪ Highly invasive method ▪ Non-targeted drug dispersion ▪ Ineffective volume of drug distribution ▪ Potential to cause significant neurotoxic and cognitive damage
Chemical barrier disruption	Injection of a vasoactive agent generates a temporary inflammatory reaction in the endothelial cells, disrupting the TJs	▪ Non-invasive ▪ Increases drug concentration capable of entering the brain	▪ Non-targeted drug dispersion through body and brain ▪ Potential for drugs to reach neurotoxic levels ▪ Influx of neurotoxic blood-borne molecules
Osmotic barrier disruption	Endothelial cells are exposed to hypertonic solution, causing them to shrink, placing significant stress on the TJs causing them to open.	▪ Non-invasive ▪ Increases drug concentration capable of entering the brain	▪ Non-targeted drug dispersion throughout the body and in the brain ▪ Negative effects on blood pressure and fluid balance ▪ Potential for cytotoxic agents to reach neurotoxic levels ▪ Global barrier disruption ▪ Influx of neurotoxic blood-borne molecules
Focused ultrasound disruption	Microbubbles are injected into patient and when they m pass through the ultrasound field directed at the tumour site, they oscillate at the same, causing them to expand and contract, disrupting the TJs.	▪ Non-invasive ▪ Barrier disruption occurs at the tumour site ▪ Increases concentration of chemotherapeutic capable of entering the brain	▪ Non-targeted drug dispersion throughout the body and in the brain ▪ Influx of neurotoxic blood-borne molecules
Hijacking transport mechanisms	Therapeutic modalities are substrates developed which are substrates for influx transporters present on endothelial cell membranes.	▪ Non-invasive ▪ BBB remains intact ▪ Increased drug delivery as a result of circumventing efflux capacity of the BBB	▪ Non-targeted drug dispersion in the brain

Hijacking transport mechanisms

A promising technique being developed which could be employed to deliver chemotherapeutics and treat BMs is the development of therapeutic modalities which are substrates for influx transporters present on endothelial cell membranes. Through designing drugs and transport modalities for these transporters they attain the ability to pass through the BBB without disruption, in addition to circumventing the efflux capacity of the BBB, ultimately increasing drug delivery to brain (Deeken et al, 2007).

Given the strong expression of the TfR on the BBB, numerous therapeutics have been generated in an attempt to improve the therapeutic treatment of Alzheimer's disease and glioblastoma (Xu et al, 2011; Yu et al, 2011). In 2011, Xu et al reported the anti-tumour effects of an anti-Tf monoclonal antibody on glioma cells *in vitro*, both in combination with a chemotherapeutic drug and alone (Xu et al, 2011). Interestingly, results from this study showed that the antibody alone had an anti-proliferative effect through induced S phase accumulation and apoptosis, and when used in combination with the chemotherapeutic the effect was further enhanced, which suggested that combination therapy was more effective (Xu et al, 2011). In the same year, Yu et al reported the development of a bi-specific antibody capable of exploiting the TfR and targeting an enzyme associated with Alzheimer's disease (Yu et al, 2011). The reduction in amyloid-β production following antibody administration, demonstrates that, through targeting the TfR pathway, a therapeutically relevant concentration of antibody can be delivered across the BBB in an *in vivo* model (Yu et al, 2011). An important observation arose from this report in regards to antibody affinity toward the TfR. It was discovered that the antibody required a lower binding affinity (~100nM), in order for it to be released from the TfR upon internalization, an important observation for future research and development of this method (Yu et al, 2011). Recently, the ability of other antibody formats, such as single-chain fragment variables (scFv), to transcytose the BBB have been investigated (Chandramohan et al, 2013; Wang et al, 2014; Bao et al, 2016; Kim et al, 2018). Through employing a scFv of an anti-human TfR monoclonal antibody Kim et al. were able to develop BBB crossing nucleic acid encapsulating liposomes with the ability of modulating neuronal gene expression and apoptosis (Kim et al, 2018). Numerous fragments have also shown therapeutic potential for targeting glioblastoma *in vivo*, however results thus far have only been generated using highly invasive delivery techniques, including convection enhanced delivery and intracerebral injection (Chandramohan et al, 2013; Wang et al, 2014; Bao et al, 2016). Given the promise behind this delivery technique, it may be a suitable method for the treatment of BMs. However, given the severe side effects and immunological risk antibodies pose and the invasive delivery methods used thus far for scFv's a novel therapeutic strategy is required.

APTAMERS AS A TARGETED NOVEL DRUG DELIVERY METHOD FOR THE TREATMENT OF BRAIN METASTASES

Nucleic acid-based aptamers are an emerging field of novel therapeutics which have the potential to be developed for the treatment of BMs. Aptamer development began in 1990, following the ground breaking development of the polymerase chain reaction, which had a key influence on molecular biology (Mayer, 2009). This development involved the influence of three independent research groups, who each contributed independently by documenting the isolation of a single stranded nucleic acid with pre-defined functions (Ellington et al, 1990; Robertson et al, 1990). Following their discovery of RNA molecules that bound to an organic dye, Ellington and Szostak defined the molecules as aptamers, a word chimera built from the Latin 'aptus' (to fit) and the Greek 'meros' (part) (Ellington et al, 1990; Mayer, 2009). Often referred to as chemical antibodies, aptamers are chemically synthesised molecules that bind to their target through shape recognition, like antibodies.

Systematic Evolution of Ligands by Exponential Enrichment

The chemical generation of aptamers, a process known as the systematic evolution of ligands by exponential enrichment (SELEX), is a well-established technology, which generates oligonucleotides with the highest possible target affinity (Figure 4). A combinatorial chemistry technique of screening, SELEX involves iterative rounds of partition and amplification of a random large library of oligonucleotides containing 10^{14}–10^{16} candidates (Qu et al, 2017). Initially, the target ligand is incubated with the large random library, followed by the removal of non-binding and low-binding sequences, and then the bound sequences are eluted and amplified using PCR to be used in subsequent rounds of selection (Tuerk et al, 1990; Stoltenburg et al, 2007). Subsequent to the first few rounds of positive selection, negative selection steps are introduced into the cycle to eliminate non-targeted sequences. After the final round of selection, the generated aptamers are sequenced and characterised. Based on this technology, aptamers can be developed to bind to a number of different classes of targets, from single molecules to complex targets such as whole cells or organisms (Stoltenburg et al, 2007). Indeed, the ability to generate cell-specific aptamers through employing whole live cells as targets, could be employed for the treatment of brain metastases. Through targeting metastatic tumour cells rather than primary tumour cells, the phenotypic traits of the metastatic cells could be exploited to increase selectivity. While the selection process is characterised by repetition of the aforementioned steps, there is no standardised SELEX protocol, and the overall design of the selection conditions depends on a number of factors, mainly the library, the target, desired features, and aptamer application (Stoltenburg et al, 2007).

Advantages and limitations of aptamers

As aptamers function by molecular recognition, they can be developed for therapeutic applications with the same intended function as antibodies, such as drug delivery vehicles. Though analogous to their protein counterparts in regards to target recognition and application, aptamers possess numerous key advantages over antibodies and antibody fragments, the main being their size, production process and cost, stability and nucleic acid structure (Keefe et al, 2010). The significantly smaller size of aptamers to antibodies (6–10kDa vs 150kDa) allows for

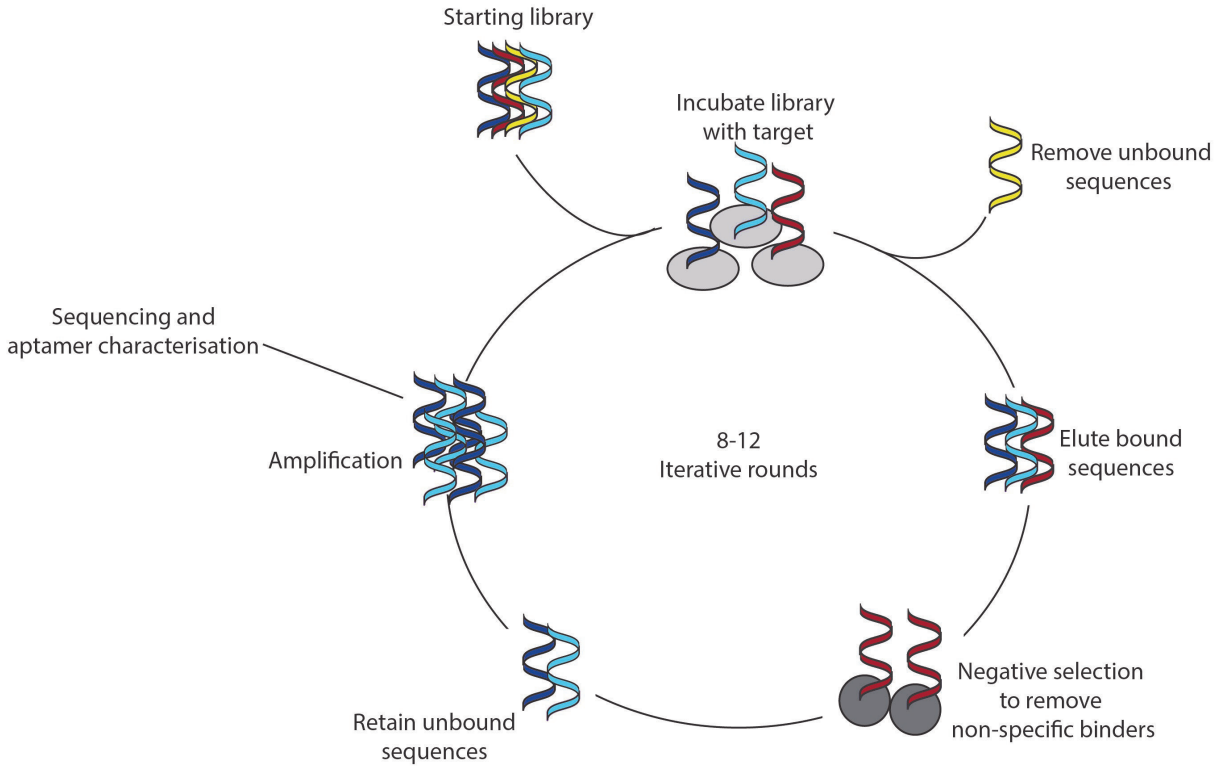

Figure 4. The Systematic Evolution of Ligands by Exponential Enrichment (SELEX). Initially a desired target ligand is exposed to a random pool of chemically synthesised oligonucleotides. Low and non-binding species are removed while the binding species are eluted and amplified using PCR subsequent to the next selection round. Prior to incubation with the target throughout selection, sequences are subjected to a negative selection step to eliminate non-specific sequences from the pool. Following the completion of the final round of SELEX aptamers can then be sequenced and characterised.

greater tissue penetration and permits their access to biological compartments inaccessible to antibodies (Zhou et al, 2012). However, given the comparable size of antibody fragments (27kDa), similar tissue penetration patterns would be observed (Razpotnik et al, 2017). Small size may also be viewed as a limitation as it restricts circulatory half-life due to filtration through the reticuloendothelial system, which has a cut-off threshold of 30–50kDa (Keefe et al, 2010). To address this issue, aptamers and antibody fragments can be conjugated with bulky molecules, such as polyethylene glycol or cholesterol, to increase their size (Keefe et al, 2010; Razpotnik et al, 2017). The chemical generation of aptamers guarantees consistent production and is relatively inexpensive in comparison to the laborious and inconsistent *in vitro* or *in vivo* production of antibodies and antibody fragments (Zhou et al, 2017). The nucleic acid composition of the aptamers offers significant advantage over the protein nature of antibodies in regards to structural stability. While antibodies are irreversibly denatured when exposed to varying physical conditions such as temperature and pH, aptamers are insensitive to these changes, retaining the ability to return to their original confirmations following exposure (Adler et al, 2008). However, in regards to serum stability and *in vivo* half-life, this composition can be a disadvantage, as both unmodified RNA and DNA are highly susceptible to nuclease degradation. Numerous modifications can be introduced to address this, majority of which concern the sugar component of the nucleic acid, as the 2'-position of it is the natural site of nucleophilic attack (Mayer, 2009). These modifications include the incorporation of fluoro, amino, alkyl and thio groups at this position and the inclusion of locked and unlocked nucleic acids (Ni et al, 2017). Issues arise when introducing these modifications along with the polymers, as introduction Post-SELEX has the potential to influence binding affinity and selectivity. Therefore, following modification, it is essential to reassess these characteristics. While it is thought that aptamers lack immunogenicity given their nucleic acid nature, they are synthetic and comprised of CpG motifs and other immunostimulatory sequences and can therefore potentially activate the innate immune system *in vivo* (Avci-Adali et al, 2013). Depending on the intended application of the aptamer, this would need to be assessed prior to *in vivo* application.

Applications of aptamers in the treatment of brain metastases

Given their similarity to antibodies, aptamers can be employed in a range of pharmacological areas, including applications such as drug discovery, diagnostics, molecular imaging, drug delivery vehicles and as protein inhibitors or modulators. At present, only one aptamer, Macugen, has been approved by the US Food and Drug Administration (FDA) while several aptamers are currently in clinical trials (Ruckman et al, 1998; Bell et al, 1999; Sundaram et al, 2013; Yu et al, 2016b). Approved by the FDA in 2004 Macugen is used for the treatment of macular degeneration, functioning through the inhibition of an isoform of vascular endothelial growth factor, thus ultimately inhibiting angiogenesis (Ruckman et al, 1998; Bell et al, 1999).

The ability to generate aptamers against cell surface markers which are internalised via receptor mediated endocytosis makes them an excellent platform for specific drug delivery. Currently, cytotoxic agents employed to treat malignancies are indiscriminate and non-specific, meaning they not only kill cancer cells but also healthy cells, leading to severe and dose-limiting side effects (Wang et al, 2012). In addition to this, the therapeutic efficiencies of these agents decreases throughout the treatment course as a result of chemo acquired resistance (Porciani et al, 2015). Doxorubicin (DOX) is one of the most widely employed chemotherapeutic agents for the treatment of several cancers, including lung and breast cancer, both which have a high incidence of metastasising to the brain (Table 1). The anticancer effect of DOX is the result of its ability to intercalate into DNA, disrupting replication and transcription (Thorn et al, 2011; Yang et al, 2014). As aptamers form tertiary conformations with short double stranded non-binding nucleic acid regions, they can be exploited to intercalate DOX, resulting in the development of a specific drug delivery vehicle (Famulok et al, 2007; Keefe et al, 2010; Zhou et al, 2017). This was first demonstrated in 2006 by Bagalkot et al who reported the specific delivery of DOX to prostate cancer cells utilising an aptamer which targets prostate specific membrane antigen expressed on prostate cancer cells (Bagalkot et al, 2006). Since then, the development of aptamer DOX conjugates have been extensively reported (Bagalkot et al, 2006; Huang et al, 2009; Subramanian et al, 2012; Xu et al, 2013; Porciani et al, 2015; Yu et al, 2016a; Xiang et al, 2017). The overexpression of the membrane glycoprotein epithelial cell adhesion molecule (EpCAM) on a number of solid cancers with a high incidence of metastasising to the brain, makes it a highly attractive target for the treatment of BMs (Shigdar et al, 2011; Soysal et al, 2013). In 2017, Xiang et al reported the development of an aptamer-DOX conjugate targeting EpCAM (Xiang et al, 2017). Through modifying the original EpCAM aptamer to increase the length of the double stranded region, they were able to intercalate 2–3 molecules of DOX per aptamer (Xiang et al, 2017). Treatment of tumour bearing xenograft mice with the aptamer-DOX conjugates compared to DOX alone, resulted in a 3-fold inhibition of tumour growth and a significantly longer survival time (Xiang et al, 2017). As DOX is intercalated into the aptamer structure, this delivery method is only effective for chemotherapeutics with a similar mechanism of action.

Given their nucleic acid structure, aptamers can easily be modified with linkers to allow attachment of drugs not capable of direct intercalation. The covalent conjugation of these drugs indirectly to the aptamer via chemical linkers makes them unlikely to influence specificity and sensitivity. Through the direct conjugation of methotrexate, a drug used to treat acute myeloid leukaemia, to a DNA aptamer targeting CD117 via an amine coupling reaction, Zhao et al were able to demonstrate a significantly increased cytotoxic effect compared to methotrexate alone (Zhao et al, 2015). More recently, through enzymatic or chemical conjugation, Yoon et al conjugated gemcitabine and 5-fluorouracil to the pancreatic cancer RNA aptamer P19 (Yoon et al, 2017). Within this study the aptamer-drug conjugates were shown to be internalised and induce DNA damage, even in a gemcitabine resistant cell line (Yoon et al, 2017). While these aptamer-drug conjugates do not target cancers with a high incidence of metastasising to the brain, or have the ability to transcytose the BBB, the drugs described can easily be attached to aptamers which do.

Over the past decade nanoparticles have been developed as anti-cancer drug delivery vehicles given their high loading drug capacity. While alone these vehicles lack specific targeting, once combined with an active targeting mechanism, such as aptamers, highly specific drug delivery vehicles are developed. This has recently been explored through the functionalisation of drug encapsulating biodegradable polymeric nanoparticles for glioblastoma targeting (Monaco et al, 2017). Platelet derived growth factor receptor β (PDGFRβ) is a highly attractive target for glioblastoma treatment given its expression in different glioblastoma subtypes and high expression on endothelial cells of the BBB (Kim et al, 2012). Through conjugating an aptamer targeting PDGFRβ with the drug encapsulating nanoparticles, Monaco et al were able to develop an active targeting mechanism capable of crossing the BBB via receptor mediated transcytosis and targeting glioblastoma cells (Monaco et al, 2017). A similar drug delivery vehicle to this could be developed for the treatment of BMs.

TRANSFERRIN RECEPTOR APTAMERS WITH THE POTENTIAL OF CROSSING THE BLOOD-BRAIN-BARRIER

Ubiquitously expressed in normal cells at low levels and more highly expressed (approximately 100-fold up regulation) in cells with a high proliferation rate and on the endothelium of the BBB, the TfR has become an ideal target for cancer diagnosis and treatment (Daniels et al, 2012). Two methods exist in which this receptor can be targeted and influence cancer cell progression. The first involves blocking the natural function of the receptor, iron homeostasis, which consequently leads to cancer cell death (Daniels et al, 2012). The second is the use of the receptor as a ferrying system to deliver therapeutic molecules into cancerous cells (Figure 3D) (Wilner et al, 2012). Tf, the natural ligand of this receptor has been widely employed as a transport vector for this method (Elliott et al, 1988; Kratz et al, 1998; Singh et al, 1998). The near saturation of the TfR from endogenous Tf in physiological conditions limits the applicability of Tf as a transport vehicle, as in order to ensure adequate delivery of the therapeutic payloads exceedingly high levels would be required (Porciani et al, 2014). Aptamers are a promising alternative to overcome this limitation given their ability to target different sites on the TfR and the possibility of generating them with higher affinities towards TfR than natural Tf (Porciani et al, 2014).

In 2008, Chen et al reported the development of DNA and RNA aptamers that selectively recognise the extracellular domain of the mouse TfR (Chen et al, 2008). Originally 64 nucleotides long, Chen and colleagues truncated the selected DNA aptamer, GS24, to 50 nucleotides while still maintaining sensitivity and selectivity (Chen et al, 2008). Using the same aptamer, Porciani et al found that through mutating the aptamer sequence, affinity towards the mouse TfR was increased and the aptamer was capable of binding human TfR albeit with a lower affinity (Porciani

et al, 2014). During the development of new medicinal therapies, a fundamental problem which arises and hinders progression from bench top to clinical trials is the efficacy of the therapy within *in vivo* animal models (White et al, 2001). Therefore, because the TfR aptamer generated by Porciani and colleagues cross reacts between mouse and human, it is highly attractive for further functionalisation and to be developed for therapeutic application. While being cross reactive, this aptamer was selected using unmodified nucleotides, making it highly susceptible to nuclease degradation (Chen et al, 2008). In 2012, Wilner at al. reported the generation of nuclease stabilised aptamers which target the human TfR and are readily internalised by human cell lines (Wilner et al, 2012).

DEVELOPING A BIFUNCTIONAL APTAMER TO TARGET BRAIN METASTASES

The concept of developing aptamers for the treatment of brain disorders is not new. There have been numerous reports of aptamers generated for the targeted treatment of specific brain diseases such as glioblastoma and Alzheimer's disease (Ylera et al, 2002; Tannenberg et al, 2013; Aptekar et al, 2015; Esposito et al, 2016). While these aptamers have been shown to be highly specific for their target and demonstrated efficient cellular uptake, only one is capable of crossing the BBB alone as a result of its target, PDGFRβ, being overexpressed on the BBB and glioblastoma cells (Esposito et al, 2016). To overcome this restrictive barrier, through numerous rounds of *in vivo* selection, Cheng and colleagues generated an RNA brain penetrating aptamer known as A15 (Cheng et al, 2013). Through *in vitro* and *in vivo* characterisation, it was confirmed that the A15 aptamer possessed the ability to enter brain endothelial cells under physiological conditions and in addition to this, could enter the brain parenchyma (Cheng et al, 2013). While the ability of this aptamer to enter the brain parenchyma is noteworthy, the aptamer has purely been generated to cross into the brain. Further functionalisation, such as drug attachment, or the addition of a second targeting mechanism, is required for this aptamer to be developed into an effective therapeutic.

The therapeutic potential of mono-specific nucleic acid aptamers can be further enhanced through the production of bifunctional aptamers. Developed by fusing two aptamer binding sequences, these aptamers are designed by two different pathways. The first entails the fusion of two aptamers with independent binding activities. Generation in this manner broadens the limited recognition capability of mono-functional aptamers, a highly attractive property for cancer therapeutics (Zhu et al, 2012). In 2011, Min et al reported the development of a bifunctional aptamer based DOX delivery vehicle (Min et al, 2011). Through the conjugation of an RNA aptamer targeting PSMA positive cells, with a peptide aptamer specific for PSMA negative cells, DOX was synchronously delivered to two types of prostate cancer cells and resultantly induced cell cytotoxicity, addressing the underlying problem of solid tumour heterogeneity (Min et al, 2011). Similarly, the following year a bifunctional aptamer targeting leukaemia subtypes, sgc8c-sgda5a aptamer, was developed which elicited bi-specific cytotoxicity (Zhu et al, 2012). The second method involves the joining of two aptamer binding sequences in which the binding of the first aptamer influences the binding of the second (Le et al, 2013). Synthesis in this manner creates the possibility of delivering drug payloads to sites within the body which may not be accessible to drug alone due to restrictive transport mechanisms. However, as mentioned previously, modification of an aptamers sequence can negatively impact binding properties and therefore, some aptamers may be less tolerant to fusion with another sequence. Therefore re-characterisation following the formation of bifunctional aptamers is essential.

Given the highly restrictive nature of the BBB, this method could be utilised to generate a bifunctional aptamer, which targets the TfR to transport chemotherapeutic agents across the barrier and specifically deliver them to BMs by targeting markers expressed on the cancer cell surface membrane. This method has been explored through the truncation and fusion of two aptamer sequences, one targeting the TfR and the other targeting EpCAM (Figure 5) (Soysal et al, 2013; Macdonald et al, 2017). Fusion of the truncated sequences resulted in the generation of an aptamer which demonstrated specificity and sensitivity to both intended targets (Macdonald et al, 2017). Given the ubiquitous expression of TfR throughout the body, the ability of the aptamer to transcytose the BBB and its distribution in non-targeted tissues was investigated (Macdonald et al, 2017). From this, the ability of the aptamer to transcytose the BBB was confirmed, with a percentage of injected dose 12 fold higher than the control sequence recorded in the brain 30minutes after administration (Macdonald et al, 2017). As expected, aptamer accumulation in highly perfused organs, such as the liver and spleen, 30minutes after administration was high (Macdonald et al, 2017). Compared to this, retention at 60minutes was markedly lower, indicating the measured levels at 30minutes are not likely the result of aptamer binding, but perfusion levels (Macdonald et al, 2017). When considering these results, it is important to note the fact that they were measured in a healthy animal model, meaning the aptamer was targeting TfR on the BBB as well throughout the body. Further experimental work in a disease model needs to be conducted to gain an accurate representation of bio-distribution. Using the same model, aptamer distribution within the brain itself needs to be investigated, given TfR expression in healthy neuronal cells (Moos et al, 2000). The results thus far highlight the potential this aptamer has to be developed as an effective modality for overcoming the BBB, opening a new window for targeted drug delivery to the brain.

Given the ability to intercalate anthracycline chemotherapeutics in aptamers structure, the bifunctional aptamer has the potential to be developed as a drug delivery vehicle without modification. The conjugation of a chemotherapeutic drug to the bifunctional aptamer has numerous benefits, to both the patient and healthcare system. Firstly, the required concentration of the drug could be reduced, benefiting both the patient and healthcare system. Secondly, intercalation into the aptamers structure, would give them the ability to circumvent the BBB, a task alone they are incapable of. Furthermore, the effective targeting of the aptamer to tumour cells and subsequent internalisation via endocytosis would reduce the indiscriminate side effects

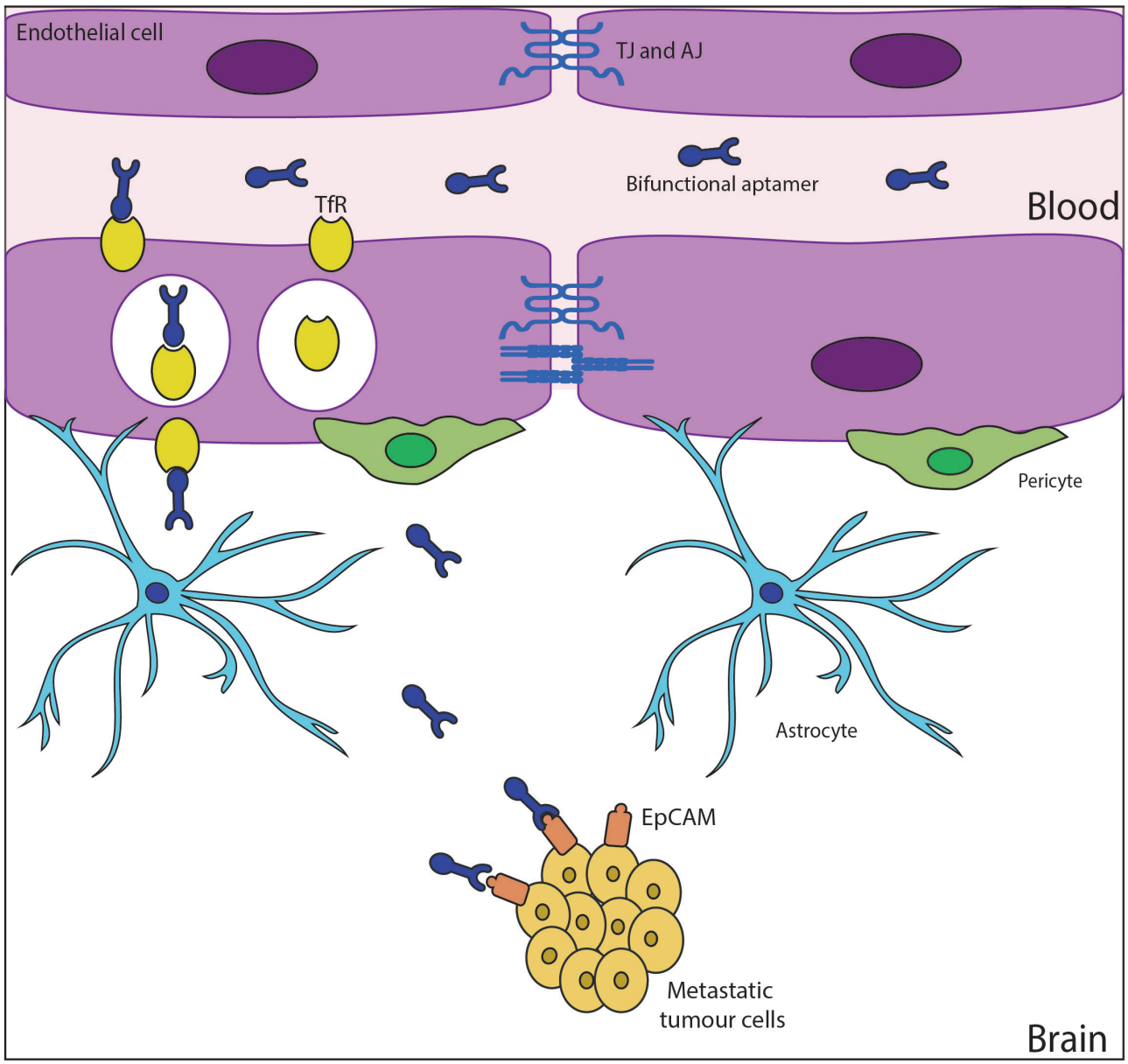

Figure 5. Schematic representation of the bifunctional aptamer mechanism. While in systemic circulation the aptamer binds to the TfR on the apical membrane where it is then invaginated into an intracellular vesicle. The aptamer is then transcytosed through the endothelial cell and released into the brain microenvironment where it targets EpCAM expressed on the metastatic tumour cells. The TfR is then recycled back to the apical membrane.

of the agent and increase drug concentration. While there is the potential for side effects in non-targeted tissues, compared to the effects experienced by patients following current treatment regimens, the extent of these would be considerably reduced.

CONCLUSIONS

While the landscape of cancer treatment has drastically changed over the last few decades, improving primary malignancy survival rates, the incidence of BMs is still increasing. This is the result of treatment advances increasing the length of patient's lives, which in turn allows a greater period of time for metastasis to occur. Treatment of these metastatic malignancies is complicated by the presence of the BBB, which isolates the brain microenvironment from systemic circulation by strictly regulating the passage of molecules. This presents a problem as practically all large molecules and 98% of small molecules are prevented from crossing the BBB, necessitating the need for more effective therapeutics (Gabathuler, 2010). Numerous strategies have been developed for circumventing the barrier, such as direct injection or barrier disruption to increase permeability, but each strategy poses great risk to the patient (Debinski et al, 2009; Lassaletta et al, 2009; Wu et al, 2014).

A promising technique being developed to reduce these risks and overcome the BBB is the development of therapeutics modalities which are substrates for influx transporters present on endothelial cell membranes. Given their specificity, safety profile and stability, nucleic acid based therapeutics are ideal for this purpose. The development of

a targeted delivery system capable of crossing the BBB and specifically delivering chemotherapeutic agents to BMs has the potential to significantly improve patient survival and quality of life.

Furthermore, through specially targeting the cancerous cells and sparing the healthy brain tissue, this system will reduce the concentration of drug required for treatment, further reducing the associated side effects and additionally reducing healthcare costs for the patient and the public healthcare system. In conclusion, the development of bifunctional aptamers for the treatment of BMs shows great promise, however, further investigation is required prior to clinical translation.

COMPETING INTERESTS

None declared.

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ABBREVIATIONS

BBB: Blood **B**rain **B**arrier
AJ: Adheren **J**unction
BMs: Brain **M**etastases
DOX: Doxorubicin
EpCAM: Epithelial **C**ell **A**dhesion **M**olecule
FDA: Food and **D**rug **A**dministration
PDGFRβ: Platelet **D**erived **G**rowth **F**actor **R**eceptor
RMT: Receptor **M**ediated **T**ranscytosis
scFV: single-**c**hain **F**ragment **V**ariables
SELEX: Systematic **E**volution of **L**igands by **EX**ponential enrichment
Tf: Transferrin
TfR: Transferrin **R**eceptor
TJ: Tight **J**unction
ZO: Zonula **O**ccluden

REFERENCES

Abbott NJ, Patabendige AAK, Dolman DEM, et al. 2010. Structure and function of the blood-brain barrier. Neurobiol Dis, 37, 13–25.

Adler A, Forster N, Homann M, et al. 2008. Post-selex chemical optimization of a trypanosome-specific RNA aptamer. Comb Chem High Throughput Screen, 11, 16–23.

Aptekar S, Arora M, Lawrence CL, et al. 2015. Selective targeting to glioma with nucleic acid aptamers. PLoS ONE, 10, e0134957.

Avci-Adali M, Steinle H, Michel T, et al. 2013. Potential capacity of aptamers to trigger immune activation in human blood. PLoS ONE, 8, e68810.

Bagalkot V, Farokhzad OC, Langer R, et al. 2006. An aptamer-doxorubicin physical conjugate as a novel targeted drug-delivery platform. Angew Chem, 45, 8149–8152.

Ballabh P, Braun A and Nedergaard M. 2004. The blood-brain barrier: An overview: Structure, regulation, and clinical implications. Neurobiol Dis, 16, 1–13.

Bao X, Pastan I, Bigner DD, et al. 2016. EGFR/EGFRVIII-targeted immunotoxin therapy for the treatment of glioblastomas via convection-enhanced delivery. Receptors Clin Investig, 3, e11430.

Barlesi F, Gervais R, Lena H, et al. 2011. Pemetrexed and cisplatin as first-line chemotherapy for advanced non-small-cell lung cancer (NSCLC) with asymptomatic inoperable brain metastases: A multicenter phase II trial (gfpc 07–01). Ann Oncol, 22, 2466–2470.

Bell C, Lynam E, Landfair DJ, et al. 1999. Oligonucleotide NX1838 inhibits VEGF165-mediated cellular responses in vitro. In Vitro Cell Dev Biol Anim, 35, 533–542.

Bernacki J, Dobrowolska A, Nierwińska K, et al. 2008. Physiology and pharmacological role of the blood-brain barrier. Pharmacol Rep, 60, 600–622.

Chandramohan V, Bao X, Kato Kaneko M, et al. 2013. Recombinant anti-podoplanin (nz-1) immunotoxin for the treatment of malignant brain tumors. Int J Cancer, 132, 2339–2348.

Chen CH, Dellamaggiore KR, Ouellette CP, et al. 2008. Aptamer-based endocytosis of a lysosomal enzyme. Proceedings of the National Academy of Sciences of the United States, 105, 15908–15913.

Cheng C, Chen YH, Lennox KA, et al. 2013. In vivo SELEX for identification of brain-penetrating aptamers. Mol Ther- Nucl Acids, 2, e67.

Cioni C, Turlizzi E, Zanelli U, et al. 2012. Expression of tight junction and drug efflux transporter proteins in an in vitro model of human blood-brain barrier. Front Psychiatry, 3, 8.

Daneman R. 2012. The blood-brain barrier in health and disease. Ann Neurol, 72, 648–672.

Daniels TR, Bernabeu E, Rodríguez JA, et al. 2012. The transferrin receptor and the targeted delivery of therapeutic agents against cancer. Biochimica Biophysica Acta, 1820, 291–317.

Debinski W and Tatter S. 2009. Convection-enhanced delivery for the treatment of brain tumors. Expert Rev Neurother, 9, 1519–1527.

Deeken JF and Loscher W. 2007. The blood-brain barrier and cancer: Transporters, treatment, and trojan horses. Clin Cancer Res, 13, 1663–1674.

Edelman MJ, Belani CP, Socinski MA, et al. 2010. Outcomes associated with brain metastases in a three-arm phase III trial of gemcitabine-containing regimens versus paclitaxel plus carboplatin for advanced non-small cell lung cancer. J Thorac Oncol, 5, 110–116.

Ellington A and Szostak J. 1990. In vitro selection of RNA molecules that bind specific ligands. Nature, 346, 818–822.

Elliott RL, Stjernholm R and Elliott MC. 1988. Preliminary evaluation of platinum transferrin (MPTC-63) as a potential nontoxic treatment for breast cancer. Cancer Detect Prev, 12, 469–480.

Esposito CL, Nuzzo S, Kumar SA, et al. 2016. A combined microRNA-based targeted therapeutic approach to eradicate glioblastoma stem-like cells. J. Controlled Release, 238, 43–57.

Famulok M, Hartig JS and Mayer G. 2007. Functional aptamers and aptazymes in biotechnology, diagnostics, and therapy. Chem Rev, 107, 3715–3743.

Fortin D. 2012. The blood-brain barrier: Its' influence in the treatment of brain tumors metastases. Curr Cancer Drug Targets, 12, 247–259.

Gabathuler R. 2010. Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases. Neurobiol Dis, 37, 48–57.

Georgieva JV, Hoekstra D and Zuhorn IS. 2014. Smuggling drugs into the brain: An overview of ligands targeting transcytosis for drug delivery across the blood-brain barrier. Pharmaceutics, 6, 557–583.

Gloor SM, Wachtel M, Bolliger MF, et al. 2001. Molecular and cellular permeability control at the blood–brain barrier. Brain Res Brain Res Rev, 36, 258–264.

Grossi F, Scolaro T, Tixi L, et al. 2001. The role of systemic chemotherapy in the treatment of brain metastases from small-cell lung cancer. Crit Rev Oncol Hematol, 37, 61–67.

Hardesty DA and Nakaji P. 2016. The current and future treatment of brain metastases. Front Surg, 3, 30.

Huang YF, Shangguan D, Liu H, et al. 2009. Molecular assembly of an aptamer-drug conjugate for targeted drug delivery to tumor cells. ChemBioChem, 10, 862–868.

Huber JD, Egleton RD and Davis TP. 2001. Molecular physiology and pathophysiology of tight junctions in the blood–brain barrier. Trends Neurosci, 24, 719–725.
Keefe AD, Pai S and Ellington A. 2010. Aptamers as therapeutics. Nat Rev Drug Discov, 9, 537–550.
Kim S-S, Rait A, Garrido-Sanabria ER, et al. 2018. Nanotherapeutics for gene modulation that prevents apoptosis in the brain and fatal neuroinflammation. Mol Ther, 26, 84–94.
Kim Y, Kim E, Wu Q, et al. 2012. Platelet-derived growth factor receptors differentially inform intertumoral and intratumoral heterogeneity. Genes and Development, 26, 1247–1262.
Kratz F, Beyer U, Roth T, et al. 1998. Transferrin conjugates of doxorubicin: Synthesis, characterization, cellular uptake, and in vitro efficacy. J Pharm Sci, 87, 338–346.
Lajoie JM and Shusta EV. 2015. Targeting receptor-mediated transport for delivery of biologics across the blood-brain barrier. Annu Rev Pharmacol Toxicol, 55, 613–631.
Langley RR and Fidler IJ. 2013. The biology of brain metastasis. Clin Chem, 59, 180–189.
Lassaletta A, Lopez-Ibor B, Mateos E, et al. 2009. Intrathecal liposomal cytarabine in children under 4 years with malignant brain tumors. J Neuro-Oncol, 95, 65–69.
Le TT, Scott S and Cass AE. 2013. Streptavidin binding bifunctional aptamers and their interaction with low molecular weight ligands. Analytica chimica acta, 761, 143–148.
Luissint A-C, Artus C, Glacial F, et al. 2012. Tight junctions at the blood brain barrier: Physiological architecture and disease-associated dysregulation. Fluids Barriers CNS, 9, 23–23.
Macdonald J, Henri J, Goodman L, et al. 2017. Development of a bifunctional aptamer targeting the transferrin receptor and epithelial cell adhesion molecule (EpCAM) for the treatment of brain cancer metastases. ACS Chem Neurosci, 8, 777–784.
Mayer G. 2009. The chemical biology of aptamers. Angewandte Chemie 48, 2672–2689.
McCarthy RC and Kosman DJ. 2015. Iron transport across the blood-brain barrier: Development, neurovascular regulation and cerebral amyloid angiopathy. Cell Mol Life Sci, 72, 709–727.
Meng WX and Takeichi M. 2009. Adherens junction: Molecular architecture and regulation. Cold Spring Harb Perspect Biol., 1, 1:a002899.
Miller DS. 2010. Regulation of p-glycoprotein and other ABC drug transporters at the blood–brain barrier. Trends Pharmacol Sci, 31, 246–254.
Min K, Jo H, Song K, et al. 2011. Dual-aptamer-based delivery vehicle of doxorubicin to both PSMA (+) and PSMA (-) prostate cancers. Biomaterials, 32, 2124–2132.
Monaco I, Camorani S, Colecchia D, et al. 2017. Aptamer functionalization of nanosystems for glioblastoma targeting through the blood-brain barrier. J Med Chem, 60, 4510–4516.
Moos T and Morgan EH. 2000. Transferrin and transferrin receptor function in brain barrier systems. Cell Mol Neurobiol, 20, 77–95.
Murrell DH, Perera F, Chambers AF, et al. 2017. Brain metastasis: Basic biology, clinical management, and insight from experimental model systems In: A Ahmad (ed.), *Introduction to cancer metastasis*, 1 edn, Academic Press, pp. 317–333.
Nayak L, Lee EQ and Wen PY. 2012. Epidemiology of brain metastases. Curr Oncol Rep, 14, 48–54.
Ni S, Yao H, Wang L, et al. 2017. Chemical modifications of nucleic acid aptamers for therapeutic purposes. Int J Mol Med, 18, 1683.
Ponka P and Lok CN. 1999. The transferrin receptor: Role in health and disease. Int J Biochem Cell B, 31, 1111–1137.
Porciani D, Signore G, Marchetti L, et al. 2014. Two interconvertible folds modulate the activity of a DNA aptamer against transferrin receptor. Mol Ther- Nucl Acids, 3, e144.
Porciani D, Tedeschi L, Marchetti L, et al. 2015. Aptamer-mediated codelivery of doxorubicin and nf-[kappa]b decoy enhances chemosensitivity of pancreatic tumor cells. Mol Ther- Nucl Acids, 4, e235.
Preusser M, Winkler F, Valiente M, et al. 2018. Recent advances in the biology and treatment of brain metastases of non-small cell lung cancer: Summary of a multidisciplinary roundtable discussion. ESMO Open, 3, 1–13.
Qu J, Yu S, Zheng Y, et al. 2017. Aptamer and its applications in neurodegenerative diseases. Cell Mole Life Sci, 74, 683–695.
Razpotnik R, Novak N, Curin Serbec V, et al. 2017. Targeting malignant brain tumors with antibodies. Front Immunol, 8, 1181.
Robertson DL and Joyce GF. 1990. Selection in vitro of an RNA enzyme that specifically cleaves single-stranded DNA. Nature, 344, 467–468.
Ruckman J, Green LS, Beeson J, et al. 1998. 2'-fluoropyrimidine RNA-based aptamers to the 165-amino acid form of vascular endothelial growth factor (VEGF165). Inhibition of receptor binding and VEGF-induced vascular permeability through interactions requiring the exon 7-encoded domain. J Biol Chem, 273, 20556–20567.
Shigdar S, Lin J, Yu Y, et al. 2011. RNA aptamer against a cancer stem cell marker epithelial cell adhesion molecule. Cancer Sci, 102, 991–998.
Singh M, Atwal H and Micetich R. 1998. Transferrin directed delivery of adriamycin to human cells. Anticancer Res, 18, 1423–1427.
Singh M, Manoranjan B, Mahendram S, et al. 2014. Brain metastasis-initiating cells: Survival of the fittest. Int J Mol Med, 15, 9117–9133.
Soffietti R, Trevisan E and Ruda R. 2012. Targeted therapy in brain metastasis. Curr Opin Oncol, 24, 679–686.
Soysal SD, Muenst S, Barbie T, et al. 2013. EpCAM expression varies significantly and is differentially associated with prognosis in the luminal B HER2(+), basal-like, and HER2 intrinsic subtypes of breast cancer. Br J Cancer, 108, 1480–1487.
Stoltenburg R, Reinemann C and Strehlitz B. 2007. SELEX--a (r)evolutionary method to generate high-affinity nucleic acid ligands. Biomol Eng, 24, 381–403.
Subramanian N, Raghunathan V, Kanwar JR, et al. 2012. Target-specific delivery of doxorubicin to retinoblastoma using epithelial cell adhesion molecule aptamer. Mol Vis, 18, 2783–2795.
Sundaram P, Kurniawan H, Byrne ME, et al. 2013. Therapeutic RNA aptamers in clinical trials. Eur J Pharm Sci, 48, 259–271.
Svetlana MS, Nikola S, Richard FK, et al. 2011. Blood-brain barrier permeability: From bench to bedside. In: MK Günel (ed.), *Management of epilepsy- research, results and treatment* Inttech, pp. 113–140.
Tannenberg RK, Shamaileh HA, Lauridsen LH, et al. 2013. Nucleic acid aptamers as novel class of therapeutics to mitigate Alzheimer's disease pathology. Curr Alzheimer Res, 10, 442–448.
Thorn CF, Oshiro C, Marsh S, et al. 2011. Doxorubicin pathways: Pharmacodynamics and adverse effects. Pharmacogenet Genom, 21, 440–446.
Tuerk C and Gold L. 1990. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science, 249, 505–510.
Walbert T and Gilbert MR. 2009. The role of chemotherapy in the treatment of patients with brain metastases from solid tumors. Int J Clin Oncol, 14, 299–306.
Wang C, Hu W, Shen L, et al. 2014. Adoptive antitumor immunotherapy in vitro and in vivo using genetically activated erbB2-specific T cells. J Immunother, 37, 351–359.
Wang S, Placzek WJ, Stebbins JL, et al. 2012. Novel targeted system to deliver chemotherapeutic drugs to EphA2-expressing cancer cells. J Med Chem, 55, 2427–2436.
White R, Rusconi C, Scardino E, et al. 2001. Generation of species cross-reactive aptamers using toggle SELEX. Mol Ther, 4, 567–573.
Wilner SE, Wengerter B, Maier K, et al. 2012. An RNA alternative to human transferrin: A new tool for targeting human cells. Mol Ther- Nucl Acids, 1, e21.
Wu L, Li X, Janagam DR, et al. 2014. Overcoming the blood-brain barrier in chemotherapy treatment of pediatric brain tumors. Pharm Res, 31, 531–540.
Xiang D, Shigdar S, Bean AG, et al. 2017. Transforming doxorubicin into a cancer stem cell killer via EpCAM aptamer-mediated delivery. Theranostics, 7, 4071–4086.
Xu G, Wen X, Hong Y, et al. 2011. An anti-transferrin receptor antibody enhanced the growth inhibitory effects of

chemotherapeutic drugs on human glioma cells. Int. Immunopharmacol., 11, 1844–1849.
Xu W, Siddiqui IA, Nihal M, et al. 2013. Aptamer-conjugated and doxorubicin-loaded unimolecular micelles for targeted therapy of prostate cancer. Biomaterials, 34, 5244–5253.
Yang F, Teves SS, Kemp CJ, et al. 2014. Doxorubicin, DNA torsion, and chromatin dynamics. Biochimica Biophysica Acta, 1845, 84–89.
Ylera F, Lurz R, Erdmann VA, et al. 2002. Selection of RNA aptamers to the Alzheimer's disease amyloid peptide. Biochem Biophys Res Commun, 290, 1583–1588.
Yoon S, Huang K-W, Reebye V, et al. 2017. Aptamer-drug conjugates of active metabolites of nucleoside analogs and cytotoxic agents inhibit pancreatic tumor cell growth. Mol Ther- Nucl Acids, 6, 80–88.
Yu G, Li H, Yang S, et al. 2016a. SsDNA aptamer specifically targets and selectively delivers cytotoxic drug doxorubicin to HepG2 cells. PLoS ONE, 11, e0147674.
Yu Y, Liang C, Lv Q, et al. 2016b. Molecular selection, modification and development of therapeutic oligonucleotide aptamers. Int J Mol Sci, 17, 358.
Yu Y, Zhang Y, Kenrick M, et al. 2011. Boosting brain uptake of a therapeutic antibody by reducing its affinity for a transcytosis target. Sci Transl Med, 3, 84ra44.
Zhao N, Pei S-N, Qi J, et al. 2015. Oligonucleotide aptamer-drug conjugates for targeted therapy of acute myeloid leukemia. Biomaterials, 67, 42–51.
Zhou J, Bobbin ML, Burnett JC, et al. 2012. Current progress of rna aptamer-based therapeutics. Front Genet, 3, 234.
Zhou J and Rossi J. 2017. Aptamers as targeted therapeutics: Current potential and challenges. Nat Rev Drug Discov, 16, 181–202.
Zhu G, Meng L, Ye M, et al. 2012. Self-assembled aptamer-based drug carriers for bispecific cytotoxicity to cancer cells. Chem Asian J, 7, 1630–1636.