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Advancing Drug Therapy for Brain Tumours: A Current Review of the Pro-inflammatory Peptide Substance P and its Antagonists as Anti-cancer Agents

Kimberley Mander, Elizabeth Harford-Wright, Kate M. Lewis and Robert Vink*

*Adelaide Centre for Neuroscience Research, Discipline of Anatomy and Pathology, University of Adelaide, and
Division of Health Sciences, University of South Australia, Adelaide, Australia

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Abstract: Evidence for the involvement of the Substance P (SP)/NK1 receptor system in the development and progression of cancer strongly supports its potential as a therapeutic target in malignancies. Novel strategies for approaching cancer treatment are urgently required particularly with regard to tumours of the central nervous system (CNS), which are notoriously difficult to effectively treat and associated with extremely poor prognosis for many patients. This is due, in part, to the presence of the highly specialised blood-brain barrier, which is known to restrict common treatments such as chemotherapy and hinder early tumour diagnosis. Additionally, tumours of the CNS are difficult to surgically resect completely, often contributing to the resurgence of the disease many years later. Interestingly, despite the presence of the blood-brain barrier, circulating tumour cells are able to gain entry to the brain and form secondary brain tumours; however, the underlying mechanisms of this process remain unclear. Tachykinins, in particular Substance P, have been implicated in early blood-brain barrier disruption via neurogenic inflammation in a number of other CNS pathologies. Recent evidence also suggests that Substance P may play a central role in the development of CNS tumours. It has been well established that a number of tumour cells express Substance P, NK1 receptors and mRNA for the tachykinin NK1 receptor. This increase in the Substance P/NK1 receptor system is known to induce proliferation and migration of tumour cells as well as stimulate angiogenesis, thus contributing to tumour progression. Accordingly, the NK1 receptor antagonist presents a novel target for anti-cancer therapy for which a number of patents have been filed. This review will examine the role of Substance P in the development of CNS tumours and its potential application as an anti-cancer agent.

Keywords: Blood-brain barrier, CNS tumours, glioma, metastases, substance P, tight junctions.

INTRODUCTION

Cancer is currently the leading cause of death and disability worldwide with future projections estimating annual cases of cancer will rise from 14 million in 2012 to 22 million within the next two decades [1]. While improvements to treatment and early diagnosis have led to an increased survival rate for many common cancers, the incidence of cancer burden is still increasing [2]. Tumours of the central nervous system (CNS) represent one of the more clinically feared diagnoses of cancer, primarily due to the challenge they pose with regard to treatment and poor patient outcome [3]. Specifically, functional barriers unique to the CNS, such as the blood-brain barrier (BBB), and efflux transporters add further complexity with regard to diagnosis and treatment. The presence of the BBB is known to restrict the delivery of effective and safe treatment agents such as chemotherapy [4, 5]. Accordingly, alternate treatment options are often aggressive, limited and largely unsuccessful, with many patients succumbing to their CNS tumour(s) within months of diagnosis [6]. As such, a greater understanding of CNS tumours and the development of targeted treatments continues to be at the forefront of current research [7].

Primary brain tumours account for approximately 2% of all cancer incidence and despite this low rate, confer a disproportionate rate of morbidity and mortality due to their potential for progressive growth and interaction with critical neurological structures [8]. More than 256,000 new cases were diagnosed in 2012, with an alarming 189,000 deaths recorded for the same year [1]. Primary brain tumours arise from cells native to the brain [9]. Given that specialised glial cells such as astrocytes outnumber their post-mitotic neuronal counterparts and retain their proliferative capacity, it is unsurprising that neoplasms of glial origin account for the majority of primary adult CNS tumours [10]. Secondary brain tumours however, significantly outweigh the diagnosis of primary cerebral neoplasms - occurring 5-10 times more frequently [11, 12]. The development of metastases to the brain represents a crucial marker in the progression of the disease, specifically, the propensity of malignant cells to evade treatment, disseminate, survive and proliferate in a new location [6]. It is reported that approximately 20-40% of all adult patients with a systemic malignancy will present with a secondary symptomatic brain metastasis over the course of their disease [13]. Clinically, both primary and secondary tumours will often be accompanied by a myriad of neurological deficits which vary depending on tumour size, location and rate of progression [14]. Headache, nausea and vomiting are the most commonly described presenting fea-

*Address correspondence to this author at the Division of Health Sciences, University of South Australia, South Australia, 5001, Australia; Tel: +61 8 8302 2029; Fax: +61 8 8302 2030; E-mail: robert.vink@unisa.edu.au

tures and often indicate diffuse consequences of the space-occupying lesion, such as a raised intracranial pressure (ICP) [12]. As tumours develop or multiply, progressive focal neurological deficits such as visual disturbances, hemiparesis and aphasia will often become more pronounced and may elucidate tumour location [3].

The diagnosis of a brain tumour, either primary or secondary, presents a challenging and complex clinical situation in terms of devising an appropriate and effective treatment protocol for patients [15]. Often treatment will be multidisciplinary involving a combination of definitive treatments directed against the tumour designed to eradicate malignancy, as well as adjunctive agents such as corticosteroids or anti-convulsants to control symptoms such as cerebral oedema and seizures. Standard treatment protocol involves surgery typically followed by radiotherapy and/or chemotherapy [5]. The total surgical resection of a tumour remains the gold-standard treatment for a localised primary intracranial neoplasm [4]. Benign lesions are the most receptive to surgery as they contain well-defined margins enabling comprehensive removal of tumour tissue and improved patient survival [15]. However, a number of factors often preclude patients from undergoing curative surgery; for example, the anatomical location of the tumour may render it inoperable due to close proximity of critical structures such as the brainstem or vital vasculature. For cases not conducive to complete surgical resection, de-bulking surgery is often performed whereby maximal tumour tissue is removed allowing for a reduction in mass effect and the immediate alleviation of symptoms associated with increased ICP or seizures [16]. Unfortunately, microscopic foci of tumour cells commonly persist post surgery allowing the potential resurgence of the disease. Consequently, a course of postoperative radiotherapy is often administered to supplement surgery.

Radiotherapy is enlisted as an adjunctive therapy or primarily when a tumour is not amenable to complete surgical resection [17]. Whole-brain radiotherapy (WBRT) is considered to be the mainstay therapy for patients with multiple intracranial metastases at diagnosis [18]. The median survival of patients with brain metastases treated with WBRT is generally 2 to 6 months, with only 10% to 15% surviving beyond a year [19, 20]. Alternatively, stereotaxic radiosurgery (SRT) will precisely deliver a high dose of radiation to the tumour area and thus requires the location and size of the tumour to be well characterised. Directed radiotherapy is advantageous as it allows for specific targeting of tumour cells whilst sparing the surrounding healthy brain tissue and reducing the side effects often associated with WBRT. Typically SRT will be reserved for patients with a low number of cerebral tumours less than 3cm in diameter that are unable to be surgically resected [21]. Large tumours or tumours compromised by oedema are often difficult to control with SRT due to an increased risk of radiation-associated necrosis and/or neurological deterioration at biologically effective doses [21]. As such, SRT administration is usually limited to one course for a particular site due to the high-risk of radiation necrosis.

The therapeutic success of chemotherapy in the treatment of both primary and metastatic CNS tumours is impeded firstly by reduced delivery of agents across the BBB and

secondly by an acquired resistance expressed by tumour cells [22]. This is particularly true during the early stages of tumour growth where evidence indicates that brain tumours are largely unresponsive to systemic chemotherapy due to the intact BBB [23]. In particular, brain metastases tend to respond poorly to chemotherapeutic agents, which may indicate a variation in chemosensitivity of metastatic tumour cells versus those of primary origin [24]. Additionally, previously treated metastatic tumours may have acquired chemoresistant colonies that reduce the efficacy of commonly administered chemotherapeutic agents; this is suggested to account for the 90% failure rate of drug therapies directed against secondary metastasis [25, 26]. Furthermore, methods for improving drug delivery as a means of bypassing the BBB or BTB are currently under investigation. More recently the biodegradable 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU) polymer wafers (GlideL) have been developed for the use in malignant high-grade gliomas and evaluated for their effectiveness against multiple metastases [27]. These wafers allow the direct delivery of high doses of chemotherapeutic agents to the tumour tissue whilst bypassing the BBB. Following surgical resection of the glioma, up to 8 GlideL wafers are placed directly on the resection cavity and release the chemotherapeutic agents over the following 2-3 weeks [28]. Clinical trials have demonstrated a statistically significant improvement in survival following treatment with GlideL wafers post surgery, however their use has been limited by a high rate of complications [29-31]. Additionally, pharmacological disruption of the BBB using hypertonic solutions such as mannitol and RMP-7 have been investigated as a means of improving chemotherapeutic penetration to the CNS [32, 33]. RMP-7 is a synthetic analogue of bradykinin, a known peptide that specifically binds to the bradykinin-P2 receptor and has a role in modulating brain endothelial tight junctions [34]. Despite positive results in both preclinical and phase I clinical trials, these results were not replicated at the phase II level, further demonstrating the need for ongoing research to identify new avenues to improve options to deliver critical antitumor agents across the restricted BBB [23, 35]. In contrast to biochemical approaches, the development of ultrasound and electromagnetic radiation methods have demonstrated transient and site specific opening of the BBB suggested to be beneficial for CNS drug delivery [36].

Molecular targeted therapy (MTT) is a relatively new approach to cancer treatment whereby surface molecules uniquely or abnormally expressed by tumour cells are targeted with the intention of sparing normal cells and subsequently reducing clinical side effects for patients [37]. The efficacy of MTT is inherently dependent on the assumption that the molecule of interest is solely expressed by cancer cells and has a primary function in the maintenance of the malignancy; thereby, an adaptive suppression of the molecule will not result in acquired resistance [38]. Glioblastoma Multiforme (GBM), the most common and lethal brain tumour in adults are known to exhibit an overexpression of a number of cell surface proteins and signalling pathway disruptions, thus highlighting potential targets for MTT [39]. Given that GBMs are rarely amenable to aggressive surgical approaches, radiotherapy or chemotherapeutic agents, and are associated with a median survival rate of only one-year

post diagnosis, the promise of specialised treatments such as MTT is eagerly anticipated. A number of MTTs have successfully entered clinical trials and received treatment approval for various malignant conditions. However, the translation of MTTs to CNS neoplasms remains limited due to the exclusion of many cerebral metastatic patients from clinical trials, thus limiting their accessibility [40].

Increasingly, research has been directed towards identifying specific mediators that can be targeted in the treatment of cancer. Of particular interest are tachykinins and their receptors, which have been implicated in many aspects of cancer growth and progression, as well as in disruption to the BBB and development of cerebral oedema [41]. Furthermore, the revelation that tachykinin receptors, and in particular, substance P (SP) receptors, are upregulated in a number of systemic and CNS malignancies, has stimulated research into the potential of SP-mediated interventions in the treatment of brain tumors. As such, this paper will build upon our previous discussion of the role of neuroinflammatory peptides in cancer progression. More specifically, the potential applications of therapies directed at modulating the inflammatory effects of SP at the BBB and the implications for limiting cancer progression within the brain.

THE BLOOD-BRAIN BARRIER

The blood-brain barrier (BBB) represents the tightest endothelial barrier of any organ and in this way is a key component for maintaining cerebral homeostasis by regulating the traffic of both solutes and cells between the peripheral vasculature and brain parenchyma [42]. It is now understood that this systematic method of regulation is much more complex and as such, the BBB represents a dynamic and vital structure which is now at the forefront of current research for a number of neurological pathologies.

Endothelial cells, astrocytes and pericytes comprise the crucial cellular elements of the BBB, however, additional cell types such as neurons, glial cells and smooth muscle cells of the vascular wall have recently been recognized to contribute to the function of the BBB [43]. Any increase in permeability of the barrier leads to a number of deleterious effects such as disordered transport of substances leading to alterations in cerebrovascular blood flow and disrupted ion content [44]. These changes will often produce additional damaging consequences such as increased ICP or seizures [44].

Both primary brain tumours and cerebral metastases are associated with impairment of the BBB, with alterations to the cerebral endothelial cells and tight junctions forming the focus of current research in tumour progression and development [45]. Commonly described characteristics of brain tumours include the opening of intercellular junctions, increased endothelial vesicles, modified endothelial cell morphology and defects in basement membrane integrity [45, 46]. Evaluation of BBB status in the presence of human cerebral metastases and malignant gliomas via MRI, demonstrate a compromised BBB at various intensities depending on the specific tumour type [47]. Interestingly, small metastatic tumours as well as those exhibiting a diffuse growth pattern have been shown to maintain an intact BBB suggesting unique neoplastic effects

for some cerebral metastases when compared to primary CNS neoplasms [46, 48].

As a CNS tumour outgrows its immediate blood supply, the process of neoangiogenesis quickly becomes critically required for additional tumour growth in both primary and metastatic lesions [49]. Current research indicates that these newly formed vessels do not maintain the physiological features of the BBB and are more commonly referred to as the 'blood-tumour barrier' (BTB) [50]. Typically, vessels of the BTB are more "leaky" when compared to the BBB due to compromised tight junction proteins, increased fenestrations and pinocytotic vacuole activity, and reduced expression of efflux transporters [51]. Consequently, when a tumour is large enough to produce a 'brain-tumour barrier' systemically administered chemotherapy can more effectively gain entry to the tumour site [23].

Indeed, the relationship between BBB permeability and the implications for both cancer growth and delivery of treatment have been well explored. Most recently however, the involvement of tachykinin neuropeptides, such as SP in BBB disruption and progression of peritumoural oedema have been investigated as potential targets to mediate brain tumour development.

THE TACHYKININ FAMILY

Tachykinins are a group of small structurally related peptides characterized by the specific C-terminal sequence, Phe-X-Gly-Lue-Met-NH₂, and include SP, neurokinin A (NKA) and neurokinin B (NKB), as well as neuropeptide K (NPK) and neuropeptide γ (NP γ) [52]. The mammalian tachykinins are encoded by two distinct genes, preprotachykinin A (PPTA) and preprotachykinin B (PPTB). The PPTB gene gives rise to NKB, whilst alternate splicing of the PPTA gene results in four different forms of mRNA: α -PPT, β -PPT, γ -PPT and δ -PPT [52]. The α -PPT and δ -PPT forms encode for SP alone, whilst β -PPT and γ -PPT encode SP, NKA, NPK and NP γ [52, 53]. Tachykinins are produced in both neuronal and glial cell bodies, and are widely distributed throughout the CNS and PNS [54]. Specifically, within the PNS, SP is found in areas of immunologic importance such as the skin, gastrointestinal and respiratory tracts [55, 56]. In the CNS, levels of SP are higher in the grey rather than white matter [57] with the highest concentrations of SP occurring in the basal ganglia, hypothalamus, amygdala, locus coeruleus and dorsal root ganglia of the spinal cord [58].

Unlike classical neurotransmitters, tachykinins are produced in the cell soma rather than in the nerve endings, and are released following calcium dependent mechanisms in response to stimuli [56]. Following release, tachykinins act on the transmembrane G-protein coupled receptors NK1, NK2 and NK3 to exert a myriad of effects, with each tachykinin demonstrating some degree of affinity for each tachykinin receptor due to the common COOH-terminal sequence [59]. However, SP preferentially binds the NK1 receptor, NKA the NK2 receptor and NKB the NK3 receptor.

Although the NK receptors are structurally closely related, they are pharmacologically distinct, which has allowed detailed investigation of the structure and function of the

Table 1. SP and NK1 receptor expression in tumours.

	SP Expression	NK1 Receptor Expression	NK1 Antagonist Treatment
Human Specimens	Present in: Astrocytoma [78, 96], Meningioma [96] Breast carcinoma [78, 97], Melanoma [67, 78]	Present in: Astrocytoma [65, 78, 98], GBM [78], Breast carcinoma [65, 78, 94, 97, 98] , Melanoma [78]	N/A
Tumour cell lines	Evident in: Breast carcinoma (ZR-75-30, BT-474, T-47D, MDA-MB-330, 184B5, CP-96 345-1, DU4475, BT 483, MDA-MB-231, MDA-MB-231 Dox, MCF7, LCC1, LCC2, SK-BR-3) [97, 99-101], Melanoma [78] Pancreatic carcinoma [102, 103]	Evident in: Astrocytoma (UC11MG, U373MG, SNB-19, DBTRG-05 MG, GAMG) [73, 75, 104-107] Breast carcinoma (ZR-75-30, BT-474, T-47D, MDA-MB-330, MDA-MB-231, 184B5, CP-96 345-1, DU4475, BT 483, MCF7, LCC1, LCC2, SK-BR-3) [81, 94, 97, 100, 101] Pancreatic carcinoma [102] Melanoma (COLO 858, MEL HO, COLO 679) [108]	Induced apoptosis in: Astrocytoma (GAMG, SNB-19, DBTRG-05 MG, U373 MG) [68, 73, 75, 107], Breast carcinoma (MDA-MB-231, T47D, MDA-MB-468, MDA-MB-453, SKBR3 MCF7, BT474) [81, 94, 109], Pancreatic carcinoma (BxPC-3, MIA PaCa-2) [102, 103] Melanoma (COLO 858, MEL HO, COLO 679) [68, 110]
Animal Models	Present in breast carcinoma [111]	Present in breast carcinoma [111]	NK1 antagonist treatment causes differential tumour response. Caused decreased tumour growth in astrocytoma (U373 MG) in the flank of female nude mice [73], but did not affect tumour growth in breast carcinoma (MDA-MB-231) in the flank of female nude mice [81].

NK1 receptor. Binding to the NK1 receptor occurs at the second and third transmembrane domains and results in rapid endocytosis and internalisation of the receptor [60]. Within the CNS, NK1 receptors are found on neurons and glia [61], with expression of NK1 receptors highest in the caudate putamen and superior colliculus [58]. The widespread expression of SP and the NK1 receptor throughout the CNS has resulted in it being implicated in a number of neurological diseases, including CNS tumours. Accordingly, recent research has been focused on the potential of this particular tachykinin as a treatment target in numerous facets of cancer development.

SUBSTANCE P IN TUMOUR GROWTH

The last decade has seen a substantial rise in research into the expression and secretion of peptides by tumours [62] and in particular the tachykinins. Tachykinins are able to facilitate cell growth via induction of DNA synthesis and cellular proliferation. In addition, tachykinins have a stimulating or potentiating effect on lymphocyte proliferation and differentiation, cytokine secretion, and immunoglobulin production [63]. More specifically, SP and NK1 receptors have been implicated in numerous facets of cancer initiation and progression, with recent research implicating the PPT-1 gene and NK receptors in breast cancer development [64]. Furthermore, NK1 receptors have also been found in intratumoural and peri-tumoural blood vessels, suggesting a potential role for SP in tumour angiogenesis [65].

An increase in SP and the NK1 receptor expression has now been reported in a number of tumour types (Table 1) including glioma, retinoblastoma, colon, metastatic melanoma, *in situ* melanoma, keratocystic odontogenic tumours and squamous cell and larynx carcinoma [66-78]. In a number of glial tumour cell lines, the presence of NK1 receptors correlated with a SP and/or NK-A mediated increase in DNA synthesis and cellular proliferation [79, 80]. This increase in SP receptors *in vivo* is therefore thought to correspond with increases in tumour growth.

Oncogenes are genes that assist in the transformation of normal cells into cancerous ones, if mutated or expressed at high levels. Under normal physiological conditions, any cellular genetic damage activates one or more of the programmed cell death pathways. In order to avoid this, tumour cells must set up ways to neutralise the multiple pathways leading to cell death. It has been proposed that the increase in the expression of NK1 receptors may play a role in malignant cells evasion of apoptosis [62, 63]. Such increased expression makes tumour cells highly dependent on SP stimulus, a known potent mitotic signal that counteracts the different death signal pathways [63]. Indeed, administration of an NK1 antagonist for 2 weeks in a nude mice model of breast cancer was found to effectively inhibit tumour growth [81], whilst administration of the SP receptor antagonist, L-733, 060, was found to inhibit the metastatic progression of NK1 expressing human glioma cancer cells [82]. Thus, antagonism of the NK1 receptor

may allow cells to become more receptive to apoptotic death signals, leading to the death of mutated cells.

As well as exhibiting mitogenic and anti-apoptotic activity, SP is known to stimulate the release of cytokines. The role of cytokines in cellular communication, coordination of cell growth and maturation, wound healing, the immune responses and neo-angiogenesis have been well documented [83]. Many of these processes can be utilised by tumour cells to facilitate their growth, thus supporting their own growth and facilitating their metastatic spread [83-85]. Furthermore, such cytokine release from malignant cells may also induce normal cells to synthesise additional cytokines perpetuating tumour progression [83]. Specifically, granulocyte-macrophage colony stimulating factor (GM-CSF) is released following SP mediated protein kinase C (PKC) activation and is responsible for the macrophage aggregation observed in malignant gliomas and the regulation of cellular growth and migration [64, 73]. Cytokines also influence the release of SP, with leukemia-inhibiting factor (LIF), a neuropoietin cytokine responsible for neurogenesis and neuronal differentiation, able to stimulate glioma proliferation by induction of SP and NK1 receptor expression [73]. LIF is produced and released by neuronal cells following exposure to proinflammatory cytokines leading to increased SP production and NK1 expression [86].

Cytokines and other mediators are also responsible for the process of tumour angiogenesis, thereby enhancing tumour growth that is dependent upon a corresponding increase in vascularization [87]. Given the presence of its NK1 receptor in tumour vessels, it is hypothesised that SP may also facilitate tumour growth by mediating angiogenesis. Indeed, an increase in SP NK1 receptors has been demonstrated in tumoural and peri-tumoural vessels in glioblastomas, which is thought to be significant due to the known vasodilative and angiogenic effects of SP [65]. It has been reported that SP enhances capillary growth in an *in vivo* rabbit cornea assay, an effect that was abolished following ad-

ministration of a SP receptor antagonist [88]. Furthermore, SP stimulates migration and proliferation of some types of endothelial cells, thus enhancing the angiogenic process in human tumours [87]. Thus, SP may play a major role in development of tumour stroma and facilitate tumoural blood supply.

Experimentally, a number of NK1 antagonists have been identified as anti-cancer agents (Table 2). However, there is only one NK1 antagonist that is currently approved for human use [89] and specifically approved for use in cancer patients. Fosaprepitant diglutemide (L-758,298) is the intravenous prodrug of aprepitant (L-754,030), and is commonly known as Emend. The application of SP *in vivo* is known to induce emesis, confirming that SP is involved in the emetic reflex pathway [90]. Emend is accordingly used as an antiemetic to ameliorate the nausea and vomiting frequently associated with the use of many chemotherapeutic agents [91-93]. Furthermore, Emend is barrier permeable and has the added benefit of being able to cross the BBB [95]. Thus, Emend may potentially be able to exert other effects on CNS tumours, given that recent evidence suggests SP may play a role in tumour growth as well as in the development of peritumoural oedema.

SUBSTANCE P IN BRAIN TUMOURS

It has been suggested that substance P and the NK1 receptors are particularly significant in the development and progression of malignant brain tumours [64]. Increased expression of NK1 receptors, compared to the normal surrounding tissue, has been well documented in human gliomas and malignant brain tumours [64, 69, 110, 114]. Indeed, in human gliomas there is a correlation between increased density of NK receptors and the degree of tumour malignancy. A study by Hennig *et al.* [65] reported the presence of NK1 receptors on 100% of glioblastoma biopsy specimens and 75% of astrocytomas using autoradiography. Furthermore, SP receptor expression was observed in

Table 2. NK1 receptor antagonists identified as anti-cancer agents

NK1 antagonists	Evidence as anticancer agents <i>in vitro</i>	Molecular Formula	Chemical name	Indication
L-733,060	glioma [68, 69, 107], neuroblastoma [68, 69], melanoma [68], gastrointestinal tract cancer [71], acute lymphoblastic leukemia [62], retinoblastoma [69, 112], laryngeal cancer [72]	C ₂₀ H ₁₉ F ₆ NO	3-((3,5-Bis(trifluoromethyl)phenyl)methoxy)-2-phenylpiperidine	Preclinical
Aprepitant	glioma [70], neuroblastoma [70], melanoma [70], breast carcinoma [113], gastrointestinal tract carcinoma [70], pancreatic carcinoma [70], retinoblastoma [70], larynx carcinoma [70]	C ₂₃ H ₂₁ F ₇ N ₄ O ₃	5-(((2R,3S)-2-((R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy)-3-(4-fluorophenyl)morpholino)methyl)-1H-1,2,4-triazol-3(2H)-one	For the prevention of nausea and vomiting associated with emetogenic chemotherapeutic agents
L-732,138	retinoblastoma [112], acute lymphoblastic leukemia [62], laryngeal cancer [76]	C ₂₀ H ₁₈ F ₆ N ₂ O ₃	<i>N</i> -Acetyl-L-tryptophan 3,5-bis(trifluoromethyl)benzyl ester	Preclinical
MEN 11467	glioma [73], breast carcinoma [81]	C ₃₈ H ₄₀ N ₄ O ₃	<i>N</i> -[2-(4-Methylphenyl)acetyl]- <i>N</i> -methyl-(3-naphthyl)-L-alanine <i>N</i> -[2-(1H-indol-3-ylcarboxamido)cyclohexyl]amide	Preclinical

blood vessels regardless of tumour grade or type. The increased expression of SP in astrocytic tumours is consistent with the widespread distribution of SP and its precursors throughout the CNS. Moreover, the high levels of NK1 receptor within primary astrocytic tumours can be attributed to the fact that astrocytes harbour functional SP receptors and retain this with malignancy [73]. SP is known to mediate tumour growth in a number of glial tumour cell lines; the presence of tachykinin NK1 receptors correlated with a SP and/or an NK-A mediated increase in DNA synthesis and cellular proliferation [79]. Following activation of the NK1 receptor, a number of biochemical reactions take place that are associated with cancer progression. Binding to the NK1 receptor leads to activation of protein kinase C (PKC) which phosphorylates a number of proteins that stimulate the induction of DNA synthesis and cell proliferation [115]. Indeed, growth regulation via PKC stimulation has been noted on the human glioma line, U373 MG [116]. The involvement of SP in tumour growth was further detailed using an experimental *in vivo* model of secondary melanoma demonstrating treatment with the NK1 antagonist Emend significantly reduced tumour growth and volume [78]. Apart from its potential role in the promotion of tumour growth, SP has been shown to be integral in the development of peri-tumoural oedema and associated BBB disruption [117]. Furthermore, SP has been implicated in increasing BBB permeability and subsequent development of oedema associated with both traumatic brain injury (TBI) and stroke [118, 119]. Given the well-documented disruption to the BBB associated with brain tumours, SP may provide a novel therapeutic target in the treatment of tumour-associated oedema Fig. (1).

SUBSTANCE P IN PERI-TUMOURAL OEDEMA

Cerebral oedema, the abnormal accumulation of fluid within the brain parenchyma that produces a volumetric enlargement of brain tissue, frequently occurs around brain tumours and significantly contributes to morbidity and mortality [120]. Tumour-associated oedema is typically vasogenic in nature, resulting from a disrupted BBB that allows the extravasation of water and other plasma constituents into the brain parenchyma. Tumour-associated oedema can also have local effects such as impairment of microcirculation, expansion of the extracellular space, and abnormalities in the fluid microenvironment which all may influence the structure and function of normal brain cells [121]. Oedematous fluid accumulates rapidly around aggressive brain tumours and once excess extracellular fluid accumulates, mechanisms must exist to allow absorption, so that the rate of fluid formation and absorption are equal. Oedematous fluid is absorbed by transepithelial flow into the ventricles while proteins are phagocytosed by astrocytes and microglia, resulting in the erosion of the osmotic gradient in the extracellular space and subsequent absorption into microvessels [122, 123].

Corticosteroids have remained the primary treatment approach for tumour-associated oedema for the past 40 years. In a broad sense, they work by reducing the permeability of a compromised BBB, with a number of mechanisms proposed to account for this action. One proposed mechanism is by inhibition of phospholipase A₂, an enzyme responsible for arachidonic acid release [124]. Arachidonic acid destabilizes membrane lysosomes and has a direct destabilizing effect on cerebral capillaries. Corticosteroids are

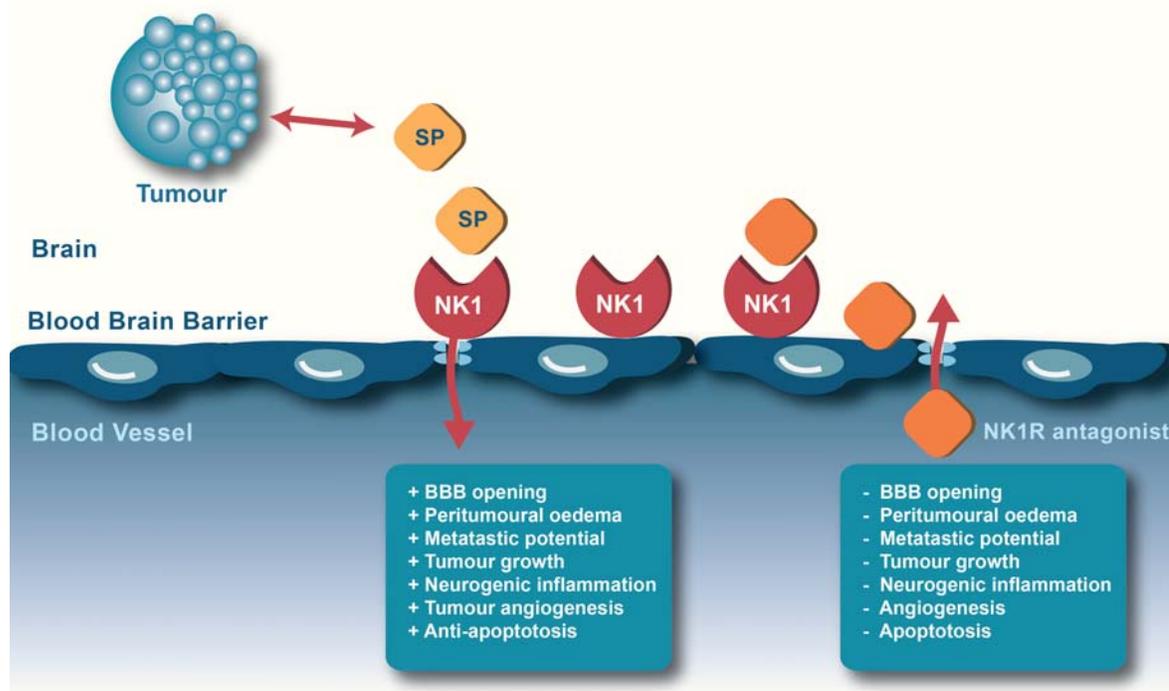


Fig. (1). Mechanism of NK1 receptor antagonists as novel anti-cancer agents. Once across the blood brain barrier (BBB), NK1 receptor antagonists bind to the NK1 receptor and prevent binding of substance P (SP). The pharmacological inhibition of the NK1 receptor is known to elicit a number of beneficial anti-tumoral effects including reduced peritumoural oedema formation, attenuation of neurogenic inflammation and inhibition of tumour growth.

also known to have the ability to reduce vascular endothelial growth factor (VEGF)-induced BBB permeability, an action reversed by a glucocorticoid receptor antagonist [125]. Therefore, corticosteroids may act to reduce the response of the cerebral capillary endothelial cells (CCEC) to VEGF or reduce the secretion of VEGF by tumour cells [125]. Nonetheless, the exact mechanism of action of corticosteroids is yet to be fully elucidated. Dexamethasone is the most commonly used steroid for tumour-associated oedema. It has a long half-life, which allows for twice daily dosing, and improves generalised symptoms such as headache and altered mental status. The majority of patients with brain metastases show marked clinical improvement within 24 to 72 hours after beginning dexamethasone treatment [126]. Nonetheless, dexamethasone is associated with a large number of potentially serious side effects, the severity of which depends on the dose and duration of steroid treatment [127]. Adverse side effects include immunosuppression, hypertension, fluid retention and mood disturbances. These complications have consequently prompted investigation into alternative treatments for tumour associated brain oedema. Given its role in the development of oedema following other insults to the CNS, the tachykinin SP has become a potential target [128, 129].

Tachykinins are known to contribute to cerebral oedema in a number of brain pathologies through a process known as neurogenic inflammation, a neurally elicited inflammatory response characterised by vasodilation, plasma extravasation, mast cell degranulation and tissue swelling [130]. It results from the stimulation of capsaicin sensitive C-fibres causing the release of neuropeptides such as SP and calcitonin gene related peptide (CGRP). Numerous studies have provided evidence for a role for neuropeptides in most immunologic and inflammatory states within the periphery. Activation or damage to neurons can lead to changes in neuropeptide synthesis, which results from the induction of neuropeptide gene expression [53]. Such changes to neuropeptide expression in sensory neurons have been observed in models of acute and chronic inflammation. Furthermore, upregulation of NK1 receptor and PPT mRNA has also been shown in the periphery during noxious stimulation or neurogenic inflammation [54].

SP is thought to be the most potent initiator of neurogenic inflammation because of its association with increased vascular permeability and subsequent protein plasma extravasation [131]. It may also potentiate inflammatory responses by stimulating the production of inflammatory mediators such as histamine, nitric oxide and kinins, interacting with adhesion molecules and the extracellular matrix causing leukocyte migration [131]. The role of SP in neurogenic inflammation in the periphery has been well documented, particularly in the skin, gastrointestinal and respiratory tracts where administration of SP has been found to induce neurogenic inflammation [132, 133]. This role has been confirmed by findings that NK1 receptor antagonists completely abolished the inflammatory response [134]. In contrast, the concept of neurogenic inflammation in the CNS has remained largely unexplored until relatively recently.

Interest in the role of neurogenic inflammation in a number of brain pathologies is on the rise. As the cerebral

arteries have a dense supply of neuropeptide containing sensory neurons, an increase in neuropeptides post CNS damage may be involved in injury pathways [135]. Vink and colleagues (2003) were the first to investigate neurogenic inflammation in the brain following traumatic brain injury (TBI) [135]. They found that depletion of neuropeptides with capsaicin completely attenuated the changes in BBB permeability and subsequent oedema formation usually observed following TBI. Subsequent abolition of neuropeptides also significantly improved functional outcome post trauma. Such findings were rationalised in terms of the high levels of NK receptors found in areas associated with both motor control and memory and learning [136]. Furthermore, successive research has demonstrated a significant upregulation of SP immunoreactivity in perivascular tissue and within the parenchyma occurs by 5 hours post trauma. This increase in SP is associated with significant increases in cerebral oedema and marked functional deficits [118, 137]. Administration of an NK1 antagonist was found to attenuate this oedema formation and improve neurological outcome in both male and female rats [118, 137]. These results are consistent with what is observed in the human condition, with SP immunoreactivity reportedly increased following human TBI [138]. A role for neuropeptides, namely SP, has also been demonstrated in other neurological diseases. Following ischaemic stroke, SP immunoreactivity was increased in the infarcted hemisphere and was associated with profound oedema formation. As was observed in TBI, administration of a SP antagonist resulted in marked improvement in functional outcome following stroke [119]. Several patents have described the use of NK1 antagonists to ameliorate SP induced vasogenic cerebral oedema, particularly following TBI and ischemic reperfusion stroke [118, 139, 140]. Furthermore, these efforts to reduce cerebral oedema through NK1 antagonist treatment have led to the filing of patents that also promote this treatment to prevent subsequent increases in ICP [141].

Numerous studies have also demonstrated that SP and NK1 receptor immunoreactivity is increased surrounding brain tumours, particularly within the perivascular region [36, 78]. This localization is suggestive of a role in the formation of tumour-associated oedema and was further explored through an experimental *in vivo* model of secondary metastasis [117]. Results of this study demonstrate the involvement of SP in the development of tumour-associated BBB disruption and subsequent oedema formation [117]. Furthermore, administration of the NK1 antagonist Emend was found to significantly reduce BBB disruption and brain water content indicating that the targeting the SP/NK1 pathway may be a beneficial approach to alleviating deleterious symptoms associated with tumour growth. This was found to be as effective as the current clinical treatment of dexamethasone in reducing tumour associated brain oedema [117]. Interestingly, these results conflict with previously reported data, which detailed that treatment with an NK1 antagonist failed to exert any significant effect on oedema formation in an *in vivo* model of metastatic breast cancer [111]. However, it has been highlighted that this apparent contradiction may be a reflection of the differing tumor cell lines, further reinforced by the findings observed in the study of human tumors indicating

that SP expression can differ between different tumor types. As such, continuation of this work will endeavor to further ascertain and characterise the differing role of SP in specific tumour types.

SUBSTANCE P IN THE EXTRAVASATION PROCESS

A key event in the development of any brain metastasis is the migration of cancer cells through the blood-brain barrier (BBB) [42]. Invasion of the brain is a multifaceted process of tightly controlled mechanisms which enable tumour cells to breach the cerebral microvessels and invade surrounding parenchyma to form micro and macrometastasis [142]. To date, the precise mechanisms underpinning this detrimental process remain unclear. As such, cancer cell interaction with the BBB and potential mediating substances form a valuable target for further investigation into the critical early stages of tumour extravasation [18]. Identification of such agents may prevent metastatic tumour formation; however to date there has been little success.

The quality of the surrounding stroma and ECM in terms of nutrients and immune cell presence is known to play a role in the survival and rate of growth for circulating tumour cells. The mere presence of tumour cells is known to induce biochemical alterations within the immediate brain microenvironment, namely catalyse paracrine signalling between endothelial cells, stromal cells and the invading metastatic cells leading to an inflammatory state [143]. Cellular remodelling of cerebral endothelial cells is apparent in the early extravasation stage of metastasis, with tumour cells shown to promote a rearrangement of the endothelial cytoskeleton resulting in an overt phenotypic change to cells which has downstream effects on their function [144]. Furthermore, histological analysis of resected human brain metastases revealed tumour cells have a clear interaction with activated microglia and astrocytes further supporting the tumour induced neuroinflammatory state of the parenchymal microenvironment [145]. It is suggested that this pro-inflammatory state is beneficial for the growth and proliferation of metastatic cells. Tumour cell-derived factors such as interleukin-1 (IL-1), macrophage inhibitory factor, and plasminogen activator inhibitor 1 are known to activate local astrocytes which consequently produce metastatic proliferative factors such as interleukin-6 (IL-6), interleukin-1beta (IL-1 β) and tumour necrosis factor alpha (TNF- α) *in vitro*. Consistent with these findings, *in vitro* co-culture model of metastasis found the presence of reactive microglia induced a five-fold increase in metastatic cellular proliferation, suggesting that the activated microenvironment advances tumour growth and invasion [146]. Additionally, SP has been detected in cerebral capillary endothelial cells and is secreted by these cells in response to treatment with high dose of cytokines such as IL-1 β and TNF- α . As a result of SP release, the concentration of calcium ions within endothelial cells of the BBB is increased by approximately 10 times above normal, and hence leads to increased permeability of the BBB through endothelial cell contraction. As previously stated, increases in SP and its NK1 receptor have been reported for a variety of different tumour cell lines, further supporting the potential of this system as a target to inhibit the entry of cancer cells into the brain [74, 78]. Furthermore, *in vitro* studies of the BBB indicate that in the presence of SP, expression of crucial TJ

proteins, claudin-5 and zona occludin-1 is decreased, thus correlating with increased permeability of the BBB [147]. Recently, it has been demonstrated that SP expression is increased locally surrounding tumour-invaded microvessels, in a rodent model of brain metastatic breast cancer (BCC). The localised elevation of SP correlated with increased permeability of the BBB and the loss of endothelial barrier antigen staining, indicative of a disrupted BBB (See Fig. 1). Additionally, it was confirmed that BCCs actively secrete SP that ultimately contribute to BBB TJ protein alteration. Moreover, treatment with the SP inhibitor, spantide III demonstrated an inhibition of changes to the BBB TJs and the subsequent reduction of BCC brain colonisation *in vivo*. These results indicate the secretion of SP directly from tumour cells may increase the permeability of the BBB, thereby allowing for the movement of tumour cells into the brain, and subsequent development of metastasis. As such, NK1 antagonists are a potentially promising preventative treatment for metastatic brain tumours, particularly for patients diagnosed with primary tumours known to preferentially spread to the brain.

The extensive research on the role of SP in cancer growth and the potential antitumoral action of NK1 antagonists in various cancer types has led to the filing of several patents. One patent by Munoz and colleagues [112] describes the induction of apoptosis in cancer cells by NK1 antagonists in a number of cancer types, but in particular gliomas, melanoma, lung and breast carcinomas. Similarly, the invention of an antibody specific against SP has been suggested for the treatment of cancers expressing NK1 or epidermal growth factor family receptors [148]. In contrast, several patents have also been filed in which SP is proposed as a treatment or preventative strategy for cancer, although these are largely targeting peripheral tissues. Aerosolized SP has been described as a prophylactic treatment for the prevention of cancer in the lung, particularly in response to environmental toxins [149, 150]. Similarly, SP in combination with an immunogenic composition has been proposed for use as a cancer vaccine, acting through tumour antigen manipulation [151]. Clearly, treatment with either an NK1 antagonist or SP must be applied selectively based on tissue location of the cancer as well as the cancer type.

CURRENT & FUTURE DEVELOPMENTS

As the burden of cancer continues to rise worldwide, many of the underlying mechanisms contributing to cancer development and progression remain unclear. Consequently, prevention, early diagnosis and treatment options continue to be hampered by such limitations in our understanding. The pursuit of improved treatment strategies has led to the investigation of multiple targets as a means of achieving improved outcome and prognosis for patients. Recent research has indicated that SP may play multiple roles in the pathology of CNS tumours, including tumour cell growth, the genesis of peri-tumoural oedema and the extravasation of tumour cells into the brain. Given that the NK1 antagonist is already administered clinically as part of cancer patient management, it remains the frontrunner in terms of further analysing the role of SP in pathways of cancer progression. Furthermore, a retrospective review of patient data may reveal administration of NK1 antagonist providing beneficial effects on tumour growth and reduced metastatic potential for some can-

cer types. To date there has been no such investigation into the effect of the treatment on cancer growth or progression in humans. Additionally, SP is expressed in varying quantities for a number of different tumour cells, thus further investigation of whether these levels correlate with tumour aggressiveness or propensity to colonise certain organs, such as the brain is required. Similarly, additional research to elucidate the effect of NK1 antagonist treatment on BBB dysfunction in the setting of experimental brain tumours will be of further benefit. Finally, there is sufficient evidence to suggest both SP and NK1 play a key role in tumour development and progression, particularly with regard to tumour dissemination to the brain. As such further research is encouraged in order to harness the benefit of targeting this pathway in cancer biology.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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DISCLOSURE

This is an extended and updated version of the authors' previous manuscript published in *Recent Pat. CNS Drug Disc.* 6 (1), 31-40 (2011) entitled "Towards drug discovery for brain tumours: interactions of kinins and tumours at the blood brain barrier interface".

REFERENCES

- [1] Ferlay JSI, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F. In: *Cancer Incidence and Mortality Worldwide*. IARC CancerBase No 11 Lyon, France: GLOBOCAN 2012: 2013.
- [2] Siegel R, Ma J, Zou Z, Jemal A. *Cancer statistics, 2014*. CA: *Canc J Clinicians* 2014; 64(1): 9-29.
- [3] Norden AD, Wen PY, Kesari S. Brain metastases. *Curr Opin Neurol* 2005; 18(6): 654-61.
- [4] Behin A, Hoang-Xuan K, Carpentier AF, Delattre JY. Primary brain tumours in adults. *Lancet* 2003; 361(9354): 323-31.
- [5] Buckner JC, Brown PD, O'Neill BP, Meyer FB, Wetmore CJ, Uhm JH. Central nervous system tumors. *Mayo Clin Proc* 2007; 82(10): 1271-86.
- [6] Gavrilovic IT, Posner JB. Brain metastases: epidemiology and pathophysiology. *J Neuro-oncol* 2005; 75(1): 5-14.
- [7] Kefford RF. Drug treatment for melanoma: progress, but who pays? *Medical J Aus* 2012; 197(4): 198-9.
- [8] Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, *et al.* The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol* 2007; 114(2): 97-109.
- [9] Pereira A, Jr., Furlan FA. Astrocytes and human cognition: modeling information integration and modulation of neuronal activity. *Prog Neurobiol* 2010; 92(3): 405-20.
- [10] Furnari FB, Fenton T, Bachoo RM, Mukasa A, Stommel JM, Stegh A, *et al.* Malignant astrocytic glioma: genetics, biology, and paths to treatment. *Genes Develop* 2007; 21(21): 2683-710.
- [11] Bafaloukos D, Gogas H. The treatment of brain metastases in melanoma patients. *Canc Treat Rev* 2004; 30(6): 515-20.
- [12] DeAngelis LM. Brain tumors. *New Eng J Med* 2001; 344(2): 114-23.
- [13] Vecht CJ. Clinical management of brain metastasis. *J Neuro* 1998; 245(3): 127-31.
- [14] Ricard D, Idhahh A, Ducray F, Lahutte M, Hoang-Xuan K, Delattre JY. Primary brain tumours in adults. *Lancet* 2012; 379(9830): 1984-96.
- [15] Drappatz J, Schiff D, Kesari S, Norden AD, Wen PY. Medical management of brain tumor patients. *Neurologic Clinics* 2007; 25(4): 1035-71, ix.
- [16] Silberman AW. Surgical debulking of tumors. *Surg Gyne Obs* 1982; 155(4): 577-85.
- [17] Kondziolka D, Flickinger JC, Lunsford LD. Radiosurgery for brain metastases. *Prog Neuro Surg* 2012; 25: 115-22.
- [18] Arshad F, Wang L, Sy C, Avraham S, Avraham HK. Blood-brain barrier integrity and breast cancer metastasis to the brain. *Pathol Res Int* 2010; 2011: 920509.
- [19] Khan AJ, Dicker AP. On the merits and limitations of whole-brain radiation therapy. *J Clin Oncol* 2013; 31(1): 11-3.
- [20] Patchell RA. The management of brain metastases. *Canc Treat Rev* 2003; 29(6): 533-40.
- [21] Ranjan T, Abrey LE. Current management of metastatic brain disease. *Neurotherapeut* 2009; 6(3): 598-603.
- [22] Fortin D. The blood-brain barrier should not be underestimated in neuro-oncology. *Revue Neurologique* 2004; 160(5 Pt 1): 523-32.
- [23] Deeken JF, Loscher W. The blood-brain barrier and cancer: transporters, treatment, and Trojan horses. *Clin Canc Res* 2007; 13(6): 1663-74.
- [24] Regina A, Demeule M, Laplante A, Jodoin J, Dagenais C, Berthelet F, *et al.* Multidrug resistance in brain tumors: roles of the blood-brain barrier. *Canc Metastas Rev* 2001; 20(1-2): 13-25.
- [25] Longley DB, Johnston PG. Molecular mechanisms of drug resistance. *J Pathol* 2005; 205(2): 275-92.
- [26] Abdullah LN, Chow EK. Mechanisms of chemoresistance in cancer stem cells. *Clin Translat Med* 2013; 2(1): 3.
- [27] Abel TJ, Ryken T, Lesniak MS, Gabikian P. Gliadel for brain metastasis. *Surg Neuro Int* 2013; 4(Suppl 4): S289-93.
- [28] Westphal M, Hilt DC, Bortey E, Delavault P, Olivares R, Warnke PC, *et al.* A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. *Neuro-onco* 2003; 5(2): 79-88.
- [29] Bregy A, Shah AH, Diaz MV, Pierce HE, Ames PL, Diaz D, *et al.* The role of Gliadel wafers in the treatment of high-grade gliomas. *Expert Rev Anticanc Ther* 2013; 13(12): 1453-61.
- [30] Nagpal S. The role of BCNU polymer wafers (Gliadel) in the treatment of malignant glioma. *Neurosurg Clin North Am* 2012; 23(2): 289-95, ix.
- [31] Brem H, Ewend MG, Piantadosi S, Greenhoot J, Burger PC, Sisti M. The safety of interstitial chemotherapy with BCNU-loaded polymer followed by radiation therapy in the treatment of newly diagnosed malignant gliomas: phase I trial. *J Neuro-onco* 1995; 26(2): 111-23.
- [32] Jain KK. Nanobiotechnology-based strategies for crossing the blood-brain barrier. *Nanomed (Lond)* 2012; 7(8): 1225-33.
- [33] Black, K.L., Ningaraj, N.S. Potassium channel mediated delivery of agents through the blood-brain barrier. WO2005025511 (2005).
- [34] Liu LB, Xue YX, Liu YH, Wang YB. Bradykinin increases blood-tumor barrier permeability by down-regulating the expression levels of ZO-1, occludin, and claudin-5 and rearranging actin cytoskeleton. *J Neurosci Res* 2008; 86(5): 1153-68.
- [35] Emerich DF, Dean RL, Marsh J, Pink M, Lafreniere D, Snodgrass P, *et al.* Intravenous cereport (RMP-7) enhances delivery of hydrophilic chemotherapeutics and increases survival in rats with metastatic tumors in the brain. *Pharmaceut Res* 2000; 17(10): 1212-9.
- [36] Tajés M, Ramos-Fernandez E, Weng-Jiang X, Bosch-Morato M, Guivernau B, Eraso-Pichot A, *et al.* The blood-brain barrier: structure, function and therapeutic approaches to cross it. *Mol Memb Biol* 2014; 31(5): 1-16.
- [37] Hochwald SN. Molecular-targeted therapy for cancer and nanotechnology. *Meth Mol Biol* 2010; 624: 11-23.
- [38] Rahma OKS. Biological Basis of Molecular Targeted Therapy. In: Roland T, Skeel SNK, Ed. *Handbook of Cancer Chemotherapy*. Philadelphia: Lippincott Williams & Wilkins; 2011.
- [39] Mischel PS, Cloughesy TF. Targeted molecular therapy of GBM. *Brain Pathol* 2003; 13(1): 52-61.
- [40] Atallah E, Flaherty L. Treatment of metastatic malignant melanoma. *Curr Treatm Opt Onco* 2005; 6(3): 185-93.
- [41] Palma C. Tachykinins and their receptors in human malignancies. *Curr Drug Targ* 2006; 7(8): 1043-52.
- [42] Wilhelm I, Molnar J, Fazakas C, Hasko J, Krizbai IA. Role of the blood-brain barrier in the formation of brain metastases. *Int J Mol Sci* 2013; 14(1): 1383-411.

- [43] Nag S, Kapadia A, Stewart DJ. Review: molecular pathogenesis of blood-brain barrier breakdown in acute brain injury. *Neuropathol App Neurobiol* 2011; 37(1): 3-23.
- [44] Banks WA. Physiology and pathology of the blood-brain barrier: implications for microbial pathogenesis, drug delivery and neurodegenerative disorders. *J Neurovirol* 1999; 5(6): 538-55.
- [45] Jia W, Martin TA, Zhang G, Jiang WG. Junctional adhesion molecules in cerebral endothelial tight junction and brain metastasis. *Anticanc Res* 2013; 33(6): 2353-9.
- [46] Harford-Wright E, Lewis KM, Vink R. Towards drug discovery for brain tumours: interaction of kinins and tumours at the blood brain barrier interface. *Rec Pat CNS Drug Discov* 2011; 6(1): 31-40.
- [47] Nduom EK, Yang C, Merrill MJ, Zhuang Z, Lonser RR. Characterization of the blood-brain barrier of metastatic and primary malignant neoplasms. *J Neurosurg* 2013.
- [48] Zhang RD, Price JE, Fujimaki T, Bucana CD, Fidler IJ. Differential permeability of the blood-brain barrier in experimental brain metastases produced by human neoplasms implanted into nude mice. *Am J Pathol* 1992; 141(5): 1115-24.
- [49] Jain RK, di Tomaso E, Duda DG, Loeffler JS, Sorensen AG, Batchelor TT. Angiogenesis in brain tumours. *Nat Rev Neurosci* 2007; 8(8): 610-22.
- [50] Lockman PR, Mittapalli RK, Taskar KS, Rudraraju V, Gril B, Bohn KA, *et al.* Heterogeneous blood-tumor barrier permeability determines drug efficacy in experimental brain metastases of breast cancer. *Clin Canc Res* 2010; 16(23): 5664-78.
- [51] Groothuis DR. The blood-brain and blood-tumor barriers: a review of strategies for increasing drug delivery. *Neuro-oncol* 2000; 2(1): 45-59.
- [52] Maggi CA. The mammalian tachykinin receptors. *Gen Pharmacol* 1995; 26(5): 911-44.
- [53] Hokfelt T, Pernow B, Wahren J. Substance P: a pioneer amongst neuropeptides. *J Intern Med* 2001; 249(1): 27-40.
- [54] McCarson KE. Central and peripheral expression of neurokinin-1 and neurokinin-3 receptor and substance P-encoding messenger RNAs: peripheral regulation during formalin-induced inflammation and lack of neurokinin receptor expression in primary afferent sensory neurons. *Neurosci* 1999; 93(1): 361-70.
- [55] Pernow B. Substance P. *Pharmacological Rev* 1983; 35(2): 85-141.
- [56] Severini C, Improta G, Falconieri-Erspamer G, Salvadori S, Erspamer V. The tachykinin peptide family. *Pharmacological Rev* 2002; 54(2): 285-322.
- [57] Helke CJ, Phillips ET. Substance P antagonist-induced spinal cord vasoconstriction: effects of thyrotropin-releasing hormone and substance P agonists. *Peptides* 1988; 9(6): 1307-15.
- [58] Shults CW, Quirion R, Chronwall B, Chase TN, O'Donohue TL. A comparison of the anatomical distribution of substance P and substance P receptors in the rat central nervous system. *Peptides* 1984; 5(6): 1097-128.
- [59] Hardwick JC, Mawe GM, Parsons RL. Tachykinin-induced activation of non-specific cation conductance via NK3 neurokinin receptors in guinea-pig intracardiac neurones. *J Physiol* 1997; 504(Pt 1): 65-74.
- [60] Malcangio M, Bowery NG. Effect of the tachykinin NK1 receptor antagonists, RP 67580 and SR 140333, on electrically-evoked substance P release from rat spinal cord. *Brit J Pharmacol* 1994; 113(2): 635-41.
- [61] Schaffer M, Beiter T, Becker HD, Hunt TK. Neuropeptides: mediators of inflammation and tissue repair? *Archiv Surg* 1998; 133(10): 1107-16.
- [62] Munoz M, Martinez-Armesto J, Covenas R. NK-1 receptor antagonists as antitumor drugs: a survey of the literature from 2000 to 2011. *Expert Opin Therapeut Pat* 2012; 22(7): 735-46.
- [63] Esteban F, Munoz M, Gonzalez-Moles MA, Rosso M. A role for substance P in cancer promotion and progression: a mechanism to counteract intracellular death signals following oncogene activation or DNA damage. *Canc Metastas Rev* 2006; 25(1): 137-45.
- [64] Lazarczyk M, Matyja E, Lipkowski A. Substance P and its receptors -- a potential target for novel medicines in malignant brain tumour therapies (mini-review). *Folia Neuropathologica Assoc Polish Neuropathol Med Res Centre, Polish Acad Sci* 2007; 45(3): 99-107.
- [65] Hennig IM, Laissue JA, Horisberger U, Reubi JC. Substance-P receptors in human primary neoplasms: tumoral and vascular localization. *Int J Canc* 1995; 61(6): 786-92.
- [66] Brener S, Gonzalez-Moles MA, Tostes D, Esteban F, Gil-Montoya JA, Ruiz-Avila I, *et al.* A role for the substance P/NK-1 receptor complex in cell proliferation in oral squamous cell carcinoma. *Anticanc Res* 2009; 29(6): 2323-9.
- [67] Khare VK, Albino AP, Reed JA. The neuropeptide/mast cell secretagogue substance P is expressed in cutaneous melanocytic lesions. *J Cutaneous Pathol* 1998; 25(1): 2-10.
- [68] Munoz M, Perez A, Rosso M, Zamarrigo C, Rosso R. Antitumoral action of the neurokinin-1 receptor antagonist L-733 060 on human melanoma cell lines. *Melanoma Res* 2004; 14(3): 183-8.
- [69] Munoz M, Rosso M, Perez A, Covenas R, Rosso R, Zamarrigo C, *et al.* The NK1 receptor is involved in the antitumoural action of L-733,060 and in the mitogenic action of substance P on neuroblastoma and glioma cell lines. *Neuropep* 2005; 39(4): 427-32.
- [70] Munoz M, Rosso M, Robles-Frias MJ, Salinas-Martin MV, Rosso R, Gonzalez-Ortega A, *et al.* The NK-1 receptor is expressed in human melanoma and is involved in the antitumor action of the NK-1 receptor antagonist aprepitant on melanoma cell lines. *Lab Investig* 2010; 90(8): 1259-69.
- [71] Rosso M, Robles-Frias MJ, Covenas R, Salinas-Martin MV, Munoz M. The NK-1 receptor is expressed in human primary gastric and colon adenocarcinomas and is involved in the antitumor action of L-733,060 and the mitogenic action of substance P on human gastrointestinal cancer cell lines. *Tumour Biol* 2008; 29(4): 245-54.
- [72] Esteban F, Gonzalez-Moles MA, Castro D, Martin-Jaen MM, Redondo M, Ruiz-Avila I, *et al.* Expression of substance P and neurokinin-1-receptor in laryngeal cancer: linking chronic inflammation to cancer promotion and progression. *Histopathol* 2009; 54(2): 258-60. Epub 2009/02/12.
- [73] Palma C, Bigioni M, Irrissuto C, Nardelli F, Maggi CA, Manzini S. Anti-tumour activity of tachykinin NK1 receptor antagonists on human glioma U373 MG xenograft. *Brit J Canc* 2000; 82(2): 480-7.
- [74] Munoz M, Covenas R. Involvement of substance P and the NK-1 receptor in cancer progression. *Peptides* 2013; 48: 1-9.
- [75] Munoz M, Rosso M, Perez A, Covenas R, Rosso R, Zamarrigo C, *et al.* Antitumoral action of the neurokinin-1-receptor antagonist L-733,060 and mitogenic action of substance P on human retinoblastoma cell lines. *Investig Ophthalmol Visual Sci* 2005; 46(7): 2567-70.
- [76] Gonzalez MMA, Esteban F, Ruiz-Avila I, Gil MJA, Brener S, Bascones-Martinez A, *et al.* A role for the substance P/NK-1 receptor complex in cell proliferation and apoptosis in oral lichen planus. *Oral Dis* 2009; 15(2): 162-9.
- [77] Munoz M, Covenas R. NK-1 receptor antagonists: a new generation of anticancer drugs. *Mini Rev Medic Chem* 2012; 12(7): 593-9.
- [78] Harford-Wright E, Lewis KM, Vink R, Ghabriel MN. Evaluating the role of substance P in the growth of brain tumors. *Neurosci* 2014; 261: 85-94.
- [79] Luo W, Sharif TR, Sharif M. Substance P-induced mitogenesis in human astrocytoma cells correlates with activation of the mitogen-activated protein kinase signaling pathway. *Canc Res* 1996; 56(21): 4983-91.
- [80] Friess H, Zhu Z, Liard V, Shi X, Shrikhande SV, Wang L, *et al.* Neurokinin-1 receptor expression and its potential effects on tumor growth in human pancreatic cancer. *Lab Investig* 2003; 83(5): 731-42.
- [81] Bigioni M, Benzo A, Irrissuto C, Maggi CA, Goso C. Role of NK-1 and NK-2 tachykinin receptor antagonism on the growth of human breast carcinoma cell line MDA-MB-231. *Anti-canc Drugs* 2005; 16(10): 1083-9.
- [82] Lang K, Drell TLT, Lindecke A, Niggemann B, Kaltschmidt C, Zaenker KS, *et al.* Induction of a metastatogenic tumor cell type by neurotransmitters and its pharmacological inhibition by established drugs. *Int J Canc* 2004; 112(2): 231-8.
- [83] Dunlop RJ, Campbell CW. Cytokines and advanced cancer. *J Pain Symptom Manag* 2000; 20(3): 214-32.
- [84] Jaiswal M, LaRusso NF, Burgart LJ, Gores GJ. Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. *Canc Res* 2000; 60(1): 184-90.
- [85] Vidal-Vanaclocha F, Fantuzzi G, Mendoza L, Fuentes AM, Anasagasti MJ, Martin J, *et al.* IL-18 regulates IL-1beta-dependent

- hepatic melanoma metastasis via vascular cell adhesion molecule-1. *Proc Nat Acad Sci USA* 2000; 97(2): 734-9.
- [86] Hu CP, Feng JT, Tang YL, Zhu JQ, Lin MJ, Yu ME. LIF upregulates expression of NK-1R in NHBE cells. *Med Inflamm* 2006; 2006(5): 84829.
- [87] Ribatti D, Conconi MT, Nussdorfer GG. Nonclassic endogenous novel [corrected] regulators of angiogenesis. *Pharmacol Rev* 2007; 59(2): 185-205.
- [88] Ziche M, Morbidelli L, Pacini M, Geppetti P, Alessandri G, Maggi CA. Substance P stimulates neovascularization *in vivo* and proliferation of cultured endothelial cells. *Microvascul Res* 1990; 40(2): 264-78.
- [89] Hesketh PJ, Grunberg SM, Gralla RJ, Warr DG, Roila F, de Wit R, et al. The oral neurokinin-1 antagonist aprepitant for the prevention of chemotherapy-induced nausea and vomiting: a multinational, randomized, double-blind, placebo-controlled trial in patients receiving high-dose cisplatin—the Aprepitant Protocol 052 Study Group. *J Clin Oncol* 2003; 21(22): 4112-9.
- [90] Gardner CJ, Twissell DJ, Dale TJ, Gale JD, Jordan CC, Kilpatrick GJ, et al. The broad-spectrum anti-emetic activity of the novel non-peptide tachykinin NK1 receptor antagonist GR203040. *Brit J Pharmacol* 1995; 116(8): 3158-63.
- [91] Vankawala, P., Elati, R., Kolla, N.K., Chlamala, S.R., Gangula, S. Preparation of aprepitant. US20128133994 (2012).
- [92] Jain, P., Barabde, U.V., Kodipyaka, R., Bhushan, I., Mathad, V.T., Vankawala, P.J., Kumar, K.N., Muthulingam, A., Scrinivas, G., Rao, C.S., Chandrasekhar, E.R.R. Aprepitant compositions US20090209541 (2009).
- [93] Ganeshbhatt, N., Raskilaltrivedi, N., Khedekar, M., Sinha, S., Ahmed, K.M., Yadav, R. Fosaprepitant Dimeglumine Intermediate, Neutral Fosaprepitant and amorphous Fosaprepitant Dimeglumine and processes for their preparations. WO2010018595 (2010).
- [94] Huang SC, Korlipara VL. Neurokinin-1 receptor antagonists: a comprehensive patent survey. *Expert Opin Therapeut Pat* 2010; 20(8): 1019-45.
- [95] Van Laere K, De Hoon J, Bormans G, Koole M, Derdelinckx I, De Lepeleire I, et al. Equivalent dynamic human brain NK1-receptor occupancy following single-dose i.v. fosaprepitant vs. oral aprepitant as assessed by PET imaging. *Clin Pharmacol Therapeut* 2012; 92(2): 243-50.
- [96] Allen JM, Hoyle NR, Yeats JC, Ghatei MA, Thomas DG, Bloom SR. Neuropeptides in neurological tumours. *J Neuro-oncol* 1985; 3(3): 197-202.
- [97] Singh D, Joshi DD, Hameed M, Qian J, Gascon P, Maloof PB, et al. Increased expression of preprotachykinin-I and neurokinin receptors in human breast cancer cells: implications for bone marrow metastasis. *Proc Nat Acad Sci USA* 2000; 97(1): 388-93.
- [98] Schulz S, Stumm R, Rocken C, Mawrin C, Schulz S. Immunolocalization of full-length NK1 tachykinin receptors in human tumors. *J Histochem Cytochem* 2006; 54(9): 1015-20.
- [99] Aalto Y, Forsgren S, Kjorell U, Bergh J, Franzen L, Henriksson R. Enhanced expression of neuropeptides in human breast cancer cell lines following irradiation. *Peptides* 1998; 19(2): 231-9.
- [100] Castro TA, Cohen MC, Rameshwar P. The expression of neurokinin-1 and preprotachykinin-1 in breast cancer cells depends on the relative degree of invasive and metastatic potential. *Clin Experim Metastas* 2005; 22(8): 621-8.
- [101] Ramkissoon SH, Patel PS, Tabora M, Rameshwar P. Nuclear factor-kappaB is central to the expression of truncated neurokinin-1 receptor in breast cancer: implication for breast cancer cell quiescence within bone marrow stroma. *Canc Res* 2007; 67(4): 1653-9.
- [102] Li X, Ma G, Ma Q, Li W, Liu J, Han L, et al. Neurotransmitter substance P mediates pancreatic cancer perineural invasion via NK-1R in cancer cells. *Mol Canc Res* 2013; 11(3): 294-302.
- [103] Munoz M, Covenas R. Involvement of substance P and the NK-1 receptor in pancreatic cancer. *World J Gastroenter* 2014; 20(9): 2321-34.
- [104] Fowler CJ, Brannstrom G. Substance P enhances forskolin-stimulated cyclic AMP production in human UC11MG astrocytoma cells. *Meth Findings Experimental Clin Pharmacol* 1994; 16(1): 21-8. Epub 1994/01/01.
- [105] Johnson CL, Johnson CG. Characterization of receptors for substance P in human astrocytoma cells: radioligand binding and inositol phosphate formation. *J Neurochem* 1992; 58(2): 471-7.
- [106] Eistetter HR, Mills A, Brewster R, Alouani S, Rambosson C, Kawashima E. Functional characterization of neurokinin-1 receptors on human U373MG astrocytoma cells. *Glia* 1992; 6(2): 89-95.
- [107] Akazawa T, Kwatra SG, Goldsmith LE, Richardson MD, Cox EA, Sampson JH, et al. A constitutively active form of neurokinin 1 receptor and neurokinin 1 receptor-mediated apoptosis in glioblastomas. *J Neurochem* 2009; 109(4): 1079-86.
- [108] Munoz M, Rosso M, Covenas R. A new frontier in the treatment of cancer: NK-1 receptor antagonists. *Curr Medic Chem* 2010; 17(6): 504-16.
- [109] Mayordomo C, Garcia-Recio S, Ametller E, Fernandez-Nogueira P, Pastor-Arroyo EM, Vinyals L, et al. Targeting of substance P induces cancer cell death and decreases the steady state of EGFR and Her2. *J Cell Physio* 2012; 227(4): 1358-66.
- [110] Munoz M, Rosso M. The NK-1 receptor antagonist aprepitant as a broad spectrum antitumor drug. *Investig New Drugs* 2010; 28(2): 187-93.
- [111] Lewis KM, Harford-Wright E, Vink R, Nimmo AJ, Ghabriel MN. Walker 256 tumour cells increase substance P immunoreactivity locally and modify the properties of the blood-brain barrier during extravasation and brain invasion. *Clin Experim Metastas* 2013; 30(1): 1-12.
- [112] Munoz, S.M. Use of non-peptidic NK1 receptor antagonist for the production of apoptosis in tumour cells. EP1803456 (2007).
- [113] Lewis KM, Harford-Wright E, Vink R, Ghabriel MN. NK1 receptor antagonists and dexamethasone as anticancer agents *in vitro* and in a model of brain tumours secondary to breast cancer. *Anti-cancer Drugs* 2013; 24(4): 344-54.
- [114] Lee CM, Kum W, Cockram CS, Teoh R, Young JD. Functional substance P receptors on a human astrocytoma cell line (U-373 MG). *Brain Res* 1989; 488(1-2): 328-31.
- [115] Otsuka M, Yoshioka K. Neurotransmitter functions of mammalian tachykinins. *Physiol Rev* 1993; 73(2): 229-308.
- [116] Yamaguchi K, Richardson MD, Bigner DD, Kwatra MM. Signal transduction through substance P receptor in human glioblastoma cells: roles for Src and PKCdelta. *Canc Chemother Pharmacol* 2005; 56(6): 585-93.
- [117] Harford-Wright E, Lewis KM, Ghabriel MN, Vink R. Treatment with the NK1 antagonist emend reduces blood brain barrier dysfunction and edema formation in an experimental model of brain tumors. *PLoS One* 2014; 9(5): e97002.
- [118] Donkin JJ, Nimmo AJ, Cernak I, Blumbergs PC, Vink R. Substance P is associated with the development of brain edema and functional deficits after traumatic brain injury. *J Cerebral Blood Flow Metabol* 2009; 29(8): 1388-98.
- [119] Turner RJ, Helps SC, Thornton E, Vink R. A substance P antagonist improves outcome when administered 4 h after onset of ischaemic stroke. *Brain Res* 2011; 1393: 84-90.
- [120] Unterberg AW, Stover J, Kress B, Kiening KL. Edema and brain trauma. *Neurosci* 2004; 129(4): 1021-9.
- [121] Zhang M, Olsson Y. Hematogenous metastases of the human brain—characteristics of peritumoral brain changes: a review. *J Neuro-oncol* 1997; 35(1): 81-9.
- [122] Thapar K, Rutka, JT, Laws, ER. Brain Edema, increased intracranial pressure, vascular effects, and other epiphenomena of human brain tumours. In: Livingston C, Ed. *Brain Tumours: An Encyclopedic Approach*. Edinburgh: 1995.
- [123] Reulen HJ, Huber P, Ito U, Groger U. Peritumoral brain edema. *Adv Neuro* 1990; 52: 307-15.
- [124] Ohnishi T, Sher PB, Posner JB, Shapiro WR. Capillary permeability factor secreted by malignant brain tumor. Role in peritumoral brain edema and possible mechanism for anti-edema effect of glucocorticoids. *J Neurosurg* 1990; 72(2): 245-51.
- [125] Heiss JD, Papavassiliou E, Merrill MJ, Nieman L, Knightly JJ, Walbridge S, et al. Mechanism of dexamethasone suppression of brain tumor-associated vascular permeability in rats. Involvement of the glucocorticoid receptor and vascular permeability factor. *J Clin Invest* 1996; 98(6): 1400-8.
- [126] Soffietti R, Ruda R, Mutani R. Management of brain metastases. *J Neuro* 2002; 249(10): 1357-69.
- [127] Dietrich J, Rao K, Pastorino S, Kesari S. Corticosteroids in brain cancer patients: benefits and pitfalls. *Exp Rev Clin Pharmacol* 2011; 4(2): 233-42.
- [128] Vink R, Young A, Bennett CJ, Hu X, Connor CO, Cernak I, et al. Neuropeptide release influences brain edema formation after

- diffuse traumatic brain injury. *Acta Neurochirurgica Supplement* 2003; 86: 257-60.
- [129] Fromm L, Heath DL, Vink R, Nimmo AJ. Magnesium attenuates post-traumatic depression/anxiety following diffuse traumatic brain injury in rats. *J Am College Nutr* 2004; 23(5): 529S-33S.
- [130] Campos MM, Calixto JB. Neurokinin mediation of edema and inflammation. *Neuropeptides* 2000; 34(5): 314-22.
- [131] Holzer P. Neurogenic vasodilatation and plasma leakage in the skin. *Gen Pharmacol* 1998; 30(1): 5-11.
- [132] Harrison S, Geppetti P. Substance p. *Int J Biochem Cell Biol* 2001; 33(6): 555-76.
- [133] Scholzen TE, Stander S, Riemann H, Brzoska T, Luger TA. Modulation of cutaneous inflammation by angiotensin-converting enzyme. *J Immuno* 2003; 170(7): 3866-73.
- [134] Emanuelli C, Grady EF, Madeddu P, Figini M, Bunnett NW, Parisi D, *et al.* Acute ACE inhibition causes plasma extravasation in mice that is mediated by bradykinin and substance P. *Hypertens* 1998; 31(6): 1299-304.
- [135] Nimmo AJ, Cernak I, Heath DL, Hu X, Bennett CJ, Vink R. Neurogenic inflammation is associated with development of edema and functional deficits following traumatic brain injury in rats. *Neuropeptides* 2004; 38(1): 40-7.
- [136] Hasenohrl RU, Frisch C, Nikolaus S, Huston JP. Chronic administration of neurokinin SP improves maze performance in aged *Rattus norvegicus*. *Behav Neural Biol* 1994; 62(2): 110-20.
- [137] Corrigan F, Leonard A, Ghabriel M, Van Den Heuvel C, Vink R. A substance P antagonist improves outcome in female Sprague Dawley rats following diffuse traumatic brain injury. *CNS Neurosci Therapeut* 2012; 18(6): 513-5.
- [138] Zacest AC, Vink R, Manavis J, Sarvestani GT, Blumbergs PC. Substance P immunoreactivity increases following human traumatic brain injury. *Acta Neurochirurgica Supplement* 2010; 106: 211-6.
- [139] Vink R, Nimmo, A.J. Brain, spinal and nerve injury treatment. WO200152844 (2001).
- [140] Nimmo, A.J., Whitfield, K.M., Turner, R.J., Vink, R. Method and composition for reducing reperfusion injury. US20100305183 (2010).
- [141] Vink, R., Nimmo, A.J. Method for reducing intracranial pressure. US20100184819 (2010).
- [142] Rahmathulla G, Toms SA, Weil RJ. The molecular biology of brain metastasis. *J Oncol* 2012; 2012: 723541..
- [143] Caffo M, Barresi V, Caruso G, Cutugno M, La Fata G, Venza M, *et al.* Innovative therapeutic strategies in the treatment of brain metastases. *Int J Mol Sci* 2013; 14(1): 2135-74.
- [144] Kumar S, Weaver VM. Mechanics, malignancy, and metastasis: the force journey of a tumor cell. *Canc Metastas Rev* 2009; 28(1-2): 113-27.
- [145] Steeg PS, Camphausen KA, Smith QR. Brain metastases as preventive and therapeutic targets. *Nat Rev Canc* 2011; 11(5): 352-63.
- [146] Eichler AF, Chung E, Kodack DP, Loeffler JS, Fukumura D, Jain RK. The biology of brain metastases-translation to new therapies. *Nat Rev Clin Oncol* 2011; 8(6): 344-56.
- [147] Rodriguez PL, Jiang S, Fu Y, Avraham S, Avraham HK. The pro-inflammatory peptide Substance P promotes blood-brain barrier breaching by breast cancer cells through changes in microvascular endothelial cell tight junctions. *Int J Canc* 2013.
- [148] Almendro, N., Gascon, V.P. Antibodies for treating cancer. US20110027296 (2011).
- [149] Witten, M., Harris, D.T. Substance P treatment for immunostimulation. EP0957930 (1999).
- [150] Harris DT, Witten M. Aerosolized substance P protects against cigarette-induced lung damage and tumor development. *Cell Mol Biol (Noisy-le-grand)* 2003; 49(2): 151-7.
- [151] Wilhelm, M., Siegel, H.N., Larrengia, A.T., Morelli, A.E. Substance P and analogs thereof as a cancer immunogenic composition adjuvant. WO2009129498 (2009).