Towards Drug Discovery for Brain Tumours: Interaction of Kinins and Tumours at the Blood Brain Barrier Interface

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

Adelaide Centre for Neuroscience Research, and the Discipline of Anatomy and Pathology, University of Adelaide, Adelaide, South Australia

Received: September 12, 2010; Accepted: October 27, 2010; Revised: October 29, 2010

Abstract: Cancers of the brain are intrinsically more complicated to treat than systemic malignancies due to the unique anatomical features of the brain. The blood-brain barrier prevents chemotherapeutic agents from reaching brain neoplasms, and angiogenesis occurs as the metabolic needs of the tumour increase, thus further complicating treatment. The newly formed blood vessels form the blood-tumour barrier and are distinct from the blood-brain barrier in that they are more permeable. Being more permeable, these abnormal blood vessels lead to the formation of peri-tumoural edema, which is the cause of much morbidity and mortality associated with central nervous system neoplasms. While the cause of the increased permeability is unclear, kinins have been implicated in regulating the permeability of normal vasculature. Kinins are also known to exert many inflammatory actions affecting both normal and angiogenic blood vessels, as well as tumour cells. The vasodilatory and vascular permeabilizing effects of kinins, and particularly bradykinin and substance P, have been investigated with regard to delivery of chemotherapeutic agents to neoplastic brain tissue through both vascular barriers. In contrast, kinin receptor antagonists have been found to exert effects on tumour cells that result in decreased angiogenesis, tumour cell motility and growth. Thus, many recent patents describe kinin activity on brain vasculature, which may play an integral role in the development of treatments for malignancies in the central nervous system through amelioration of angiogenesis. In conjunction, patents that discuss the ability of kinins to decrease tumour cell migration and proliferation demonstrate that kinins may offer novel approaches to brain tumour therapy in the future.

Keywords: CNS tumours, glioma, kinins, blood-brain barrier, substance P, bradykinin.

INTRODUCTION

Few disease states have received as much attention over the past decades as cancer. Worldwide, 10 million new cases and more than 6 million deaths occur due to cancer each year [1]. With the increasing incidence of cancer worldwide, research has been mainly focused on better understanding and developing effective treatments for this complex disorder. The multifaceted nature of cancer means that many mechanisms and genetic mutations contribute to carcinogenesis. Although this makes the disease inherently difficult to treat, it does present numerous potential treatment targets, many of which are under consideration today.

Primary brain tumours arise predominantly from the glial cells of the brain, whereas secondary brain tumours are malignancies that invade the brain from outside the central nervous system (CNS). Typically, primary brain neoplasms are more infiltrative in nature, whilst metastatic brain tumours compress the surrounding brain tissue, rather then invade it. Both primary and secondary brain tumours have a poor prognosis, despite advances in the diagnosis and treatment of CNS tumours; the majority of cases cannot be cured. At present, the standard treatment for brain tumours is surgical resection followed by radiation therapy. Surgery has long been advocated for the treatment of single brain tumours or in metastases patients with controlled systemic disease [2]. It is typically considered in any patient with a single brain malignancy in an accessible location, particularly when it is large and the mass effect of the lesion is significant. Complete surgical resection of a single tumour allows immediate relief of neurological symptoms such as intracranial hypertension, seizures and a reduction in focal neurological deficits. However, unless the tumour is extremely well encapsulated, it is difficult to completely remove all of it. Often microscopic foci of tumour cells not visible on MRI are left behind. Surgery thus tends to be followed by a course of postoperative radiotherapy.

Whole brain radiation therapy (WBRT) is the standard treatment for most patients with brain malignancies, as it remains the best alternative for patients with single metastases that are not surgically or radiosurgically resectable [3]. The majority of patients treated with WBRT have multiple metastases at the time of diagnosis making surgical or focal treatments ineffective [4]. The median survival of patients with brain metastases treated with WBRT is generally 3 to 6 months, with only 10% to 15 % surviving beyond a year [3]. However, most patients treated with WBRT die of a progressive systemic disease and not as a direct result of secondary brain tumours [5]. Complications of WBRT include temporary loss of hair and short term transient worsening of neurological symptoms. Unfortunately, due to the short survival time, long-term effects remain unknown.

Stereotactic radiosurgery (SRS) is increasingly being used in the treatment of brain tumours. SRS delivers a single,

*Address csorrespondence to this author at the Discipline of Anatomy and Pathology, School of Medical Sciences, The University of Adelaide, South Australia, 5005, Australia; Tel: +61 8 8303 4533; Fax: +61 8 8303 5384; E-mail: Robert.Vink@adelaide.edu.au

1574-8898/11 $100.00+.00

relatively high dose of radiation to a tumour with great accuracy. It aims to maximize the dose to the tumour whilst minimizing the effect on surrounding tissue [2]. Typically, SRS is reserved for patients with between one and three cerebral tumours that are less than 3cm in diameter, highly radioresistant and which are unable to be surgically resected [6]. Large tumours or tumours with extensive edema have been found to be difficult to control with SRS because of a high risk of radiation associated necrosis or neurological deterioration at biologically effective doses [6]. Thus, more than one course of SRS is rarely administered to the same site because of the increased risk of radiation necrosis.

Both primary and metastatic brain tumours are inherently difficult to treat by pharmacological means because the blood brain barrier (BBB) prevents most chemotherapeutic agents from reaching the cancerous tissue. In particular, brain metastases tend to respond poorly to chemotherapeutic agents, which may in part be due to the differences in chemosensitivity between primary and metastatic tumours. In metastases of previously treated tumours, clonal selection of chemoresistant tumour cells may already have occurred [7]. In small cell lung carcinoma, the response rate of cerebral metastases to chemotherapeutic agents without radiotherapy is only 53% when compared to a 79% response rate in the primary tumour [8]. Many methods of drug delivery that bypass the BBB and blood-tumour barrier have been investigated. Intraventricular administration of drugs is traumatic and further complicated by the blood cerebrospinal fluid barrier. Furthermore, intracerebral implants do not allow diffusion of medicants throughout the tumour, which is especially important for treatment of the highly infiltrative glioblastoma multiforme [9]. At present, arterial infusion of mannitol is used to create a controllable increase in BBB permeability and subsequent increase of drug delivery to tumours [10]. Mannitol causes the endothelial cells to shrink thereby disrupting the structure of the tight junctions and allowing spaces to form in between the cells [11]. However, this procedure not only allows entry of chemotherapeutic agents into the brain tumours, but also into the brain parenchyma, causing neural toxicity. In addition, this would increase the permeability of the BBB to larger blood proteins like albumin [12], which may result in further formation of brain edema and associated complications.

Corticosteroids have remained the primary treatment of tumour-associated edema for the past 40 years. By reducing the permeability of a compromised BBB, corticosteroids effectively reduce edema. It has been suggested one of the mechanisms by which this occurs is due to inhibition of phospholipase A_2, an enzyme responsible for arachidonic acid release [13]. Arachidonic acid destabilizes membrane lysosomes and has a direct destabilizing effect on cerebral capillaries. Corticosteroids are also known to have the ability to reduce VEGF-induced BBB permeability, an action reversed by a glucocorticoid receptor antagonist [14]. 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 [14]. Nonetheless, the exact mechanism of action of corticosteroids is yet to be elucidated.

Increasingly, research has been directed towards finding specific mediators that can be targeted in the treatment of cancer. Kinins and their receptors have been implicated in many aspects of cancer growth and progression, as well as in the development of cerebral edema. The revelation that bradykinin and tachykinin receptors are upregulated on brain tumour cells has stimulated research into the potential of kinin-mediated interventions in the treatment of brain malignancies. The aim of the current review is to give a broad outline of some of the kinin directed approaches to treating various aspects of CNS cancer. In particular, this review focuses on the interactions of kinins with the BBB and their role in neoplastic progression, as well as their potential for improving current treatments and developing novel interventions.

THE BLOOD BRAIN BARRIER

Brain malignancies are inherently different to their systemic counterparts due to the unique microenvironment of this organ. Unlike other tissues the brain is surrounded by the protective BBB, lacks lymphatic drainage and is bathed in an interstitial fluid high in chloride [3]. Consequently, trials of novel cancer treatments often exclude CNS neoplasms due to the complicating factors associated with the BBB [15].

The BBB is made up of brain capillary endothelial cells with tight junctions between them thus separating the blood within the cerebral capillaries from the brain parenchyma (Fig. **1**). It is a selective barrier, with nutrients such as glucose, amino acids, oxygen, carbon dioxide and some electrolytes passing through the endothelial cell membranes, while most other large molecules are excluded. It is, however, ineffective against lipophilic molecules like fatty acids that diffuse easily through the plasma membrane [16]. Macrophages are also able to infiltrate the brain parenchyma through an intact BBB that remains impermeable to many other solutes [17]. Finally, the barrier is also a dynamic system, the properties of which may change in response to external stimuli.

It is generally accepted that opening of tight junctions, fenestrations, gap junctions and increases in pinocytotic vesicles are the basis for barrier permeability in brain tumours (see Fig. (**1**)) [18-20]. Typically, the breakdown is confined to the pathologic margins of the tumour (see Fig. (**2**)), with no morphological evidence of increased permeability of the peritumoural capillaries. Unfortunately, breakdown of the BBB is associated with the development of cerebral edema, a significant contributor to morbidity and mortality in patients with brain tumours [21, 22]. Despite this, the BBB has been found to be intact in all small brain metastasis and all secondary tumours with a diffuse growth pattern [23] in studies using the protein dye sodium fluorescein. Indeed, the permeability in these lesions was not increased when compared to normal tissue. Accordingly, it has been suggested that the integrity of the barrier around small metastatic lesions may be restored after passage of metastatic cells into the brain parenchyma [23].

In the case of metastatic brain tumours these blood vessels do not retain the qualities of the BBB and have the characteristics of the tissue of origin [24]. Furthermore, the

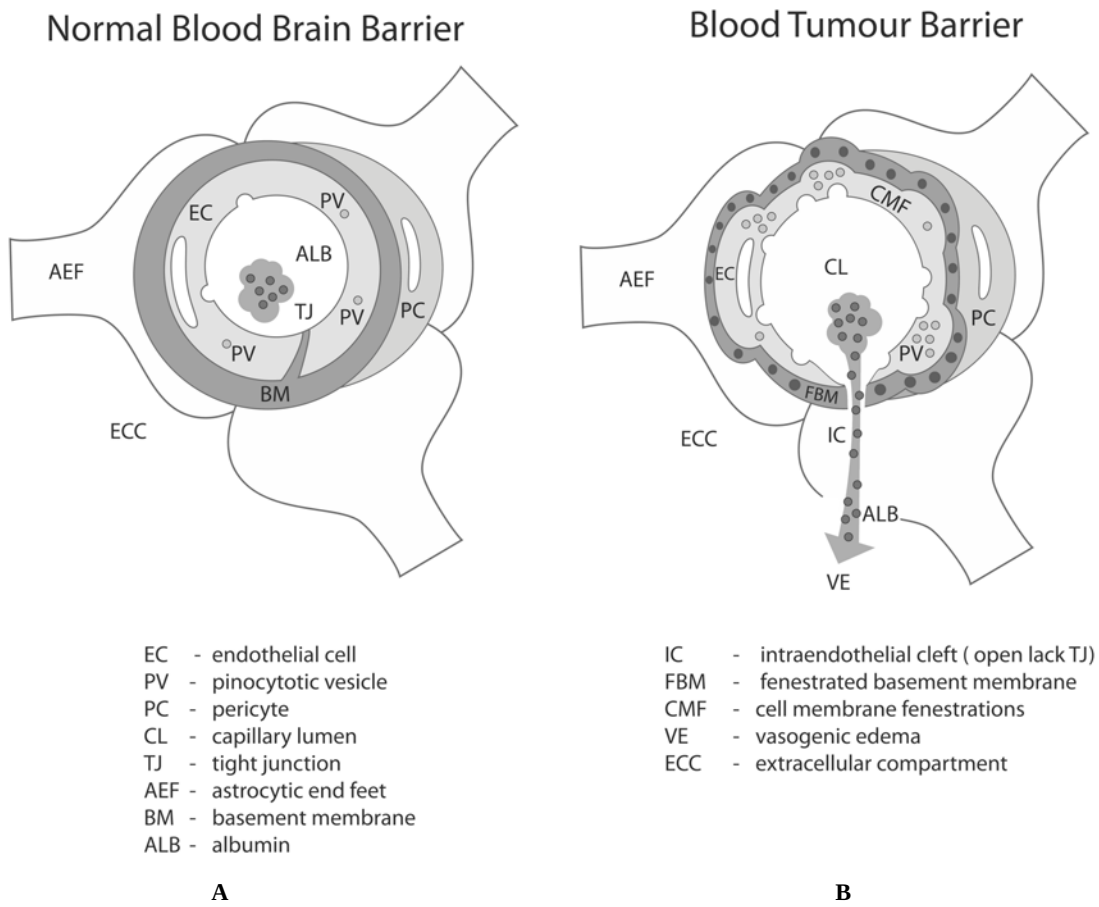

Fig. (1). Blood Brain Barrier Structure. A) Normal structure of the Blood Brain Barrier, **B**) as compared to the disrupted blood-brain barrier adjacent to tumours.

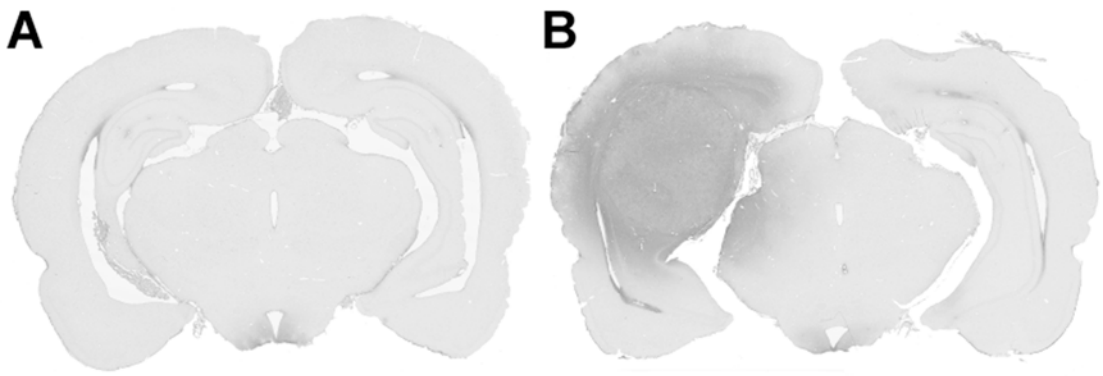

Fig. (2). Albumin immunoreactivity following tumour cell inoculation. A) Brain injected with culture medium (vehicle controls) reveals little albumin immunoreactivity indicating an intact blood-brain barrier. **B**) The brain from a tumour inoculated animal demonstrates increased albumin staining in the peritumoural region indicating increased permeability of the blood vessels.

rapid and continued proliferation of tumours means that angiogenic vessels formed within them are unable to go through a maturation phase. Consequently, the altered characteristics of the new tumour blood vessels do not resolve during this phase. Interestingly, some angiogenic vessels formed within brain tumours have been found to have altered reactions to vasoconstrictive agents, being insensitive to endothelin-1 and angiotensin II [25]. Tumour cell expression of vascular endothelial growth factor (VEGF) has been directly correlated with the rate of angiogenesis [26].

THE KININ FAMILY OF PEPTIDES

Kinins are peptide autocoids that have been implicated in a number of pathological processes. They include bradykinin, kallidin, tachykinins and urotensin 2, and their role in inflammation has been well established, including their ability to promote smooth muscle contraction, increase

vessel permeability, and promote vascular dilation and prostaglandin synthesis. Recent research has demonstrated that kinins also have proliferative and angiogenic effects, and thus may have a role in cancer growth and progression [27, 28]**.** In particular, the bradykinins and tachykinins have been the focus of much anti-cancer research.

Two biochemical cascades produce the kinins bradykinin and kallidin, one occurring within the plasma whilst the other takes place in tissues [29]. In the blood plasma, kallikrein acts on high molecular weight kininogen (HMWK) to generate bradykinin, whilst in body tissues low molecular weight kininogen (LMWK) is converted to kallidin. HMWK and LMWK are derived from alternate splicing of a single gene [30], whilst the amino acid sequence of kallidin differs from bradykinin only by the addition of a lysine molecule at the N-terminus. Bradykinin and kallidin exert their effects *via* the B1 and B2 receptors, with bradykinins predominantly acting on the B2 receptor. Both receptors are G-protein coupled receptors that share a seven transmembrane domain [30], and while the B1 receptor is typically expressed in very low levels, it can be upregulated in inflammatory conditions by cytokines. Within the central nervous system (CNS), the bradykinins have been implicated in nociception as well as a number of neurological disorders including epilepsy, stroke and multiple sclerosis [31-34].

Tachykinins are a group of small structurally related peptides characterized by the specific C-terminal sequence Phe-X-Gly-Lue-Met-NH_2. The tachykinin family includes substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) and are produced in both neuronal and glial cells of the CNS and peripheral nervous systems (PNS). The mammalian tachykinins are encoded for by two distinct genes, preprotachykinin A (PPTA) and preprotachykinin B (PPTB) [35]. Alternate splicing of the PPTA gene results in four different forms of mRNA: α-PPT, β-PPT, γ-PPT and δ-PPT [35]. The α-PPT and δ-PPT forms encode for SP alone, whilst β-PPT and γ-PPT encode both SP and NKA [36]. The PPTB gene gives rise to NKB and is expressed in the CNS but not in sensory neurons. Tachykinins are released from the ends of sensory nerve fibers and act on the transmembrane G-protein coupled receptors NK_1, NK_2 and NK_3 to exert a myriad of effects. Although tachykinins demonstrate the ability to interact which each kind of receptor, SP, NKA and NKB preferentially bind to the NK_1, NK_2 and NK_3 receptors, respectively. After binding with the appropriate ligand, tachykinin receptors undergo endocytosis, with chronic exposure of SP mediating NK_1 receptor downregulation [37, 38]. Like the bradykinins, tachykinins have been implicated in a variety of conditions in the CNS including traumatic brain injury, stroke, Parkinson's disease and depression [39-43].

The CNS contains all the components of the kallikrein-kinin system [29] with both bradykinins and tachykinins of increasing interest in the setting of CNS tumours.

KININS IN BRAIN TUMOURS

Kinins and their receptors are thought to be heavily involved in the development and progression of many neoplasms, as they are able to generate biochemical pathways associated with acceleration of cell growth. With respect to bradykinin, the B2 receptors are expressed in many tissue types under normal conditions, and a number of recent patents propose the use of B2 receptor antagonists in the treatment of cancer [44-47]. Indeed, these disclosures depict cancer as a bradykinin-mediated disease and accordingly propose B2 receptor antagonists for treatment of these disease processes, including glioma, astrocytoma and CNS metastases of lung and breast origin.

Kallikrein has also been the subject of a number of recent patent disclosures, particularly since it has been associated with tumour progression. There are 15 known kallikrein genes located at chromosome 19q13.3, which is altered in many cancerous conditions including human astrocytomas [48]. More specifically, the tissue kallikrein 5 (KLK5) and kallikrein 7 to 15 genes have all been implicated in hormone dependent tumour development in the brain [49, 50]. Some specific kallikreins also have functions that are unrelated to the kallikrein-kinin system [51]. Both human kallikrein 6 (hK6) and human kallikrein 8 (hK8) are typically expressed in glial cells in normal brain tissue [52]. Kallikrein is known to be expressed in both breast and lung cancer, with 22-30% of patients diagnosed with breast cancer and 15% of people with lung cancer will have a brain metastasis at the time of diagnosis [53-55]. As a result, many patents have focused on the fact that kallikreins are differentially expressed in cancerous tissues and the potential benefits of using this marker for diagnostic and prognostic purposes [51, 56-58]. Recently, a patent filed by Ladner *et al*. [59] described a preventative method for reducing ischemia by administering a kallikrein inhibitory polypeptide**.**

Tachykinins are known to have a stimulating or potentiating effect on lymphocyte proliferation and differentiation, cytokine secretion, and immunoglobulin production [60]. SP and NK_1 receptors have been implicated in numerous facets of cancer initiation and progression, with recent research linking the PPT-A gene and NK receptors in breast cancer development [61, 62]. Furthermore, examination of human gliomas has revealed a correlation between the increased density of NK_1 receptors and the degree of tumour malignancy [63]. NK_1 receptors have also been identified in intratumoral and peritumoural blood vessels, suggesting a potential role for SP in tumour angiogenesis [64]. Consequently, there is now considerable interest in developing novel pharmaceutical agents that target SP receptors in order to attenuate progression of brain malignancies.

KININS IN TUMOUR PROGRESSION

The increased presence of kinins and their receptors within CNS malignancies has stimulated considerable interest in the potential role they play in cancer progression. Bradykinin B1 receptors have been implicated in tumour growth despite the fact that their expression is normally low [65, 66]. Indeed, many glioma, astrocytic tumours, lung cancer, melanoma and breast cancer cell types express B1 receptors [67-69] and they show an increase in migratory activity when exposed to bradykinin, most likely mediated through a COX-2 pathway [70, 71]. Furthermore, administration of a bradykinin agonist resulted in B1 mediated tumour cell growth and proliferation, which was abolished following treatment with a B1 receptor antagonist [72].

Notably, the antagonist did not affect the basal level of proliferation seen before the application of the bradykinin agonist. Further supporting a role for bradykinin in tumour growth is the observation that B2 receptor antagonists are effective in reducing tumour growth and angiogenesis, but are not effective in rats unable to produce endogenous bradykinin [73]. Although both bradykinin B1 and B2 receptors are present on astrocytic tumour cells, B2 receptors are concentrated at the tumour border, whilst the B1 receptors are more ubiquitously expressed throughout the tumour [69]. As B1 receptors are more prevalent in tumour tissue compared with normal brain parenchyma, treatment with B1 antagonists is less likely to cause high levels of neuronal and glial toxicity.

With respect to the tachykinins, human gliomas and malignant brain tumours have an increased expression of NK_1 receptors compared to the normal surrounding tissue [63]. SP can also increase tumour growth in a number of glial tumour cell lines; the presence of neurokinin receptors correlated with a SP and/or an NKA mediated increase in DNA synthesis and cellular proliferation [74, 75]. The high number of NK_1 receptors increases the amount of mitotic signals directed at the tumour cell, which in turn counteracts the apoptotic pathways activated to remove mutated cells [60]. As such, the neoplastic cells are able to continue to divide and the disease allowed to progress. As SP is known to promote tumour cell growth, it was thought that this growth might be inhibited by administration of an NK_1 antagonist, and indeed, administration of L-733,060 was found to inhibit the progression of NK_1 expressing human glioma cancer cells [76]. Consequently, a number of patents have focused on the anti-proliferative properties of SP in brain tumour treatment [77, 78].

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 neutralize the multiple pathways leading to cell death. It has been suggested that the increase in the expression of NK_1 receptors may play a role in the malignant cell's evasion of apoptosis [60]. Such increased expression makes tumour cells highly dependent on SP stimuli, a known potent mitotic signal that counteracts the different death signal pathways [60, 79]. Consistent with this, it has been reported that administration of an NK_1 antagonist for two weeks in a nude mice model of cancer effectively inhibited tumour growth [80]. Blockade of the NK_1 receptor caused the cells to become more receptive to apoptotic death signals and die. Similarly, administration of the B2 receptor antagonist B204 showed 100% tumour growth inhibition in a model of small cell lung carcinoma, presumably by inducting apoptosis [46, 81].

Aside from mitogenic activity, the kinin SP is known to stimulate the release of cytokines [62]. Cytokines are involved in the communication between cells, coordinating cell growth and maturation, wound healing, the immune responses and contributing to neoangiogensis [82]. Tumour cells have the ability to secrete and produce cytokines, and thus support their own growth and facilitate metastatic spread. Such cytokine release from malignant cells may also induce normal cells to synthesize additional cytokines to further tumour progression [82]. Leukemia inhibiting factor (LIF) is a neuropoietin cytokine responsible for neurogenesis and neuronal differentiation and is able to stimulate glioma proliferation by induction of SP and NK_1 receptor expression [83]. LIF is produced and released by neuronal cells following proinflammatory cytokines leading to increased SP production and NK_1 expression [84].

KININS & ANGIOGENESIS

It has been well established that tumour growth is dependent upon angiogenesis, with any increase in tumour growth requiring a corresponding increase in vascular growth [85]. Tumour angiogenesis is an uncontrolled process, which is potentiated by interactions between numerous mediators and cytokines with both pro and anti-angiogenic activity [85]. As kinins are known to significantly effect vasculature, it follows that they may also have a role in tumour angiogenesis and tumour growth as detailed in Table **1**.

Hypoxia often results within rapidly growing tumours where angiogenesis does not occur sufficiently to meet the increased metabolic demand. This would stimulate bradykinin release, which in turn, elicits an angiogenic response *via* activation of VEGF [86]. It has been suggested that tumour growth and angiogenesis is mediated by B2 rather

Table 1. Kinin Actions on Tumour Cells and the Blood-tumour Barrier.

Kinins	Actions on Tumor Cells	Actions on the Blood-Tumor Barrier
Bradykinin	Acts on B1 and B2 receptors to increase tumour growth B1 and B2 receptors differentially expressed by tumour cells; the blocking of these receptors leads to decreased mitogenesis and in some cases apoptosis	Increase permeability for chemotherapy delivery to tumour cells Blocking B1 or B2 receptors may reduce angiogenesis depending on the level of receptor expression
Substance P	Acts on NK_1 receptors to increase tumour cell mitogenesis and migration NK_1 receptors expressed by some tumour cells, the blockade of which results in tumour cell apoptosis	NK_1 receptors expressed on tumour blood vessels, where Substance P acts to increase angiogenesis
Kallikreins	Expression may be altered in tumour cells, which is useful as a diagnostic tool	Inhibit endothelial cell migration and thus angiogenesis

than B1 receptors, as treatment with a B2 antagonist resulted in reduced tumour mass in a mouse S-180 sarcoma model, whilst B1 antagonism did not [87]. In contrast, B1 receptor antagonists and not B2 receptor antagonists were effective in suppressing blood vessel growth in an alternative model of angiogenesis [88]. It may be that the differential effect of endogenous bradykinin is dependent upon the relative levels of B1 receptor upregulation in the different models of cancer.

Kallikreins, particularly human kallikrein 3 (hK3), have been identified as inhibitors of endothelial cell migration and proliferation and subsequently angiogenesis *in vitro* and *in vivo* [89]. Thus, they may be a useful treatment for cancers that rely on angiogenesis for expansion of tumour mass. Different kalikreins can also be used in combination with each other to produce the desired anti-angiogenic effect, with human kallikrein (hK5), human kallikrein 6 (hK6), human kallikrein 10 (hK10) and human kallikrein 13 (hK13) suppressing capillary formation when applied together, at doses that had no effect when administered individually [90].

With respect to the tachykinins, an increase in SP receptors has been demonstrated in tumoral and peritumoural vessels in glioblastomas, which is thought to be significant due to the known vasodilative and angiogenic effects of SP [63]. Thus, SP may play a major role in development of tumour stroma and facilitate tumoral blood supply. Indeed, SP was found to enhance capillary growth *in vivo*, an effect that was abolished following administration of a NK_1 antagonist [64]. Furthermore, SP has the ability to enhance capillary growth, stimulate migration and proliferation of some types of endothelial cells, thus enhancing the angiogenic process in human tumours [85].

KININS IN PERITUMOURAL EDEMA

Peritumoural edema is a common symptom of brain tumours and is the cause of much morbidity and mortality. Edema in the brain is typically vasogenic in nature and results from a compromised BBB. Vasogenic edema is characterised by the abnormal increase in the volume of interstitial fluid and may be attributed to increased blood pressure, increased capillary permeability and decreased plasma protein concentration within the capillaries [22]. Furthermore, it is thought that vasogenic edema may provide a favourable environment for the proliferation of malignant cells [22].

Peritumoural edema is known to have local effects such as impairment of microcirculation, expansion of extracellular space and abnormalities in the fluid microenvironment, all of which may influence the structure and function of normal brain cells [22]. Edematous fluid accumulates rapidly around aggressive brain tumours [91]. Once excess extracellular fluid accumulates, mechanisms must exist to allow absorption so that the rate of fluid formation and absorption are equal. Edematous fluid is absorbed by transependymal flow into the ventricles, and interstitial proteins are phagocytosed by astrocytes and microglia, which results in the erosion of the osmotic gradient in the extracellular space and subsequent fluid absorption into microvessels [16].

Upregulation of the expression of water channel proteins is also associated with enhanced edematous fluid absorption. Aquaporins (AQPs) are a family of molecular water channels that are membrane spanning proteins expressed on the plasma membranes of many cells involved in water transport [92]. In particular, AQP-4 and AQP-1 are found in high concentration in brain tissue and are thought to be associated with alterations to normal brain water homeostasis. AQP-4 is abundant on the astrocytic foot processes near blood vessels, and as a bidirectional water channel, it is able to facilitate or reduce edema formation [92]. In rats, the AQP-4 channels are expressed in perimicrovessel astrocyte foot processes and alterations to AQP-4 are associated with disturbances to normal brain water homeostasis [93]. In addition, the pattern of expression of AQP-4 is correlated with BBB permeability and has been found to be upregulated in edematous astrocytomas, meningiomas and metastatic tumours, with a significant correlation between their expression level and edema [92, 94].

AQP-1 has also been implicated in peritumoural edema. When both melanoma tumour cells and breast cancer cells were transfected with AQP-1 and injected into the vasculature of mice, there was an increase in membrane permeability by 5-10 fold [95]. Furthermore, expression of AQP-1 increased the rate of tumour cell extravasation into lung tissue by 1.5 fold [95]. Deletion of AQP-1 has also been reported to result in less endothelial cell migration, and thus angiogenesis [96]. Although somewhat unclear, any AQP-1 enhanced migration may be mediated, in part, by the unequal rates of osmotic water flow across the membrane of the migrating cell, propelling the cell towards hypo-osmolality and augmenting the actions of the actin cytoskeleton [95, 96].

Tachykinins are also known to contribute to cerebral edema in a number of brain pathologies through a process known as neurogenic inflammation. Neurogenic inflammation is characterised by fluid movement from cerebral vasculature into the intercellular space as a result of vasodilation and plasma extravasation [97]. In a model of traumatic brain injury, depletion of sensory neuropeptides with capsaicin completely attenuated the changes in BBB permeability and subsequent post-traumatic edema formation [98]. Subsequent reports demonstrated that an upregulation of perivascular SP occurs after injury, which is associated with increased BBB permeability and the development of cerebral edema [99]. Moreover, administration of an NK_1 antagonist was shown to attenuate these increases [99]. Similarly, bradykinin has been shown to perpetuate cerebral edema in ischemic stroke, reperfusion injury and TBI [100, 101]. Blocking both B2 and B1 receptors has been effective in ameliorating increases in BBB permeability and in reducing edematous fluid accumulation in a model of ischemia and reperfusion injury, with B2 receptor blockade being the most effective of the two treatments [101]. Equally, B2 receptor knockout mice showed significantly decreased brain edema, contusion size and improved functional outcome 7 days after controlled cortical impact, when compared to their wild type counterparts [100]. Accordingly, it has been proposed that these peptide mediators of vascular permeability may play a critical role in the development of pertumoural edema [22].

KININS & CURRENT TREATMENTS

As previously discussed, the use of chemotherapeutic agents in the treatment of brain tumours can be challenging due to the protective nature of the BBB. Accordingly, there has been considerable interest in the use of kinin agonists to aid in the delivery of chemotherapeutic agents to brain tumours. Bradykinin is known to reduce cerebral blood flow and increase BBB permeability through increased pinocytotic activity in cerebral endothelial cells and opening of tight junctions [102]. However, bradykinin is degraded on the order of seconds by kinases in the blood. Consequently, its actions are short lived and lead to a refractory period in which B2 receptors are internalised and bradykinin no longer affects the blood-tumour barrier [103]. Thus, non-peptide bradykinin agonists have been developed to allow for a longer period of action [103]. The intravenous administration of bradykinin agonists allows low dose intermittent infusions over a long period of time, thereby prolonging action. Additionally, if patient sensitivity occurs, the infusion can be easily terminated. However, it should be noted that due to the integral role of bradykinin in blood pressure, intravenous bradykinin administration could result in an adverse hypotensive outcome [9].

In contrast with this overall increase in BBB permeability, the patent disclosure by Black *et al.* [104] reported that carotid artery infusion of bradykinin or a bradykinin agonist selectively increases the permeability of the abnormal blood vessels of the blood-tumour barrier. Experimental treatment with a bradykinin agonist and the chemotherapeutic agent cisplatin resulted in an increase in survival in brain tumour subjects when compared to cisplatin treatment alone [105]. Similar results were observed in glioma bearing rats treated with a different bradykinin agonist and a chemotherapeutic agent [106]. Some variability in response to bradykinin treatment has been noted with different administrative routes, although this may be due to differences in B2 receptor density in the angiogenic blood vessels within brain tumours. Expression of B2 receptors in gliomas corresponds with the grade of tumour and subsequent degree of blood-tumour barrier opening [107]. The mechanism of bradykinin-induced increase in blood-tumour barrier permeability is most likely through the actions of nitric oxide (NO), cyclic guanosine monophosphate (cGMP) and calcium dependant potassium channels [108-110].

Subsequent studies have moved away from looking at bradykinin agonists, which have broad vascular effects, to investigating more specific downstream bradykinin initiated events. In order to eliminate side effects, calcium activated potassium channels, cGMP and nitric oxide synthase have been investigated as potential targets for refinement of bradykinin mediated effects on CNS tumour vasculature. In pathological tissues, bradykinin increases production of cGMP resulting in increased microvessel permeability [109]. Simultaneous administration of GMP phosphodiesterase inhibitors and bradykinin agonists allows for a further and more prolonged increase in permeability of abnormal vessels, beyond that seen with the bradykinin agonist alone [111]. Vardenafil, which blocks the phosphodiesterase-5 (PDE5) mediated increase in cGMP, increases the concentration of vesicles in brain tumour endothelial cell cytoplasm, but has no effect on the degree of opening of the tight junctions between these cells [109]. Treatment of brain tumour bearing mice with vardenafil along with the chemotherapeutic agent herceptin, resulted in 20% longer survival and than herceptin alone [112]. A further advantage of vardenafil is that it can be administered orally.

Bradykinin is thought to increase calcium levels, *via* stimulation of B2 receptors, and thus activate calcium dependant potassium channels and nitric oxide synthase. Notably, increased expression of calcium dependant potassium channels has been demonstrated in brain tumours [110]. Exposure to bradykinin causes in an increase in calcium flux resulting in loosening of tight junctions and an increase in pinocytotic activity [110]. However, bradykinin has been recently implicated in side effects that include reduced cerebral blood flow due to excessively low blood pressure and associated brain edema. Therefore, the use of potassium channel agonists other than bradykinin has been patented [113]. The effect of calcium activated potassium channel agonists on the vessels of the blood-tumour barrier lasts three times longer than that elicited by application of a bradykinin agonist to the same system [110]. Another patent disclosure describes the use of a potassium channel agonists to induce selective apoptosis in glioma cells, leaving normal brain cells unaffected [114]. A more recent invention relates to ATP-dependent potassium channel agonists, which have been found to possess anti-neoplastic properties. The inventors suggest that administration of this drug may be associated with prolonged survival time of cancer patients [115].

Radiotherapy is currently the standard treatment following surgery for brain tumours. Radiopharmaceuticals are highly specific drugs that contain atoms of a radioactive element, and deliver relatively high doses of ionizing radiation to disease sites. The success of peptide receptor radiotherapy in somatostatin receptor positive cancers has led to the development of this treatment targeting other peptides known to have increased expression in other cancers [116, 117]. Recently, Merlo *et al.* [118] have disclosed the use of SP as a radiopharmaceutical in the diagnosis and treatment of cerebral malignancies. Numerous studies have demonstrated increased expression of the NK_1 receptor expressed in cerebral tumours, as well as in the tumoural and peritumoural vessels [63, 79, 118]. Another approach uses SP as a radiopharmaceutical in combination with surgery to further improve outcome. The purpose of a study by Cordier *et al.* [119] was to reduce and define the main glioma tumour mass, whilst targeting tumour cells in adjacent areas prior to surgery. In subjects that received intratumoural injections of radiolabelled SP, the extent of tumour resection was in the range of 90 to 100%. Higher extent of resection has been shown to positively correlate with a prolonged time to recurrence [119] and thus, may provide a novel treatment that can be used in conjunction with the conventional treatments for brain tumours. Another novel invention was presented recently by Schoor *et al.* [120] related to immunotherapeutic peptides and their use for the treatment of glioblastoma. The inventors reveal that a composition of tumour-associated peptides can be used in conjunction with anti-cancer vaccines to elicit an anti-tumour response.

CURRENT & FUTURE DEVELOPMENTS

Research over the past decade has emphasized the importance of peptide receptor expression in various human cancers. Cancer is a complex and multifaceted disorder with many mechanisms and processes contributing to its development. Accordingly, there are numerous points of potential interventions, as well as many markers of malignancy. The revelation that kinin receptors are upregulated in brain tumours may present new avenues for the development of clinical interventions. Furthermore, the implication that brain tumour interactions with the blood-brain barrier may be mediated, at least in part, by kinins have led to important developments in anti-angiogenic treatments and methods to aid drug delivery to brain tumours. Interestingly, current research has moved away from using kinin agonists to aid drug delivery to the brain and is now focusing on the downstream events mediated by the kinin system as potential treatment targets for improved chemotherapeutic delivery. As the kinin system produces a variety of different effects, by targeting more specific molecules only the blood tumour barrier will be affected and deleterious side effects may be minimised. Furthermore, future research should be directed towards clarifying the role of the B_1 and B_2 receptors in angiogenesis and tumour growth, as the evidence in the literature for the role of each receptor remains somewhat contradictory.

ACKNOWLEDGEMENTS

Supported, in part, by the Neurosurgical Research Foundation (Adelaide). We thank Tavik Morgenstern for designing the biomedical illustration.

CONFLICT OF INTEREST

Authors do not have any conflicts of interest.

REFERENCES

[1] World Health Organization. National Cancer Control Programmes: Policies and Managerial Guidelines. Geneva, Switzerland: World Health Organization 2002.

[2] Bafaloukos D, Gogas H. The treatment of brain metastases in melanoma patients. Cancer Treat Rev 2004; 30: 515-20.

[3] Greenberg HS, Chandler, WF, Sandler HM. Brain Tumours. New York: Oxford Univerity Press 1999.

[4] Patchell RA, Tibbs PA, Walsh JW, Dempsey RJ, Maruyama Y, Kryscio RJ, *et al.* A randomized trial of surgery in the treatment of single metastases to the brain. N Engl J Med 1990; 322: 494-500.

[5] Berk L. An overview of radiotherapy trials for the treatment of brain metastases. Oncology 1995; 9: 1205-12.

[6] Ranjan T, Abrey LE. Current management of metastatic brain disease. Neurotherapeutics 2009; 6: 598-603.

[7] 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. Cancer Metastasis Rev 2001; 20: 13-25.

[8] Twelves CJ, Souhami RL, Harper PG, Ash CM, Spiro SG, Earl HM, *et al*. The response of cerebral metastases in small cell lung cancer to systemic chemotherapy. Br J Cancer 1990; 61: 147-50.

[9] Black KL, Ningaraj NS. Modulation of brain tumor capillaries for enhanced drug delivery selectively to brain tumor. Cancer Control 2004; 11: 165-73.

[10] Neuwelt EA, Barnett PA, McCormick CI, Remsen LG, Kroll RA, Sexton G. Differential permeability of a human brain tumor xenograft in the nude rat: Impact of tumor size and method of administration on optimizing delivery of biologically diverse agents. Clin Cancer Res 1998; 4: 1549-55.

[11] Farkas A, Szatmari E, Orbok A, Wilhelm I, Wejksza K, Nagyoszi P, *et al.* Hyperosmotic mannitol induces Src kinase-dependent phosphorylation of beta-catenin in cerebral endothelial cells. J Neurosci Res 2005; 80: 855-61.

[12] Kroll RA, Pagel MA, Muldoon LL, Roman-Goldstein S, Fiamengo SA, Neuwelt EA. Improving drug delivery to intracerebral tumor and surrounding brain in a rodent model: A comparison of osmotic versus bradykinin modification of the blood-brain and/or blood-tumor barriers. Neurosurgery 1998; 43: 879-86.

[13] 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: 245-51.

[14] 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: 1400-8.

[15] Puduvalli VK. Brain metastases: Biology and the role of the brain microenvironment. Curr Oncol Rep 2001; 3: 467-75.

[16] Thapar K, Rutka JT, Laws ER. Brain Edema, Increased Intracranial Pressure, Vascular Effects, and other Epiphenomena of Human Brain Tumours. Brain Tumours: An Encyclopedic Approach. Edinburgh: Churchill Livingston 1995: 163-90.

[17] Schackert G, Simmons RD, Buzbee TM, Hume DA, Fidler IJ. Macrophage infiltration into experimental brain metastases: Occurrence through an intact blood-brain barrier. J Natl Cancer Inst 1988; 80: 1027-34.

[18] Strugar J, Rothbart D, Harrington W, Criscuolo GR. Vascular permeability factor in brain metastases: Correlation with vasogenic brain edema and tumor angiogenesis. J Neurosurg 1994; 81: 560-6.

[19] Shibata S. Ultrastructure of capillary walls in human brain tumors. Acta Neuropathol 1989; 78: 561-71.

[20] Hirano A, Matsui T. Vascular structures in brain tumors. Hum Pathol 1975; 6: 611-21.

[21] Criscuolo GR. The genesis of peritumoral vasogenic brain edema and tumor cysts: A hypothetical role for tumor-derived vascular permeability factor. Yale J Biol Med 1993; 66: 277-314.

[22] Zhang M, Olsson Y. Hematogenous metastases of the human brain--characteristics of peritumoral brain changes: A review. J Neurooncol 1997; 35: 81-9.

[23] 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: 1115-24.

[24] Long DM. Capillary ultrastructure in human metastatic brain tumors. J Neurosurg 1979; 51: 53-8.

[25] Andrade SP, Beraldo WT. Pharmacological reactivity of neoplastic and non-neoplastic associated neovasculature to vasoconstrictors. Int J Exp Pathol 1998; 79: 425-32.

[26] Yano S, Shinohara H, Herbst RS, Kuniyasu H, Bucana CD, Ellis LM, *et al.* Expression of vascular endothelial growth factor is necessary but not sufficient for production and growth of brain metastasis. Cancer Res 2000; 60: 4959-67.

[27] Silvestre JS, Bergaya S, Tamarat R, Duriez M, Boulanger CM, Levy BI. Proangiogenic effect of angiotensin-converting enzyme inhibition is mediated by the bradykinin B(2) receptor pathway. Circ Res 2001; 89: 678-83.

[28] Duchene J, Schanstra JP, Pecher C, Pizard A, Susini C, Esteve JP, *et al.* A novel protein-protein interaction between a G protein-coupled receptor and the phosphatase SHP-2 is involved in bradykinin-induced inhibition of cell proliferation. J Biol Chem 2002; 277: 40375-83.

[29] Walker K, Perkins M, Dray A. Kinins and kinin receptors in the nervous system. Neurochem Int 1995; 26: 1-16.

[30] Rodi D, Couture R, Ongali B, Simonato M. Targeting kinin receptors for the treatment of neurological diseases. Curr Pharm Des 2005; 11:1313-26.

[31] Calixto JB, Medeiros R, Fernandes ES, Ferreira J, Cabrini DA, Campos MM. Kinin B1 receptors: Key G-protein-coupled receptors and their role in inflammatory and painful processes. Br J Pharmacol 2004; 143: 803-18.

[32] Pereira MG, Gitai DL, Paco-Larson ML, Pesquero JB, Garcia-Cairasco N, Costa-Neto CM. Modulation of B1 and B2 kinin receptors expression levels in the hippocampus of rats after

audiogenic kindling and with limbic recruitment, a model of temporal lobe epilepsy. Int Immunopharmacol 2008; 8: 200-5.

[33] Francel PC. Bradykinin and neuronal injury. J Neurotrauma 1992; 9 (Suppl 1): S27-45.

[34] Prat A, Biernacki K, Saroli T, Orav JE, Guttmann CR, Weiner HL, *et al.* Kinin B1 receptor expression on multiple sclerosis mononuclear cells: Correlation with magnetic resonance imaging T2-weighted lesion volume and clinical disability. Arch Neurol 2005; 62: 795-800.

[35] Hokfelt T, Pernow B, Wahren J. Substance P. A pioneer amongst neuropeptides. J Intern Med 2001; 249: 27-40.

[36] Schaffer M, Beiter T, Becker HD, Hunt TK. Neuropeptides: Mediators of inflammation and tissue repair? Arch Surg 1998; 133: 1107-16.

[37] Tsao P, Cao T, von Zastrow M. Role of endocytosis in mediating downregulation of G-protein-coupled receptors. Trends Pharmacol Sci 2001; 22: 91-6.

[38] Cottrell GS, Padilla B, Pikios S, Roosterman D, Steinhoff M, Gehringer D, *et al.* Ubiquitin-dependent down-regulation of the neurokinin-1 receptor. J Biol Chem 2006; 281: 27773-83.

[39] Harford-Wright E, Thornton E, Vink R. Angiotensin-converting enzyme (ACE) inhibitors exacerbate histological damage and motor deficits after experimental traumatic brain injury. Neurosci Lett 2010; 481: 26-9.

[40] Vink R, van den Heuvel C. Substance P antagonists as a therapeutic approach to improving outcome following traumatic brain injury. Neurotherapeutics 2010; 7: 74-80.

[41] Turner RJ, Blumbergs PC, Sims NR, Helps SC, Rodgers KM, Vink R. Increased substance P immunoreactivity and edema formation following reversible ischemic stroke. Acta Neurochir Suppl 2006; 96: 263-6.

[42] Thornton E, Tran TT, Vink R. A substance P mediated pathway contributes to 6-hydroxydopamine induced cell death. Neurosci Lett 2010; 481: 64-7.

[43] Kramer MS, Cutler N, Feighner J, Shrivastava R, Carman J, Sramek JJ, *et al.* Distinct mechanism for antidepressant activity by blockade of central substance P receptors. Science 1998; 281: 1640-5.

[44] Katsu, Y.T., Kawai, M., Koike, H., Nukui, S. N-benzenesulfonyl L-proline compounds as bradykinin antagonists. US6734306 (**2004**).

[45] Shaw, C.C., Hirst, D., Chen, T., O'rourke, M., Rao, P. Bradykinin B2 receptor antagonist peptide from amphibian skin. US20080044463 (**2008**).

[46] Stewart, J.M.D., Chan, D.C., Whalley, E.T., Gera, L. Cytolytic bradykinin antagonists. US5849863 (**1998**).

[47] Stewart, J.M.D., Gera, L. Bradykinin antagonists containing pentafluorophenylalanine. US5935932 (**1999**).

[48] Goyal J, Smith KM, Cowan JM, Wazer DE, Lee SW, Band V. The role for NES1 serine protease as a novel tumor suppressor. Cancer Res 1998; 58: 4782-6.

[49] Clements JA, Willemsen NM, Myers SA, Dong Y. The tissue kallikrein family of serine proteases: Functional roles in human disease and potential as clinical biomarkers. Crit Rev Clin Lab Sci 2004; 41: 265-312.

[50] Li B, Goyal J, Dhar S, Dimri G, Evron E, Sukumar S, *et al.* CpG methylation as a basis for breast tumor-specific loss of NES1/kallikrein 10 expression. Cancer Res 2001; 61: 8014-21.

[51] Golz, S.E., Bruggemeter, U., Geerts, A., Summer, H. Diagnostics and Therapeutics for Diseases Associated with Kallikrein 2 (KLK2). US20070218047 (**2007**).

[52] Singh J, Naran A, Misso NL, Rigby PJ, Thompson PJ, Bhoola KD. Expression of kallikrein-related peptidases (KRP/hK5, 7, 6, 8) in subtypes of human lung carcinoma. Int Immunopharmacol 2008; 8: 300-6.

[53] Hines SL, Vallow LA, Tan WW, McNeil RB, Perez EA, Jain A. Clinical outcomes after a diagnosis of brain metastases in patients with estrogen- and/or human epidermal growth factor receptor 2-positive versus triple-negative breast cancer. Ann Oncol 2008; 19: 1561-5.

[54] Crivellari D, Pagani O, Veronesi A, Lombardi D, Nole F, Thurlimann B, *et al.* High incidence of central nervous system involvement in patients with metastatic or locally advanced breast cancer treated with epirubicin and docetaxel. Ann Oncol 2001; 12: 353-6.

[55] Hochstenbag MM, Twijnstra A, Wilmink JT, Wouters EF, ten Velde GP. Asymptomatic brain metastases (BM) in small cell lung cancer (SCLC): MR-imaging is useful at initial diagnosis. J Neurooncol 2000; 48: 243-8.

[56] Dong, Y.Q., Clements, J.A. Diagnosis of cancer or benign tumor using the aberrant expression product of the klk4 gene. US20040137455 (**2004**).

[57] Yousef, G.S.J., Diamandis, E.P. Methods for detecting breast and ovarian cancer. US20060073525 (**2006**).

[58] Mack, D.H.M.P., Gish, K.C., Afar, D. Methods of diagnosis of breast cancer, compositions and methods of screening for modulators of breast cancer US20040029114 (**2004**).

[59] Ladner, R.C., Ley, A.C., Hirani, S., Williams, A., Simoni, M.G., Bergamaschini, L. Kallikrein-inhibitor therapies. US7704949 (**2010**).

[60] 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. Cancer Metastasis Rev 2006; 25: 137-45.

[61] Singh AS, Caplan A, Corcoran KE, Fernandez JS, Preziosi M, Rameshwar P. Oncogenic and metastatic properties of preprotachykinin-I and neurokinin-1 genes. Vascul Pharmacol 2006; 45: 235-42.

[62] 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 Neuropathol 2007; 45: 99-107.

[63] Hennig IM, Laissue JA, Horisberger U, Reubi JC. Substance-P receptors in human primary neoplasms: Tumoral and vascular localization. Int J Cancer 1995; 61: 786-92.

[64] Ziche M, Morbidelli L, Pacini M, Geppetti P, Alessandri G, Maggi CA. Substance P stimulates neovascularization *in vivo* and proliferation of cultured endothelial cells. Microvasc Res 1990; 40: 264-78.

[65] Tung, J.S.B., Garofalo, A.W., Pleiss, M.A., Wu, J., Wone, D.W.G., Guinn, A.C., Dressen, D.B., Neitz, J.R., Marugg, J., Neitzel, M. Substituted pyrazole derivatives and related compounds as bradykinin B_1 receptor antagonists. US7432379 (**2008**).

[66] Su, D., Bock, M.G. Novel quinoxalinone derivatives as bradykinin B1 antagonists. US20040132733 (**2004**).

[67] Wu J, Akaike T, Hayashida K, Miyamoto Y, Nakagawa T, Miyakawa K, *et al.* Identification of bradykinin receptors in clinical cancer specimens and murine tumor tissues. Int J Cancer 2002; 98(1): 29-35.

[68] Andoh T, Akira A, Saiki I, Kuraishi Y. Bradykinin increases the secretion and expression of endothelin-1 through kinin B2 receptors in melanoma cells. Peptides 2010; 31: 238-41.

[69] Raidoo DM, Sawant S, Mahabeer R, Bhoola KD. Kinin receptors are expressed in human astrocytic tumour cells. Immunopharmacology 1999; 43: 255-63.

[70] Lu DY, Leung YM, Huang SM, Wong KL. Bradykinin-induced cell migration and COX-2 production mediated by the bradykinin B1 receptor in glioma cells. J Cell Biochem 2010; 110: 141-50.

[71] Ifuku M, Farber K, Okuno Y, Yamakawa Y, Miyamoto T, Nolte C, *et al.* Bradykinin-induced microglial migration mediated by B1-bradykinin receptors depends on Ca^{2+} influx *via* reverse-mode activity of the Na^+/Ca^{2+} exchanger. J Neurosci 2007; 27: 13065-73.

[72] Nantel, F.S., Chammas, R., Sirois, P., Battistini, B.J. Method of treating cancer and neuropathic complications induced by chemotherapeutic agents using selective Bradykinin B1 receptor antagonists. US20070015715 (**2007**).

[73] Ikeda Y, Hayashi I, Kamoshita E, Yamazaki A, Endo H, Ishihara K, *et al.* Host stromal bradykinin B2 receptor signaling facilitates tumor-associated angiogenesis and tumor growth. Cancer Res 2004; 64: 5178-85.

[74] 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. Cancer Res 1996; 56: 4983-91.

[75] 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 Invest 2003; 83: 731-42.

[76] Munoz M, Rosso M, Perez A, Covenas R, Rosso R, Zamarriego 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. Neuropeptides 2005; 39: 427-32.

[77] Burman, A.C.G., Prasad, S., Mukherjee, R., Jaggi, M., Singh, A.T. Substance P analogs for the treatment of cancer. US6596692 (**2003**).

[78] Maier, N.C.I.P., Jarstfer, A.G. System and method for inhibiting cellular proliferation with tachykinins. US20107691817 (**2010**).

[79] 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: 1079-86.

[80] 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. Anticancer Drugs 2005; 16: 1083-9.

[81] Chan D, Gera L, Helfrich B, Helm K, Stewart J, Whalley E, *et al.* Novel bradykinin antagonist dimers for the treatment of human lung cancers. Immunopharmacology 1996; 33: 201-4.

[82] Dunlop RJ, Campbell CW. Cytokines and advanced cancer. J Pain Symptom Manage 2000; 20: 214-32.

[83] Palma C, Maggi CA. The role of tachykinins *via* NK1 receptors in progression of human gliomas. Life Sci 2000; 67: 985-1001.

[84] Hu CP, Feng JT, Tang YL, Zhu JQ, Lin MJ, Yu ME. LIF upregulates expression of NK-1R in NHBE cells. Mediators Inflamm 2006; 2006: 84829.

[85] Ribatti D, Conconi MT, Nussdorfer GG. Nonclassic endogenous novel regulators of angiogenesis. Pharmacol Rev 2007; 59: 185-205.

[86] Stewart JM. Bradykinin antagonists as anti-cancer agents. Curr Pharm Des 2003; 9: 2036-42.

[87] Ishihara K, Hayash I, Yamashina S, Majima M. A potential role of bradykinin in angiogenesis and growth of S-180 mouse tumors. Jpn J Pharmacol 2001; 87: 318-26.

[88] Hu DE, Fan TP. [Leu8]des-Arg9-bradykinin inhibits the angiogenic effect of bradykinin and interleukin-1 in rats. Br J Pharmacol 1993; 109:14-7.

[89] Holaday, J.W.B., Fortier, A.H. Compositions and methods for inhibiting endothelial cell proliferation and regulating angiogenesis using cancer markers. US20036544947 (**2003**).

[90] Chu, C., Shao, Z., Yang, Q., Huppe, G. Therapeutic uses of kallikreins. US20060287231 (**2006**).

[91] Reulen HJ, Huber P, Ito U, Groger U. Peritumoral brain edema. A keynote address. Adv Neurol 1990; 52: 307-15.

[92] Ng WH, Hy JW, Tan WL, Liew D, Lim T, Ang BT, *et al.* Aquaporin-4 expression is increased in edematous meningiomas. J Clin Neurosci 2009; 16: 441-3.

[93] Saadoun S, Papadopoulos MC, Davies DC, Krishna S, Bell BA. Aquaporin-4 expression is increased in oedematous human brain tumours. J Neurol Neurosurg Psychiatry 2002; 72: 262-5.

[94] Saadoun S, Papadopoulos M, Bell B, Krishna S, Davies D. The aquaporin-4 water channel and brain tumour oedema. J Anat 2002; 200: 528.

[95] Hu J, Verkman AS. Increased migration and metastatic potential of tumor cells expressing aquaporin water channels. FASEB J 2006; 20: 1892-4.

[96] Papadopoulos MC, Saadoun S, Verkman AS. Aquaporins and cell migration. Pflugers Arch 2008; 456: 693-700.

[97] Turner R, Vink R. Inhibition of neurogenic inflammation as a novel treatment for ischemic stroke. Timely Top Med Cardiovasc Dis 2007; 11: E24.

[98] 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 Neurochir Suppl 2003; 86: 257-60.

[99] Nimmo, A.J., Vink, R. Brain, spinal, and nerve injury treatment. US20056841551 (**2005**).

[100] Trabold R, Eros C, Zweckberger K, Relton J, Beck H, Nussberger J, *et al.* The role of bradykinin B(1) and B(2) receptors for secondary brain damage after traumatic brain injury in mice. J Cereb Blood Flow Metab 2010; 30:130-9.

[101] Su J, Cui M, Tang Y, Zhou H, Liu L, Dong Q. Blockade of bradykinin B2 receptor more effectively reduces postischemic blood-brain barrier disruption and cytokines release than B1 receptor inhibition. Biochem Biophys Res Commun 2009; 388: 205-11.

[102] 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: 1153-68.

[103] Dodey PF, Luccarini J, Martinez J, Amblard M, Daffix I. Peptide agonists of bradykinin B2 receptor. US20016316413 (**2001**).

[104] Black, K.L. Enhanced opening of abnormal brain tissue capillaries. US19966043223 (**1996**).

[105] Malfroy-Camine B. Method for increasing blood-brain barrier permeability by administering a bradykinin agonist of blood-brain barrier permeability. US5112596 (**1992**).

[106] Matsukado K, Inamura T, Nakano S, Fukui M, Bartus RT, Black KL. Enhanced tumor uptake of carboplatin and survival in glioma-bearing rats by intracarotid infusion of bradykinin analog, RMP-7. Neurosurgery 1996; 39: 125-33.

[107] Zhao Y, Xue Y, Liu Y, Fu W, Jiang N, An P, *et al.* Study of correlation between expression of bradykinin B2 receptor and pathological grade in human gliomas. Br J Neurosurg 2005; 19: 322-6.

[108] Nakano S, Matsukado K, Black KL. Increased brain tumor microvessel permeability after intracarotid bradykinin infusion is mediated by nitric oxide. Cancer Res 1996; 56: 4027-31.

[109] Black KL, Yin D, Ong JM, Hu J, Konda BM, Wang X, *et al.* PDE5 inhibitors enhance tumor permeability and efficacy of chemotherapy in a rat brain tumor model. Brain Res 2008; 1230: 290-302.

[110] Hu J, Yuan X, Ko MK, Yin D, Sacapano MR, Wang X, *et al.* Calcium-activated potassium channels mediated blood-brain tumor barrier opening in a rat metastatic brain tumor model. Mol Cancer 2007; 6: 22.

[111] Black, K.L. Enhanced opening of abnormal brain tissue capillaries. US20006043223 (**2000**).

[112] Hu J, Ljubimova JY, Inoue S, Konda B, Patil R, Ding H, *et al.* Phosphodiesterase type 5 inhibitors increase Herceptin transport and treatment efficacy in mouse metastatic brain tumor models. PLoS One 2010; 5: e10108.

[113] Black, K.L., Ningaraj, N.S. Method for using potassium channel agonists for delivering a medicant to an abnormal brain region and/or a malignant tumor. US20067018979 (**2006**).

[114] Black, K.L, Ningaraj, N.S. Induction of apoptosis of malignant cells by activation of calcium-activated potassium channels. US20060280730 (**2006**).

[115] Black, K.L., Wheeler, C. Use of minoxidil sulfate as an anti-tumor drug. US7705010 (**2010**).

[116] Reubi JC, Macke HR, Krenning EP. Candidates for peptide receptor radiotherapy today and in the future. J Nucl Med 2005; 46 (Suppl 1): 67S-75S.

[117] Khan IU, Beck-Sickinger AG. Targeted tumor diagnosis and therapy with peptide hormones as radiopharmaceuticals. Anticancer Agents Med Chem 2008; 8: 186-99.

[118] Merlo, A.M., Macke, H., Reubi, J., Good, S. Radiopharmaceuticals for cancer diagnosis and treatment. US20070053837 (**2007**).

[119] Cordier D, Forrer F, Kneifel S, Sailer M, Mariani L, Macke H, *et al.* Neoadjuvant targeting of glioblastoma multiforme with radiolabeled DOTAGA-substance P-results from a phase I study. J Neurooncol 2010; 100: 129-36.

[120] Schoor, O., Hilf, N., Weinschenk, T., Trautwein, C., Walter, S., Singh, H. Composition of tumor-associated peptides and related anti-cancer vaccine for the treatment of glioblastoma (GBM) and other cancers. EP2172211 (**2010**).