Review

The substance P/NK-1 receptor system: NK-1 receptor antagonists as anti-cancer drugs

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The substance P (SP)/neurokinin (NK)-1 receptor system plays an important role in cancer. SP promotes the proliferation of tumour cells, angiogenesis and the migration of tumour cells. We review the involvement of SP, the NK-1 receptor and NK-1 receptor antagonists in cancer. Tumour cells overexpress NK-1 receptors, which are involved in their viability. This overexpression suggests the possibility of specific treatment against tumour cells using NK-1 receptor antagonists, thus promoting a considerable decrease in the side effects of the treatment. This strategy opens up new approaches for cancer treatment, since these antagonists, after binding to their molecular target, induce the death of tumour cells by apoptosis, exert an antiangiogenic action and inhibit the migration of tumour cells. The use of NK-1 receptor antagonists such as aprepitant (used in clinical practice) as antitumour agents could be a promising innovation. The value of aprepitant as an antitumour agent could be determined faster than for less well-known compounds because many studies addressing its safety and characterization have already been completed. The NK-1 receptor may be a promising target in the treatment of cancer; NK-1 receptor antagonists could act as specific drugs against tumour cells; and these antagonists could be new candidate anti-cancer drugs.

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1. Introduction

In 2008, some 12.7 million cancer cases and 7.6 million cancer deaths were recorded (Siegel *et al.* 2012). The current treatment of choice for cancer is chemotherapy, but all too often its results are unsatisfactory. According to a study on the contribution of chemotherapy for adult malignancies with respect to 5-year survival (Morgan *et al.* 2004), the overall contribution of curative and adjuvant cytotoxic chemotherapy is close to 2%. Nevertheless, some practitioners remain optimistic that cytotoxic chemotherapy will significantly improve cancer survival. However, despite the use of new and expensive single and combination drugs to improve response rates and other agents to allow for dose escalation, there has been no change in some of the regimens used, and there has been little impact from the use of newer regimens; for example, in lung cancer, the median survival has increased by only 2 months during the last 20 years (Breathnach *et al.* 2001). Over the past two decades, in the search for more effective treatments, research efforts into cancer have increase exponentially. This effort, however, has not yet resulted in greatly improved prospects regarding the problem, although several areas of research are promising (the Human Genome Project, gene therapy, new cytostatic

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agents and stem cell research). Thus, it remains necessary to explore other initiatives in cancer research, to achieve the ultimate goal of discovering one or more compounds capable of destroying the tumour specifically targeted (parasitotropic), while producing no prejudicial side effects in the host (non-organotropic). Paul Erlich termed this ambition the 'Magic Bullet'. Unfortunately, the anti-cancer agents (cytotoxic chemotherapy) currently used in clinical practice are 'far from being a Magic Bullet'. They present a very low safety profile and often provoke severe side effects in the host. These clinical effects occur because the drugs are not specific against tumour cells; as a consequence, the emphasis in research has long been to find a drug with the same or greater antitumour potential but which produces fewer side effects. One such solution might be the molecularly targeted anti-cancer therapy based upon drugs or therapeutic strategies targeting tumour-specific molecular derangements.

The expression and secretion of peptides by tumours has attracted growing interest, and novel possibilities are emerging for translational research, with the potential to improve the diagnosis and treatment of tumours (Muñoz *et al.* 2010c, 2011). Moreover, evidence suggests that neuropeptides could be implicated in cancer progression. Substance P (SP) is a neuropeptide that has been shown to act through the neurokinin-1 (NK-1) receptor as a mitogen on several human cancer cell lines. NK-1 receptor antagonists present antitumour activity against such cell lines, and these antagonists have been shown to induce the apoptosis of tumour cells (Muñoz *et al.* 2010a, e, 2011, 2012a). These findings suggest that the SP/NK-1 receptor system could play an important role in the development of cancer and that NK-1 receptor antagonists may act as broad-spectrum antitumour agents. In this review, we describe a new therapeutic target in cancer, the NK-1 receptor, and a new generation of anticancer drugs, the NK-1 receptor antagonists.

2. Substance P/NK-1 receptor system and cancer

Three receptors, termed NK-1, NK-2 and NK-3, mediate the biological actions of the tachykinins SP, neurokinin A and neurokinin B. NK-1 is a mediator of the biological activities encoded by the C-terminal sequence of tachykinins, for which SP is a more potent agonist than either neurokinin A or neurokinin B (Quartara and Maggi 1997). Thus, because SP is the natural ligand with the highest affinity for NK-1, the biological action of SP is mainly mediated by this receptor. The latter is functionally coupled with G-protein. Diverse techniques (e.g. autoradiography, expression of the mRNA encoding the NK-1 receptor protein, immunocytochemistry) have demonstrated the widespread distribution of NK-1 in the mammalian central nervous system and peripheral tissues (Muñoz *et al.* 2010c, 2011). After binding to the NK-1 receptor, SP is a very relevant factor in movement control, sensory perception, neuronal survival and degeneration, gastric motility, salivation, micturition, neurogenic inflammation, pain, depression and in the regulation of the cardiovascular system and of respiratory mechanisms. Furthermore, it induces NF-κB activation which is involved in the control of proinflammatory cytokine expression (Hökfelt *et al.* 2001; Muñoz *et al.* 2010c, 2011). In addition, the activation of NK-1 by SP or by its agonists produces the following actions in human astrocytoma cell lines: the activation of phospholipase D, the formation of inositol phosphate and the release of interleukins (IL-8 and IL-6), taurine and glutamate; moreover, it mobilizes intracellular calcium, influences both glutamate and K^+ transport and stimulates glycogen breakdown (Muñoz *et al.* 2007a, 2011).

When NK-1 receptors are stimulated, this can generate second messengers, triggering a variety of effector mechanisms that regulate cellular excitability and function. Three second-messenger systems are activated following NK-1 receptor occupancy by agonists: (a) stimulation, via phospholipase C, of phosphatidyl inositol turnover, leading to calcium mobilization from both intra- and extracellular sources; (b) arachidonic acid mobilization via phospholipase A2; and c) cAMP accumulation via stimulation of adenylate cyclase (Muñoz *et al.* 2007a, 2010c, 2011). SP activates members of the mitogen-activated protein kinase (MAPK) cascade via NK-1, including extracellular signal-regulated kinases 1 and 2 (ERK1/2) and p38MAPK. The presence of a functional EGFRkinase domain is required for SP-induced MAPK activation (Mitsuhashi *et al.* 1992), and the stimulation by SP of the NK-1 receptor located in human glioblastoma cells increases the phosphorylation and activity of Akt or protein kinase B (EC 2.7.11.1), a serine-threonine protein kinase that becomes activated via phosphatidyl-3-kinase (PI3K). The activation of Akt suppresses apoptosis (Nakajima *et al.* 1992; Takeda *et al.* 1992) whereas treatment with NK-1 receptor antagonists provokes it, because the increase in phosphorylation due to SP is blocked by the antagonist. It has also been reported that the blocking of NK-1 receptors by the NK-1 receptor antagonist L-733,060 (figure 1) inhibits the basal kinase activity of Akt. This is an important finding, because in glioblastoma the basal activity of Akt is associated with a poor prognosis (Akazawa *et al.* 2009).

It is known that upon exposure to SP, this peptide and its receptor are internalized into early endosomes within minutes of binding (Bowden *et al.* 1994; Garlan *et al.* 1994; Grady *et al.* 1995; Mantyh *et al.* 1995). SP induces a clathrin-dependent internalization of the NK-1 receptor; the SP/NK-1 receptor complex dissociates into acidified endosomes, and SP is then degraded, whereas the NK-1 receptor reverts to the cell surface (Grady *et al.* 1995).

NK-1 is expressed in many human cancer cell lines (table 1; figure 2D–F) including WERI-Rb-1 and Y-79

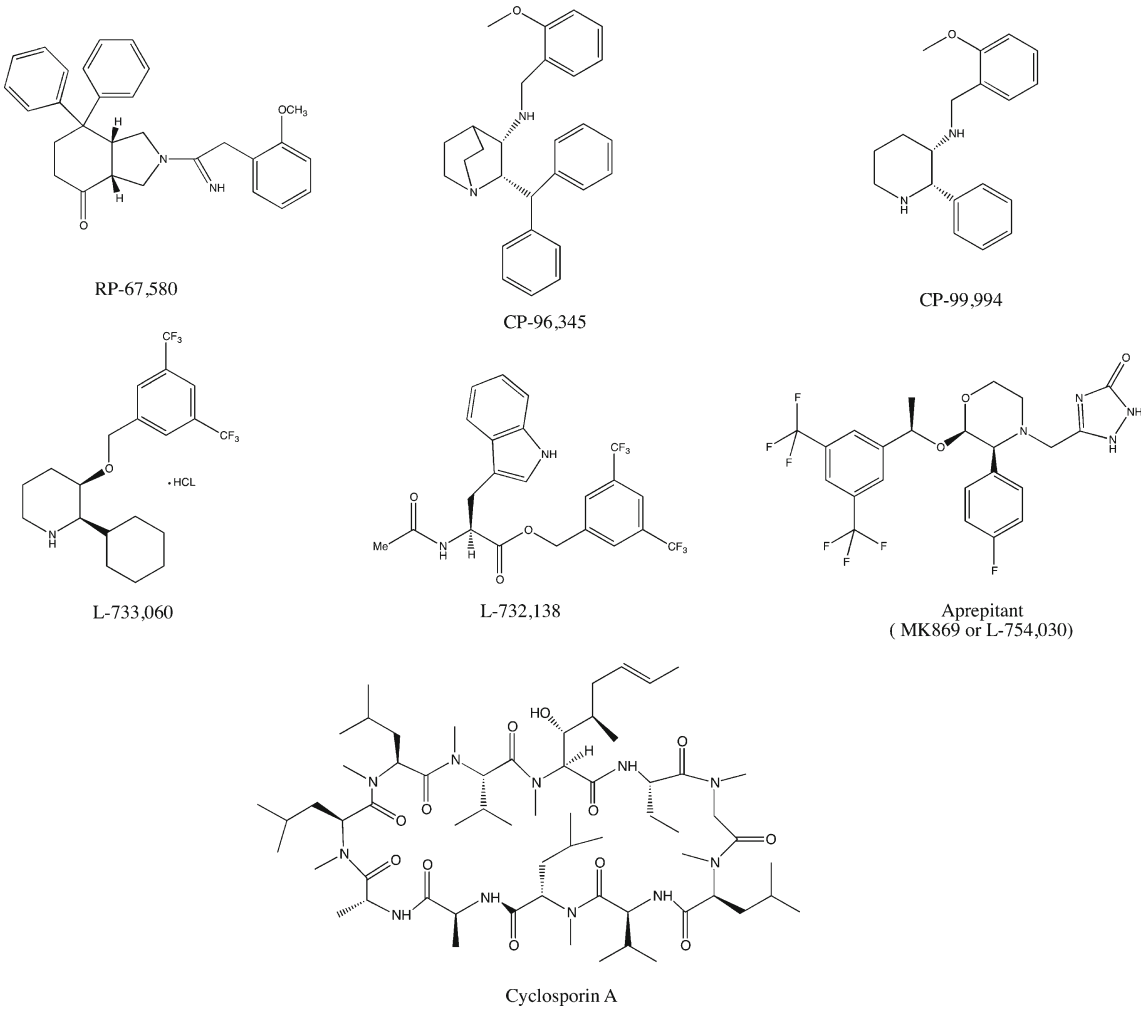

Figure 1. Non-peptide NK-1 receptor antagonists. Chemical structures of RP-67,580, CP-96,345, CP-99,994, L-733,060, L-732,138 and aprepitant. The chemical structure of cyclosporine A is also shown.

retinoblastoma, U373 MG and GAMG glioma, SKN-BE(2), Kelly and IMR-32 neuroblastoma, T-ALL BE-13 and B-ALL SD-1 leukaemia and CAPAN-1 and PA-TU 8902 pancreatic, HEp-2 larynx, 23132/87 gastric and SW-403 colon carcinomas (Muñoz *et al.* 2005a,b, 2006, 2007c, 2008, 2010c, 2011, 2012a; Rosso *et al.* 2008). It has been also demonstrated that tumour cells express mRNA for the tachykinin NK-1 receptor and that increased mRNA NK-1 receptor expression occurs in malignant tissues (e.g., breast biopsies), but not in benign ones (normal mammary epithelial cells, benign breast biopsies) (Singh *et al.* 2000). Moreover, the use of immunohistochemical techniques has revealed a high density of NK-1 receptors within human primary retinoblastomas and human gastric and colon adenocarcinomas (Muñoz *et al.* 2007c; Rosso *et al.* 2008). The expression of NK-1 has also been observed in primary human neoplastic cells of 9/12 astrocytomas, 10/10 glioblastomas, 10/12 medullary thyroid carcinomas, 8/16 breast carcinomas and 4/5 ganglioneuroblastomas, but not (or only rarely) in non-small-cell carcinomas of the lung (1/16), neuroblastomas (0/8), colon (1/21) or pancreas (1/9) adenocarcinomas, or malignant lymphomas (3/18) (Hennig *et al.* 1995). NK-1 receptors are also expressed in the cytoplasm of keratocystic odontogenic tumours, oral squamous cell carcinomas and larynx carcinomas (González-Moles *et al.* 2008; Brener *et al.* 2009; Esteban *et al.* 2009). In addition, in most cases the basal layers are NK-1 reactive (Esteban *et al.* 2009). The expression of NK-1 receptors in the cytoplasm of epithelial cells is significantly associated with the presence of dysplastic epithelium expressing Ki-67 in suprabasal layers (González-Moles *et al.* 2008). In most of the tumours investigated, NK-1 receptors have been found

in intra- and peritumoural blood vessels. The presence of NK-1 in these blood vessels could have clinical implications (see below: Tumour angiogenesis inhibition) (Hennig *et al.* 1995). In general, immunoreactive NK-1 receptors are clearly confined to the plasma membrane of tumour cells, although the immunoreactivity can also present diffuse cytoplasmic staining and, occasionally, the nuclei of tumour cells express the NK-1 receptor (González-Moles *et al.* 2008; Rosso *et al.* 2008; Muñoz *et al.* 2012b). Thus, the visualization of NK-1 receptors by immunohistochemistry (figure 2D–F) would facilitate the identification of tumours with a sufficient degree of receptor overexpression for diagnostic or therapeutic intervention using NK-1 receptor antagonists (Schulz *et al.* 2006). It should be noted that by improving the immunocytochemical protocols the expression of the NK-1 receptor has been reported in samples in which this receptor was not previously observed. This is the case for pancreatic, colon, lung and breast cancers (Friess *et al.* 2003; Rosso *et al.* 2008; Muñoz *et al.* 2012b, 2014b) where the 100% of the samples studied expressed NK-1 receptors (table 1; figure 2D–F); in contrast to that found previously (Hennig *et al.* 1995). The data suggest that all tumour samples express the NK-1 receptor.

The full-length and the truncated forms of the NK-1 receptor have been described in humans. In the truncated subtype, the C terminus of the full-length subtype is missing. The full-length form mediates the slow growth of tumour cells, whereas the truncated subtype mediates malignancy in tumour cells and increases the growth of cancer cells, stimulating the synthesis of cytokines (Patel *et al.* 2005; Ramkissoon *et al.* 2007). These latter substances activate the transcription factor called NF-κB, which in turn upregulates the truncated subtype but slightly increases the full-length subtype (Moharita *et al.* 2004; Peng 2004). The truncated form is increased in colonic epithelial cells from patients with colitis-associated cancer, whereas the full-length is not affected (Patel *et al.* 2005; Gillespie *et al.* 2011). At the present, it is unknown whether the NK-1 receptor antagonists exert or not a similar blockade of both long and short forms.

Table 1. Presence of SP and NK-1 receptors in human tumour cells and samples

Tumour cells/Samples	Substance P	NK-1 receptor
B and T cells acute lymphoblastic leukaemia	+	+
Breast carcinoma	+	+
Colon carcinoma	Not studied	+
Gastric carcinoma	Not studied	+
Glioma/Astrocytoma	+	+
Hepatoblastoma	+	+
Larynx carcinoma	+	+
Lung cancer: small- and non-small-cells	+	+
Melanoma	+	+
Neuroblastoma	+	+
Oral carcinoma	+	+
Osteosarcoma	Not studied	+
Pancreatic carcinoma	Not studied	+
Retinoblastoma	+	+
Thyroid carcinoma	Not studied	+

Previous reports have shown that different isoforms of the NK-1 receptor can be found in both human and rat tissues (with isoform sizes of 46–54 kDa) (Nakata *et al.* 1988; Organist *et al.* 1988; McGillis *et al.* 1990; van Ginkel and Pascual 1996; Caberlotto *et al.* 2003). For example, in human lymphocytes a glycosylated NK-1 receptor of 58 kDa has been observed (McGillis *et al.* 1990), and another two forms, of 38 kDa and 33 kDa, have been detected in IM-9 lymphoblasts (Organist *et al.* 1988). Moreover, in several human tumour cell lines the presence of four more abundant isoforms (75, 54–58, 46, 33–38 kDa) of the NK-1 receptor has been described (table 2). In neuroblastoma SKN-BE(2) a major 54 kDa band is observed, and an additional band of 33-38 kDa has been reported in the glioma GAMG cell line (Muñoz *et al.* 2005a). In IMR-32 and KELLY neuroblastoma cell lines, a band of 33 kDa and a major 54 kDa band have been observed, and in IMR-32 cells an additional isoform of about 75 kDa has been detected (Muñoz *et al.* 2007b). In the CAPAN-1 and PA-TU 8902 pancreatic carcinoma cell lines, bands of 75, 58, 46, 34 kDa have been reported (Friess *et al.* 2003; Muñoz *et al.* 2006). The presence of 75, 58 and 33 kDa isoforms of the NK-1 receptor in the human retinoblastoma WERI-Rb-1 and Y-79 cell lines has also been reported (Muñoz *et al.* 2007c). Moreover, isoforms of about 75, 58, 46 and 34 kDa have been observed in the human larynx carcinoma HEp-2 cell line and in the gastric adenocarcinoma 23132/87 cell line, whereas the colon adenocarcinoma SW-403 cell line and the T-ALL BE-13 and B-ALL SD-1 leukaemia cell lines express isoforms of 75, 58 and 34 kDa (Muñoz *et al.* 2008, 2012a; Rosso *et al.* 2008). It is striking that such diverse tumour cell lines as human retinoblastoma, neuroblastoma, glioma, acute lymphoblastic leukaemia, larynx and pancreas carcinomas and gastric and colon adenocarcinomas all express the same isoforms of the NK-1 receptor (table 2). Further research (e.g., the different N-glycosylation levels that this receptor undergoes after synthesis) is needed to clarify the functional roles of the different isoforms of the NK-1 receptor observed in human cancer cells (Muñoz *et al.* 2014b).

Another aspect of interest is the number of NK-1 receptors expressed by tumour cells; it has been shown that NK-1 receptor expression is increased 25- to 36-fold in human

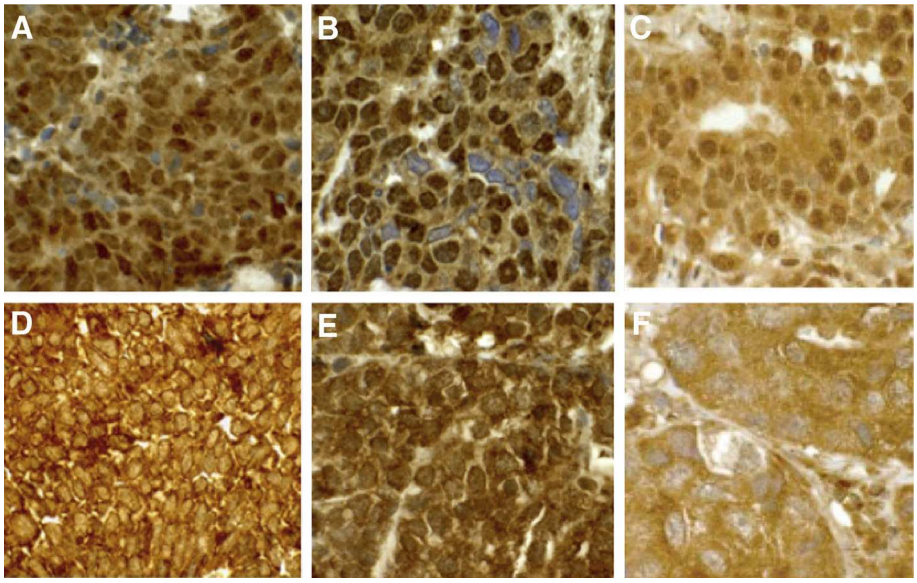

Figure 2. SP and NK-1 receptor inmunohistochemistry. SP in non-small-cell lung carcinoma (**A**) (40×), small-cell lung carcinoma (**B**) (40×) and breast carcinoma (**C**) (40×). The immunoreactivity for SP was predominantly located in the nucleus. NK-1 receptors in non-small-cell lung carcinoma (**D**) (40×), small-cell lung carcinoma (**E**) (40×) and breast carcinoma (**F**) (40×). The immunoreactivity for the NK-1 receptor was found in the cytoplasm and, occasionally, in the nucleus of tumour cells. In all cases, the sections were counterstained with hematoxylin.

pancreatic cancer cell lines in comparison with normal controls, and that tumour samples from patients with advanced tumour stages exhibit significantly higher NK-1 receptor levels (Friess *et al.* 2003). Thus, the number of NK-1 receptors expressed in normal human cells is lower (e.g., human blood T-lymphocytes express 7,000-10,000 NK-1 receptors/cell) (Payan *et al.* 1986) than that expressed in human tumour cells (e.g., astrocytoma cells express 40,000 NK-1 receptors/cell) (Fowler and Brannstrom 1994). A higher rate of NK-1 receptor expression has been reported in astrocytoma and glioblastoma tumours containing the most malignant phenotypes (Hennig *et al.* 1995). The expression of NK-1 receptors is believed to be correlated with the degree of malignancy (e.g., glioblastomas express more NK-1 receptors than do astrocytomas) (Hennig *et al.* 1995). It has also been described that astrocytoma/glioma primary tumours express more NK-1 receptors than do established astrocytoma/glioma cell lines in culture (Luo *et al.* 1996). Finally, increased mRNA NK-1 receptor expression takes place in malignant tissues (e.g., breast biopsies), but not in benign ones (Singh *et al.* 2000). Thus, the mRNA NK-1 receptor level is approximately 30-fold higher in leukaemia cell lines than in normal cells (Muñoz *et al.* 2012a). All these data suggest that the NK-1 receptor is a specific target for the treatment of tumours using NK-1 receptor antagonists (figure 1), because cancer cells overexpress NK-1 receptors (figure 2D–F).

2.1 *Substance P and cancer progression*

SP has been found in retinoblastoma, larynx carcinoma and neuroblastoma cells of the parotid gland. It is expressed in almost 90% of metastatic neuroblastoma cells in the bone marrow, in 68% of primary invasive malignant melanomas, in 40% of metastatic melanomas, in 60% of *in situ* melanomas, in 58% of atypical nevi and in 40% of spindle and epithelioid nevi. However, it has not been detected in acquired benign melanocytic nevi (Tarkkanen *et al.* 1983; Shrestha *et al.* 1994; Khare *et al.* 1998; Nowicki and Miskowiak 2002; Muñoz *et al.* 2007c, 2010e; Esteban *et al.* 2009). Moreover, it is known that breast cancer cells (figure 2C) have high levels of SP but non tumourigenic cells show very low levels of the peptide (Singh *et al.* 2000). In human glioma U373 MG xenograft, SP has been observed in the peritumoural region and in the tumour mass, especially at the periphery (Palma *et al.* 1999, 2000). It has been reported that keratocystic odontogenic tumours and oral squamous cell carcinoma and larynx carcinoma tissues express SP (table 1) in the cytoplasm and nucleus of tumour cells (González-Moles *et al.* 2008; Brener *et al.* 2009; Esteban *et al.* 2009), this SP expression being related to the positive expression of Ki-67 in dysplastic epithelium (González-Moles *et al.* 2008). The presence of SP in the nucleus of tumour cells (figure 2A–C) indicates that the peptide could act as a genetic neuromodulator (Muñoz

Table 2. Tumour cells, cytotoxicity, mitogenic effect of SP and NK-1 receptor isoforms

Tumour	Cell line	L-732,138		L-733,060		Aprepitant		SP nM	NK-1 receptor isoforms kDa
		IC_{50} μM	IC_{100} μM	IC_{50} μM	IC_{100} μM	IC_{50} μM	IC_{100} μM		
B Acute lymphoblastic leukemia	SD-1	49.7	103.5	18.4	50	29.4	59.2	5-50	75, 58, 33
Breast carcinoma	BT-474	25.4	58	10.6	20.6	31.4	59.1	5-10	48
	MCF-7	28.8	64.1	16.4	31	35.6	64		
	MDA-MB-468	27.1	56.8	13.8	27.1	29.6	57		
	MT3	27.3	57.7	8.4	18.8	40.8	75.3		
Colon carcinoma	SW-403			14.5	25.8	30.5	60.5	50	75, 58, 46, 34
Gastric carcinoma	23132-87			14.3	29.6	24.2	52.5	10	75, 58, 46, 34
Glioma	GAMG	48.1	100	21.3	43	33.1	66.2	50	54, 38, 33
Hepatoblastoma	HepT1	42		16		31.1		10-50	58, 50
	HUH6	43		11		33.18			
	HepG2	101		9		38.61			
Larynx carcinoma	HEp-2	38	77.3	21.3	42	22.7	46.5	50	75, 58, 46, 34
Melanoma	MEL-HO	76.3	140.6	27.5	54	29.6	56.5	500	75, 58, 33
Neuroblastoma	SKNBE(2)	41	80.5	11.6	21	24.6	48.8	100	54
	IMR-32	45.7	99			27.7	19.6		
	KELLY	53.2	103.4			30.4	49.5		
Non-small-cell lung cancer	COR-L23	87	102.3	22	41.5	30	60	5-100	75, 58, 46, 34
Osteosarcoma	MG-63	58.6	100	14.5	30	28.6	80	5-500	46
Pancreas carcinoma	PA-TU-8902			18.1	38.4	31.2	63	100	75, 58, 46, 33
	CAPAN-1			20	39.7	27.4	52		
Retinoblastoma	Y-79	56.8	132	12.2	25	30.4	59	100	75, 58, 33
	WERI-Rb-1	60.5	119	17.4	35	23	53.1		
Small-cell lung cancer	H-69	51.9	109	18.7	39.2	21.8	45.2	5-100	75, 58, 46, 34
T Acute lymphoblastic leukemia	BE-13	63.9	124	15.4	40	19.5	50	5-50	58, 33

et al. 2010a). SP is also located in the body fluids such as blood, cerebrospinal fluid, breast milk, i.e., SP is ubiquitous throughout human body. It is known that the peptide is rapidly degraded in blood (Ernst *et al.* 2008), but it is also known that SP could form a complex with fibronectin and this molecule protects the undecapeptide from the action of peptidases, increasing its half-life (Rameshwar *et al.* 2001).

Studies have shown that SP acts as a mitogen in amphibian limb regeneration (Globus *et al.* 1991), in mammalian connective tissue cells (Nilsson *et al.* 1985) and in regenerating planarians (Salo and Baguna 1986). Moreover, by activating NK-1 receptors, SP stimulates mitogenesis in many human tumour cell lines (e.g., neuroblastoma, astrocytoma, melanoma, retinoblastoma, pancreas carcinoma, glioma, lung carcinoma, breast carcinoma, hepatoblastoma) (table 2) and normal cell types, probably via autocrine/paracrine mechanisms (Luo *et al.* 1996; Palma *et al.* 1999; Muñoz *et al.* 2004b, 2005a, b, 2006, 2010c, 2011, 2012a, b, 2014b; Berger *et al.* 2014). In this respect, it has also been reported that the activation of NK-1 receptors by nanomolar concentrations of SP increases DNA synthesis and mitogenesis in the astrocytoma U-373 MG cell line (Eistetter *et al.* 1992; Luo *et al.* 1996). SP acts specifically through the NK-1 receptor, because the growth inhibition of human tumour cells (GAMG glioma, MEL HO, COLO 679 and COLO 858 melanoma, WERI-Rb-1 and Y-79 retinoblastoma, KELLY, SKN-BE(2) and IMR-32 neuroblastoma, larynx HEp-2, CAPAN-1 and PA-TU 8902 pancreatic, gastric 23132/87 and colon SW-403 carcinomas, T-ALL BE-13 and B-ALL SD-1 leukaemia, H-69 small-cell lung cancer cells and COR-L23 non-small-cell lung cancer cells, HepT1, HuH6 and HepG2 hepatoblastoma, MG-63 osteosarcoma, BT-474, MCF-7, MDA-MB-468 and MT3 breast carcinoma) after the administration of NK-1 receptor antagonists is partially reversed by the administration of a nanomolar dose of exogenous SP (Muñoz *et al.* 2004b, 2005b, 2006, 2007b, 2008, 2010e, 2012a, 2014a,b; Rosso *et al.* 2008; Berger *et al.* 2014). Moreover, it has been reported that after SP

stimulation the intracellular calcium levels are increased, this increase being associated with mitogenesis (Feng *et al.* 2011). The above data suggest that the activation of NK-1 receptors by SP induces mitogenesis in a large number of human tumour cells (Luo *et al.* 1996; Palma *et al.* 1999, 2000; Muñoz *et al.* 2004a, b, 2005a, b, 2006, 2010e, 2012a, 2014a, b; Berger *et al.* 2014) (table 2). As mentioned previously, this could also produce the activation of the MAPK pathway, including extracellular signal-regulated kinases 1 and 2 (ERK1/2) (Luo *et al.* 1996). The activation of NK-1 receptors by SP stimulates the formation of a scaffolding complex comprising internalized receptor, *β*-arrestin, src and ERK1/2. Once activated, ERK1/2 is translocated into the nucleus, inducing proliferation and protecting the cell from apoptosis (Eistetter *et al.* 1992; Muñoz *et al.* 2011). The expression of c-myc protein might also be involved in this mitogenic effect (Luo *et al.* 1996). In summary, these findings suggest that SP may be a universal mitogen in NK-1 receptor-expressing tumour cell types.

Moreover, in astrocytoma cells SP stimulates glycogen breakdown and increases the intracellular Ca^{2+} concentration. Both effects occur in a concentration-dependent manner. These effects are completely blocked by the NK-1 receptor antagonist CP-96,345 (Medrano *et al.* 1994) and this suggests that such effects are mediated by the NK-1 receptor. In addition, the Warburg effect occurs because most cancer cells pre-dominantly produce energy by means of a high rate of glycolysis followed by lactic acid fermentation (Warburg 1956). Growing tumour cells typically have glycolytic rates up to 200 times higher than those of their normal tissues of origin; this occurs even if oxygen is plentiful. Thus, tumour cells need to obtain a large amount of glucose for the maintenance of the Warbur effect. The release of SP from tumour cells produces glycogen breakdown and then the glucose obtained would be used by tumour cells to increase their metabolism (figure 5). By contrast, in tumour cells NK-1 receptor antagonists block glycogen breakdown (Medrano *et al.* 1994), and without glucose, the Warburg effect does not occur. Accordingly, in clinical practice it would be possible to counteract the Warburg effect by using NK-1 receptor antagonists (e.g., the aprepitant drug) (figures 1 and 5).

The presence of SP in the nuclei of tumour cells (e.g., lung and breast cancers) has been reported (Muñoz *et al.* 2012b, 2014b) (table 1; figure 2A–C). In tumour cells, SP is more strongly expressed in the nuclei than in the cytoplasm. The location of a peptide in a certain place indicates, *a priori*, that it could be involved in functions in which the structure containing the peptide is involved. Thus, the high presence of SP within the nucleus of tumour cells means that the peptide could regulate the nuclear activity of tumour cells and that the peptide could act as an epigenetic factor (Muñoz *et al.* 2010a) regulating several transcription factors involved in cancer such as extracellular signal-regulated kinases (ERK1/2), c-myc, c-fos, c-jun, AP-1, NF-κB and hypoxia-inducible factor (HIF-1α) (Luo *et al.* 1996; Walczak-Drzewiecka *et al.* 2008; Koh *et al.* 2010). In the future, this should be investigated in depth.

It should be noted that endogenous SP is widely distributed in the mammalian central and peripheral nervous systems. The demonstration that SP stimulates, *in vitro*, tumoural cell proliferation at low (nanomolar) concentrations (table 2) suggests that exists a novel mechanism for the regulation of local tumoural activity through sensory nerves containing SP. Thus, SP could modulate the growth of tumour cells, provoking a direct interaction between the latter and the neural system (Muñoz *et al.* 2006, 2010c, 2011). In this sense, SP stimulates mitogenesis by activating the NK-1 receptors expressed in tumour cells (as mentioned above, this expression is greater in tumour cells than in normal cells), probably via the following mechanisms (figure 3): (1) an autocrine mechanism, by which SP is secreted from primary tumours (e.g., neuroblastoma, keratocystic odontogenic tumours, oral squamous cell carcinoma, larynx carcinoma, melanoma); (2) a paracrine mechanism, by which SP is released from tumour cells and acts on surrounding cells (e.g., endothelial cells), thus facilitating cancer progression; (3) through the peripheral nervous system, since SP is released from peripheral nerve terminals; and (4) through an endocrine mechanism, related to emotional behaviour, by which SP reaches the whole body through the bloodstream (Muñoz *et al.* 2010c, 2011). For example, it has been demonstrated that the SP secreted by cholangiocarcinoma promotes its growth via an autocrine pathway and hence the blockade of this secretion may been important for the management of cholangiocarcinoma (Meng *et al.* 2014).

2.2 *Substance P, inflammation and cancer*

SP, released from the peripheral terminals of primary sensory neurons, has been associated with neurogenic inflammation (terminals containing SP are located close to blood vessels). The release of SP in peripheral tissues reproduces many of the physiological changes seen in acute inflammation: vasodilatation (SP is a potent vasodilator), vascular permeability (SP increases permeability), mast-cell degranulation and modulation of the immune cell function. Several lines of evidence indicate that SP plays a role in immune responses, both in peripheral tissues and in the central nervous system, while a dramatic up-regulation in the expression of NK-1 receptors during painful chronic conditions has been reported (Harrison and Geppetti 2001). In view of these findings, NK-1 receptor antagonists (e.g., L-733,060) (figures 1 and 5) have been used as antiinflammatory (Bang *et al.* 2003) or analgesic (Rupniak *et al.* 1996) agents.

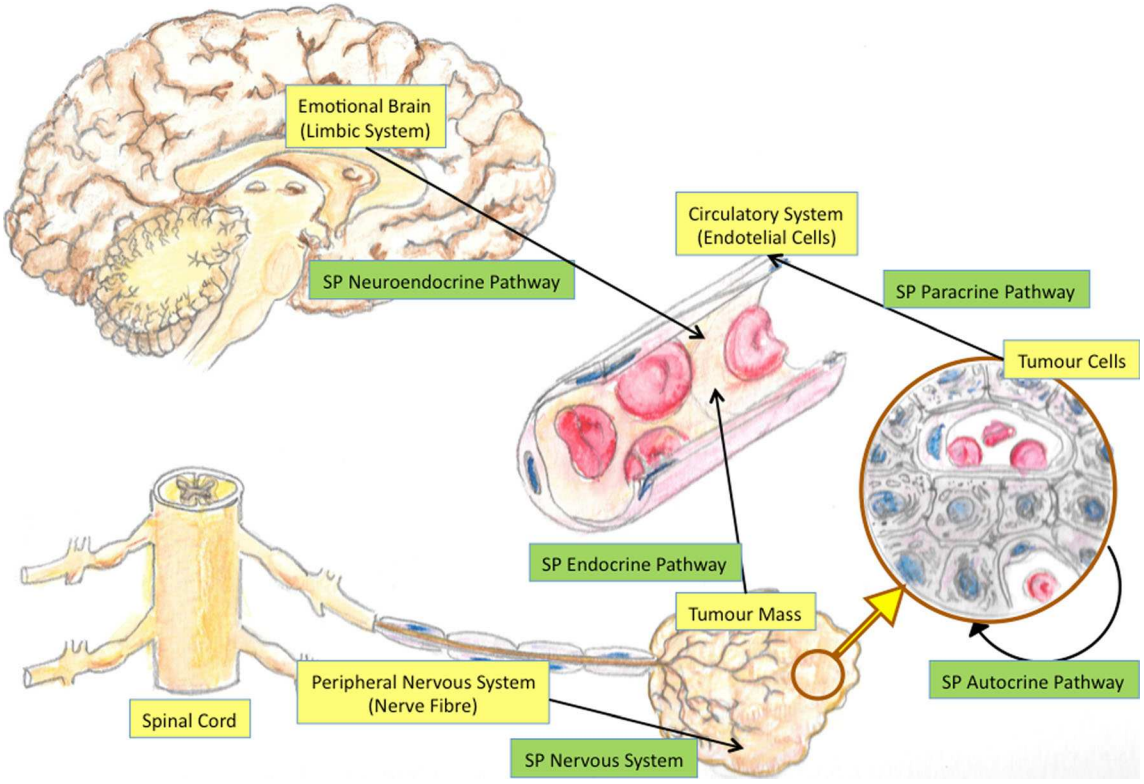

Figure 3. The autocrine, paracrine, endocrine and neuroendocrine pathways of SP. Tumour cells express SP: the undecapeptide is located in both the nucleus and cytoplasm of these cells. NK-1 receptors are overexpressed in tumour cells and are involved in the viability of these cells. After binding to the NK-1 receptor located in the plasma membrane, SP and NK-1 receptor antagonists exert opposite effects in tumour cells. SP could induce mitogenesis via autocrine (SP is secreted from tumour cells), paracrine (SP exerts a mitogenic action in other tumour cells and in endothelial cells) and/or endocrine (SP is secreted from the tumour mass into the blood vessels) mechanisms. SP is also released from nerve terminals into the tumour mass and/or the peptide reaches the whole body through the bloodstream (neuroendocrine pathway; this is regulated by the limbic system).

It is well known that the SP/NK-1 receptor system is capable of modulating the immune function and that the activation of this system produces alterations in the immune response (Pascual *et al*. 1991; Feistritzer *et al.* 2003). Furthermore, it has been reported that tachykinins modify the response of a variety of inflammatory cells, including mast cells, granulocytes, lymphocytes, monocytes and macrophages, and that oedema formation is induced by lower concentrations of SP, but blocked by NK-1 receptor antagonists (Walsh *et al.* 1995) (figure 1). Moreover, regulation of the immune cell function by SP could originate not only from neuronal sources (sensory nerves, neurogenic inflammation), but also from non-neuronal elements, such as eosinophils and macrophages, since in these cells the expression of both SP and NK-1 receptors is up-regulated during inflammation (Weinstock *et al.* 1988; Cook *et al.* 1994). Increased levels of SP during intestinal inflammation have been described, and there is significant correlation between the degree of inflammation and the clinical status of the disease (Keranen *et al.* 1996a, b). These observations suggest that the local release of SP (e.g., in lymph nodes) might be a factor contributing to the immune disorder underlying chronic inflammatory bowel disease, since the presence of NK-1 receptors in human peripheral blood T lymphocytes has been demonstrated (Payan *et al.* 1984).

Chronic inflammation is also clearly correlated with increased risk of developing cancer (Weitzman and Gordon 1990), since inflammation increases both mitogenesis and mutagenesis (Ames and Gold 1990). A dividing cell is known to be at greater risk of mutation than is a quiescent one, and cell division allows adducts to convert to mutations (Tong *et al.* 1980). The time interval for DNA repair during cell division is short, and therefore the risk of endogenous or exogenous damage is generally higher if cells are proliferating. Moreover, it is well established that cancer arises in chronically inflamed tissue, and this is particularly evident in the gastrointestinal tract (Macarthur *et al*. 2004). Inflammation may become chronic either because an

inflammatory stimulus persists or because of deregulations in the control mechanisms that would normally terminate the process. It has also been reported that many of the cells, cytokines and processes (e.g., leukocyte migration, dilatation of the local vasculature, angiogenesis) involved in the inflammation are also found in a variety of tumours. It is important to note that in the vast majority of tumours investigated, NK-1 receptors have been found in both intratumoural and peritumoural blood vessels (Hennig *et al.* 1995; Friess *et al.* 2003).

Chronic inflammation caused by intestinal flora, leading to inflammatory bowel disease, ulcerative colitis and Crohn's disease, is clearly linked with a higher incidence of colon cancer. Moreover, elevated levels of SP and up-regulated NK-1 receptor expression have been reported in the rectum and colon of patients with inflammatory bowel disease (O'Connor *et al.* 2004). In addition, the dietary intake of proinflammatory carcinogens has been associated with prostate cancer, and the chronic inflammation resulting from oesophageal reflux is known to provoke gastroesophageal reflux disease (GERD) and Barrett's oesophagus, which are also linked with a higher incidence of cancer. Chronic *Helicobacter pylori* infection produces chronic inflammation and is therefore a potential risk factor for stomach cancer (Blaser *et al.* 1995). The risk of pancreatic cancer is significantly elevated in subjects with chronic pancreatitis and appears to be independent of sex, country, or type of pancreatitis (Lowenfels *et al.* 1993). Moreover, the up-regulation of NK-1 receptor mRNA expression in chronic pancreatitis has been reported, and in these patients it is strongly related with the pain syndrome (Shrikhande *et al.* 2001). All these observations suggest that chronic inflammation could provoke cancer via the SP/NK-1 receptor system, which is up-regulated in the process of chronic inflammation, since SP stimulates mitogenesis in human tumour cells and NK-1 receptor expression is increased in cancer cells (Friess *et al.* 2003; Muñoz *et al.* 2004a, 2005a,b, 2006, 2007b, 2010c, 2011). Unlike tumour progression, where the role of inflammation in promoting cancer cell proliferation and stromal/matrix degradation is reasonably well understood, the role of inflammation in metastasis is less well defined, although it does appear to be important. SP levels rise during neurogenic inflammation, and SP is known to induce tumour cell migration (metastasis) (Muñoz *et al.* 2011). These data suggest that inflammatory processes could also be involved in metastasis through the SP/NK-1 receptor system. Moreover, it has been reported that NK-1 receptors are overexpressed in ulcerative colitis presenting a high grade of dysplasia and in colitis-associated cancer, which suggests a functional role for NK-1 receptors in malignant transformation in colitis-associated cancer. Thus, NK-1 receptors could be used as a diagnostic marker to identify patients at risk of neoplasia and may serve as a useful therapeutic target in the treatment of colitis-associated cancer (Gillespie *et al.* 2011).

2.3 *Substance P, emotional behaviour and cancer*

The possible existence of links between psychosocial factors and the incidence and progression of cancer has generated considerable scientific and public interest. Tachykinins (SP, neurokinin A and B) act as neurotransmitters in the peripheral and the central nervous systems, but tachykinins and their receptors are also expressed in several non neuronal cells contributing to the fine connections between the nervous system and the peripheral organ systems (respiratory, cardiovascular, endocrine, gastrointestinal and genitourinary). It has been proposed that psychological factors could be involved in the development and progression of cancer (Hilakivi-Clarke *et al.* 1994). Thus, mammary tumourgenesis has been associated with life-style and the exposure to diverse stressors. The possible role of life-style factors in breast cancer is important in view of the fact that mortality rates for this disease are rising in most countries and that, to date, satisfactory curative therapies for breast cancer have not been achieved. The crucial factor affecting tumour growth appears to be the interaction between stress, personality, and psychosocial support, together with the effect of this interaction on an individual's ability to cope with stress (Hilakivi-Clarke *et al.* 1994; Okamura *et al.* 2005).

Depression and cancer commonly co-occur and it has been suggested that chronic and severe depression may be associated with elevated cancer risk, that the prevalence of depression among cancer patients increases with disease severity and symptoms such as pain and fatigue, and that depression predicts cancer progression and mortality. Moreover, studies have reported that psychosocial support reduces depression, anxiety and pain, and may increase survival time with cancer. Thus, there is evidence of a bi-directional relationship between cancer and depression, which opens up new opportunities for therapeutic intervention (Spiegel and Giese-Davis 2003). Both SP and NK-1 receptors are widely distributed in the mammalian central nervous system, including the limbic system (hypothalamus, amygdala, etc.). SP may be involved in the integration of emotional responses to stress, and therefore the pathogenesis of depression could be due to an alteration of the SP/NK-1 receptor system, since increased SP levels have been observed in depression (Kramer *et al.* 1998). It has been reported that prolonged treatment with antidepressant drugs decreases SP concentrations in the striatum, the substantia nigra and the amygdala, and that expression of the genes encoding the synthesis of tachykinins and that of NK-1 receptors in selected brain areas is modified by the action of psychotropic drugs (Sivam *et al.* 1989; Humpel *et al.*

1990; Shibata *et al.* 1990). These data indicate that a reduction in SP levels in certain brain regions could contribute to a common therapeutic effect of antidepressant drugs in affective disorders (Shirayama *et al.* 1996). Moreover, the NK-1 receptor antagonist L-733,060 (figure 1) has been used as an antidepressive agent (Varty *et al.* 2003). This antagonist has antitumour activity against human cancer cell lines (Muñoz *et al.* 2004b, 2005b, 2006, 2010c, 2011; Rosso *et al.* 2008) (table 2), while another NK-1 receptor antagonist, aprepitant (figure 1), is a broad-spectrum anticancer drug (Muñoz *et al.* 2010c, e, 2011, 2012a) (table 2), which is as efficient as paroxetine in the treatment of depression. Aprepitant is well tolerated and no statistically significant difference has been observed in the frequency of adverse events compared to placebo treatment.

In view of the above findings, we conclude that depression could induce tumour cell proliferation by activating the SP/NK-1 receptor system and that treatment with NK-1 receptor antagonists could be useful not only for the depression, but also for the prevent and treatment of cancer. Thus, emotional behaviour (behaviour traits, such as depression), might be related to cancer and metastasis as a result of alterations in the SP/NK-1 receptor system (Fehder 1999; de Vane 2001; Lang *et al.* 2004). Such a system might facilitate an interrelationship between the progression of cancer and cerebral mechanisms, since SP after binding to NK-1 receptors within the limbic system produces anxiety and depression, whereas SP after binding to the same receptors located in tumour cells induces cell proliferation. Thus, NK-1 receptor antagonists (figure 1) in the limbic system exert antidepressant and anxiolytic actions, while presenting an antitumour action on tumour cells.

Chronic diseases characterized by deregulation of inflammation are known to be particularly susceptible to exacerbation by stress and emotion, and in this respect, rates of depression and anxiety are overrepresented in individuals suffering from chronic inflammatory disease. SP has been implicated both in the pathophysiology of inflammatory disease and in that of depression and anxiety (Rosenkranz 2007). In consequence, it has been hypothesized that SP deregulation may be a point of convergence underlying the overlap of chronic inflammatory disease and mood and anxiety disorders. SP is a relevant factor in emotional behaviour, in chronic inflammation and in cancer. Thus, a therapeutic intervention based upon NK-1 receptor antagonists could reduce emotional stress and chronic inflammation, contributing to prevent the development of cancer.

3. Anticancer action of NK-1 receptor antagonists

NK-1 receptor antagonists, in a concentration-dependent manner, block the pathophysiological actions of SP after binding to the NK-1 receptor (tables 2 and 3; figure 1). There are two types of NK-1 receptor antagonists: peptides and non-peptides. Peptide NK-1 receptor antagonists are chemical modifications of the SP molecule, in which an L-amino acid is replaced by a D-amino acid. These compounds are degraded for peptidases and do not cross the blood–brain barrier; in addition, some of them are toxic. However, non-peptide NK-1 receptor antagonists are lipid soluble compounds, they are not degraded by peptidases and they can cross the blood–brain barrier. Moreover, non-peptide NK-1 receptor antagonists include numerous compounds with different chemical compositions but showing similar sterochemical features (their affinity for the NK-1 receptor). Up to date, more than 300 non-peptide NK-1 receptor antagonists have been reported (Muñoz and Coveñas 2013b). This means that there are more than 300 potential anticancer drugs. Important advances have been made in the development of highly selective antagonists for the NK-1 receptor, providing a better understanding of the physiological actions of SP and the pathophysiological significance of NK-1 receptors. The development of NK-1 receptor antagonists represents an important opportunity to further exploit compounds that are active against this receptor as novel therapeutic agents. At the level of the central nervous system, NK-1 receptor antagonists (table 3; figure 1) could be used to produce analgesic, antidepressive, anxiolytic and antiemetic effects, and to treat certain forms of urinary incontinence. In the peripheral nervous system, NK-1 receptor antagonists could be used in several inflammatory diseases, including arthritis, inflammatory bowel diseases and cystitis (Quartara and Maggi 1998). Investigation into non-peptide NK-1 receptor antagonists (figure 1) is a field that is developing very quickly and a number of papers describing such antagonists have been published (Quartara and Maggi 1997, 1998). Thus, steroids (WIN- 51,708…), perhydroisoindolones (RP-67,580, RP-73,467, RPR-100,893…), benzylamino and benzylether quinuclidines (CP-96,345, L-709,210…), benzylamino piperidines (CP-

Table 3. Therapeutic effects of non-peptide NK-1 receptor antagonists

Cancer cells	Inhibit migration of tumour cells (preventing invasion and metastasis)
	Inhibit tumour cell proliferation
	Tumour cell death by apoptosis
Tumour	Decrease tumour volume
	Antiinflammatory effect
	Decrease number of tumour cells
	Inhibit angiogenesis
Patient	Antiinflammatory effect
	Decrease permeability of the blood–brain barrier (preventing brain metastases)
	Antidepressant and anxiolytic effect

99,994, GR-203,040, GR-205,171 (Vofopitant), CP-122,721…), benzylether piperidines (L-733,060, L-741,671, L-742,694…) and tryptophan-based (L-732,138…) NK-1 receptor antagonists have been reported (Quartara and Maggi 1997; Muñoz *et al.* 2010c, 2011) (figure 1).

3.1 *Tumour cell growth inhibition and tumour cell death by apoptosis*

L-733,060 is a selective and potent non-peptide NK-1 receptor antagonist (Muñoz *et al.* 2011) (figure 1). It belongs to the benzylether piperidine family of NK-1 receptor antagonists and is a potent antagonist of human tachykinin NK-1 receptors (estimated affinity=0.8 nM). The administration of L-733,060 produces analgesia and antidepressive effects, and it has been used in the treatment of various anxiety and mood disorders, as well as in inflammatory liver disease, in order to inhibit the effects of SP (table 3). Moreover, L-733,060 completely inhibits the SP-mediated increase in cancer cell migratory activity and also exerts antitumour activity against several human cancer cell lines (see below) (Muñoz *et al.* 2011) (table 2). A recent *in vitro* study demonstrated that L-733,060, combined with vinblastine or microtubule-perturbing agents, is synergistic for the growth inhibition of NK-1 receptor-possessing cancer cell lines (T98G, U87, HeLa, T24 and MDA-MB-231), but not for normal lung IMR-90 fibroblast cells (Muñoz *et al.* 2011). This combination, therefore, is more potent against NK-1 receptor-overexpressing cancer cells and thus the interaction between microtubule destabilizing agents (MDAs) and NK-1 receptor antagonists might be clinically useful. These data demonstrate the value of MDAs and NK-1 receptor antagonists in predicting novel relationships between different classes of compounds used in cancer chemotherapy (Giardina *et al.* 2003).

L-732,138 (*N*-acetyl-L-tryptophan 3,5-bis(trifluoromethyl) benzyl ester), a tryptophan derivative (figure 1), shows a competitive, selective antagonism for the NK-1 receptor (Muñoz *et al.* 2011). It is approximately 1,000 times more potent in cloned human NK-1 receptors than in cloned human NK-2 and NK-3 receptors, and approximately 200 times more potent in human NK-1 receptors than in rat NK-1 receptors. The administration of L-732,138 attenuates hyperalgesia and also antagonizes H(3) antagonist-induced skin vascular permeability. Moreover, it exerts an antitumour action against several human cancer cell lines (see below) (Muñoz *et al.* 2011) (table 2).

Aprepitant (MK-869, L-754,030), a morpholine derivative (figure 1), is a selective high-affinity antagonist of the human NK-1 receptor (Muñoz *et al.* 2011). In radioligand-binding assays, aprepitant was found to be approximately 3,000 times more selective for the human cloned NK-1 receptor (IC_{50}=0.1 nM) versus the human cloned NK-3 receptor (IC_{50}=300 nM), and 45,000 times more selective in comparison with the human cloned NK-2 receptor (IC_{50}=4500 nM). Aprepitant, used for the treatment of emesis, appears to be effective for the treatment of pain and depression and its safety is adequate; at a dose of 300 mg/day it is well tolerated with no statistically significant differences in the frequency of adverse events in comparison to placebo treatment (Muñoz *et al.* 2011). It has also been reported to be safe with respect to human fibroblast cells, with the IC_{50} for fibroblast cells being approximately three times higher than for tumour cells (Muñoz and Rosso 2010) (table 2). These findings are important, since aprepitant could be used in the treatment of cancer, as a broad-spectrum antitumour agent (Muñoz and Rosso 2010; Muñoz *et al.* 2010c, 2011; Berger *et al.* 2014). Therefore, it would be of interest to determine whether the treatment of cancer cell lines with other NK-1 receptor antagonists studied in humans, such as CP-122,721, ezlopitant (CJ-11,974), vofopitant (GR-205,171) and fosaprepitant (L-758,298) (Diemunsch and Grelot 2000), produces the same growth inhibitory action as found with L-733,060, L-732,138, or aprepitant (figure 1).

It has been reported that L-733,060, L-732,138 and aprepitant (figure 1) exert antitumour actions against human GAMG glioma, HEp-2 larynx carcinoma, KELLY, SKB-BE(2) and IMR-32 neuroblastoma, WERI-Rb-1 and Y-79 retinoblastoma, COLO 679, COLO 858 and MEL HO melanoma, 23132/87 gastric and SW-403 colon carcinoma and CAPAN-1 and PA-TU 8902 pancreas carcinoma cell lines (Muñoz *et al.* 2005a, b, 2006, 2008, 2010c, e, 2011; Rosso *et al.* 2008) (table 2). On comparing the results obtained with L-732,138 and L-733,060 on the human neuroblastoma SKN-BE (2) cell line, L-732,138 was found to exert a weaker antitumour action (L-733,060 is four/six times more potent than L-732,138). Thus, at the same concentration (10 μM), L-733,060 was found to induce 36.28% inhibition of SKN-BE(2) human neuroblastoma cells (Muñoz *et al.* 2005a), whereas L-732,138 only induced 6.34% (Muñoz *et al.* 2007b). Maximum inhibition was observed when L-733,060, at a concentration of 20 μM, was present in the cultures of SKN-BE-(2) cells (Muñoz *et al.* 2005a). However, after using the NK-1 receptor antagonist L-732,138, maximum inhibition was observed when a dose of 80 μM was used (Muñoz *et al.* 2007b). In this case, L-733,060 was four times more potent than L-732,138. It has been demonstrated that the antitumour action of L-733,060 against human cancer cell lines is stronger than that of aprepitant, and that the antitumour action of aprepitant is more potent than that of L-732,138 (Muñoz *et al.* 2010c, 2011; Berger *et al.* 2014) (table 2). In addition, the use of SP antagonists other than L-733,060/L-732,138/aprepitant inhibits the growth of small-cell lung cancer,

glioma, neuroblastoma and breast cancer and pancreatic cancer cells (Woll and Rozengurt 1988; Langdon *et al.* 1992; Reeve and Bleehen 1994; Seckl *et al.* 1997; Palma *et al.* 2000; Bigioni *et al.* 2005; Guha *et al.* 2005; Mukerji *et al.* 2005).

Moreover, it is also known that cyclosporin A (figure 1), widely used in clinical practice, is a immunosuppressor drug that shows a NK-1 receptor antagonist pharmacological profile (Gitter *et al.* 1995). This cyclic undecapeptide is a broad spectrum antitumour drug (e.g., glioma, neuroblastoma, retinoblastoma, larynx, pancreas, gastric and colon carcinomas, melanoma…); its antitumour action is exerted via the NK-1 receptor, and it has been reported that tumour cells die by apoptosis (Muñoz *et al.* 2010d; González-Ortega *et al.* 2014). However, the possible administration of cyclosporine A to patients suffering from cancer could present certain clinical limitations (e.g., nephrotoxicity), although it could be administered as an adjuvant therapy at low doses.

NK-1 receptor antagonists are known to inhibit the growth of tumour cells by apoptosis (figure 4). This has been reported, for example, in human lung cancer, colon carcinoma, A-204 rabdhomyosarcoma and SKN-BE(2), KELLY, and IMR-32 neuroblastoma cell lines (El-Salhy and Starefeldt 2003; Muñoz *et al.* 2007b, 2010c, 2011). Moreover, NK-1 receptors play a key role in glioblastoma apoptosis (Akazawa *et al.* 2009). These data suggest that the antiproliferative action of NK-1 receptor antagonists could involve a signal transduction pathway for apoptosis, after the antagonist has bound to NK-1 receptors overexpressed in tumour cells (figure 5). The death of tumour cells occurs after activation of the apoptotic machinery; therefore, the induction of apoptosis (figure 4) represents a highly suitable approach to cancer treatment. At the molecular level it is not currently known which mechanisms are responsible for inducing apoptosis in tumour cell lines. However, a number of genes, molecules or signals are often changed in cancer, and some of these are key regulators of apoptosis (Xu *et al.* 2006), while various substances other than NK-1 receptor antagonists produce apoptosis in cancer cells. For example, the COX2-selective inhibitor NS398 induces the release of cytochrome c from the mitochondria and raises caspase 9, caspase 3 and PARP enzyme activities, all of which contribute to apoptosis, and the TRAIL (the tumour necrosis factor-related apoptosis-inducing ligand) induces apoptosis in tumour cells by engaging the death receptors DR4 and DR5 (Williams *et al.* 2000; Li *et al.* 2001; Shi *et al.* 2003, 2005). It has also been suggested that any cellular genetic damage would activate one or more of the programmed cell death pathways (Esteban *et al.* 2006). Tumour cells rely on strategies to neutralize the multiple pathways leading to cell death, and it has been proposed that one of the most important is the activation and/or heightening of the phenotypic expression of the NK-1 receptor (Esteban *et al.* 2006) (figure 5). Increased expression of this receptor renders tumour cells highly dependent on the SP stimulus, which provides a potent mitotic signal. This increased mitogenic signal could counteract the different death signal pathways activated in each tumour cell by its own genetic damage, by oncogene activation, or by other causes, and it is independent of the particular genetic profile of each tumour. It has also been suggested that both the growth inhibition and the cell death induced following the use of NK-1 receptor antagonists could be secondary to the loss of these life signals induced through NK-1 receptor stimulation by SP (Esteban *et al.* 2006). The absence of these signals after the receptor has been blocked with the antagonist (figure 5) could tilt the balance within the cell to favouring apoptotic/death signals, and hence the cell dies. Each tumour bears a different set of mutations, oncogene activation and/or suppressor gene losses, and different death signals are overridden by the SP-mediated mitotic stimulus. Accordingly, by eliminating only the potent mitotic signal induced by SP, NK-1 receptor antagonists

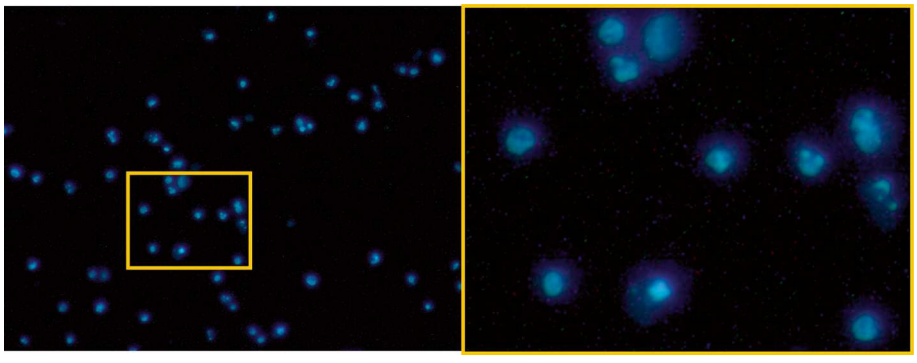

Figure 4. Culture breast carcinoma cells treated with the NK-1 receptor antagonist aprepitant. Note apoptotic figures: chromatin condensation and nuclear fragmentation are observed (40×). A high power magnification of the region delimited by the rectangle is shown on the right.

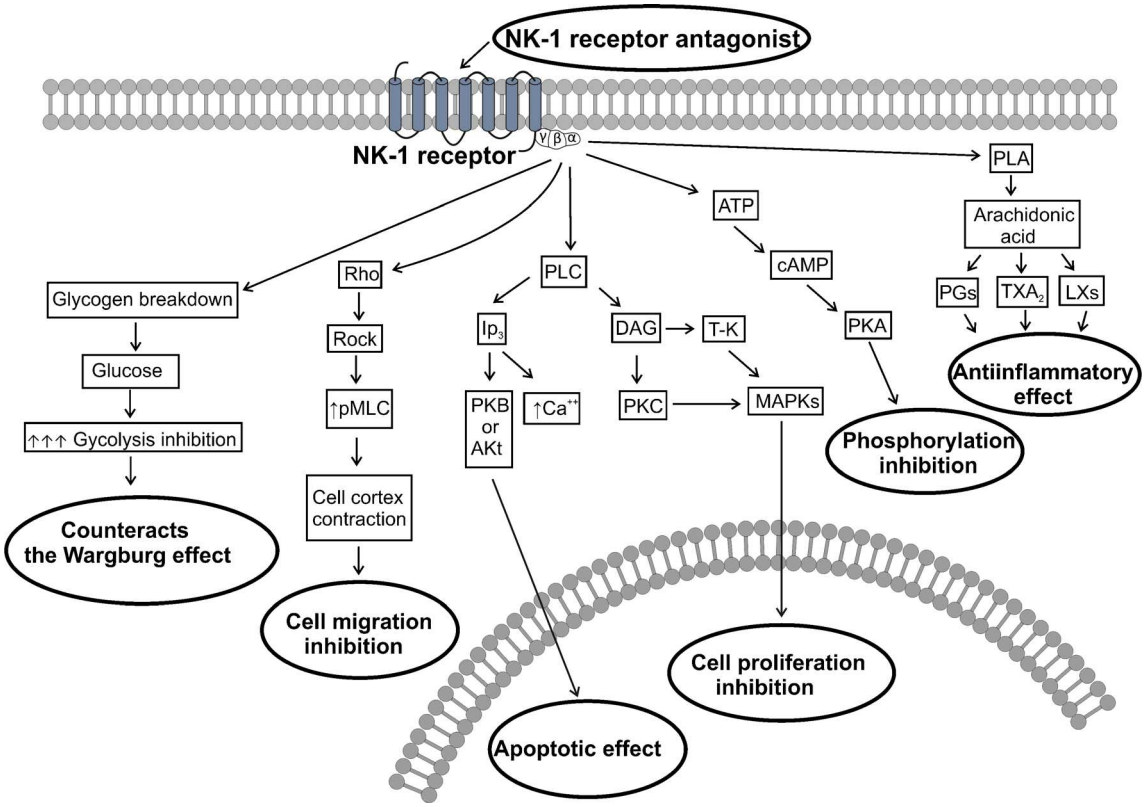

Figure 5. Model for the signalling pathways downstream of the NK-1 receptor. Activation of this receptor by SP leads to cell proliferation, antiapoptotic effect and cell migration. The pathways indicated are involved in these mechanisms. However, NK-1 receptor antagonists, after binding to the NK-1 receptor, block such pathways and inhibit both tumour cell proliferation and migration, as well as exert an apoptotic effect in tumour cells. In addition, NK-1 receptor antagonists counteract the Warburg effect. ATP: adenosine triphosphate; cAMP: cyclic adenosine monophosphate; DAG: diacilglicerol; Ip_3: inositol triphosphate; LXs: leukotrienes; MAPKs: mitogen-activated protein kinase; PGs: prostacyclin; PKA: protein kinase A; PKB or AKt: protein kinase B; PKC: protein kinase C; PLA: phospholipase A; PLC: phospholipase C; pMLC: myosin regulatory light chain phosphorylation; T-K: tyrosine-kinase; TXA_2: thromboxane A_2.

(figure 5) leave the cell alone with its death load or at least render the balance between life and death signals favourable to the latter (Esteban *et al.* 2006). Moreover, it has recently been demonstrated that the NK-1 receptor is involved in the viability of tumour cells (Muñoz *et al.* 2010e, 2012a).

The cell death observed in cancer cell lines after treatment with NK-1 receptor antagonists (L-733,060, L-732,138, aprepitant) (figures 1 and 4) is believed to be due to the general toxic effect of such antagonists or otherwise to some specific action. The following data supports the second possibility (Muñoz *et al.* 2010c,e, 2011, 2012a, 2014a,b; Berger *et al.* 2014): (1) tumour cells express the NK-1 receptor (figure 2D–F); (2) SP preferentially binds to the NK-1 receptor and induces mitogenesis (table 2); (3) the antitumour action of NK-1 receptor antagonists is dose-dependent; and (4) experiments carried out on human cancer cell lines show that exogenous SP cell proliferation is partially reversed by the administration of NK-1 receptor antagonists. This means that NK-1 receptor antagonists block NK-1 receptors overexpressed by tumour cells and that the structurally very different molecules piperidine (L-733,060), tryptophan (L-732,138) and morpholine (aprepitant) (figure 1) exert the same antitumour action (these molecules only have in common their specificity for the NK-1 receptor). NK-1 receptor antagonists are a growing family of compounds. The molecules belonging to this family are structurally unrelated compounds. However, the antitumour action of non-peptide NK-1 receptor antagonists is linked to sterochemical features and not to the chemical composition. All have one aspect in common, namely their affinity for the NK-1 receptor. SP and non-peptide NK-1 receptor antagonists bind to different regions of the NK-1 receptor (figure 6): both SP and peptide NK-1 receptor antagonists (water

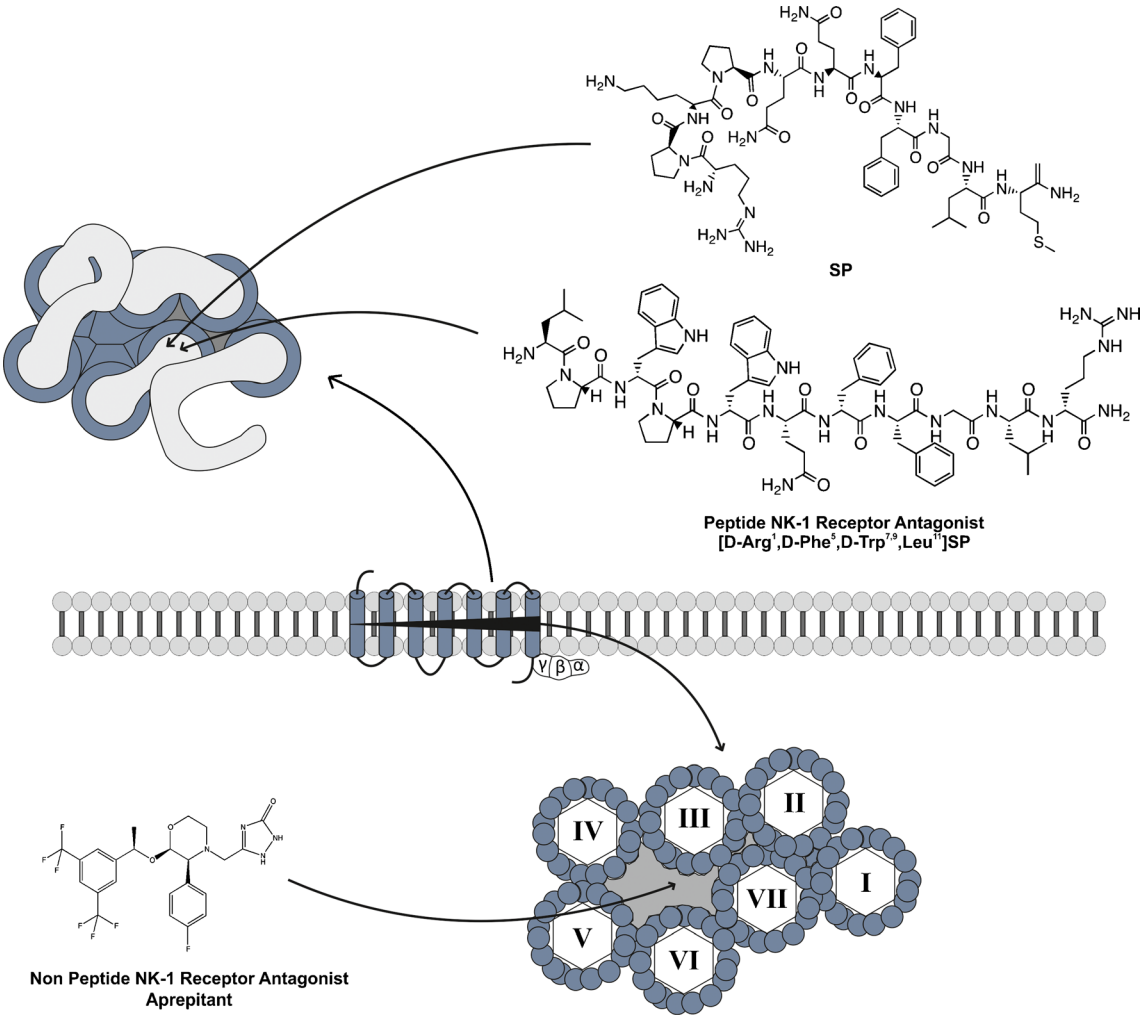

Figure 6. SP/peptide NK-1 receptor antagonists and non peptide NK-1 receptor antagonists bind to different sites of the NK-1 receptor. SP and peptide NK-1 receptor antagonists bind to the extracellular loops of the receptor, whereas NK-1 receptor antagonists (e.g. aprepitant) bind more deeply, between the transmembrane segments.

soluble) bind to the extracellular loops of the receptor, whereas the non-peptide NK-1 receptor antagonists (lipid soluble) bind more deeply between the III, IV, V and VI transmembrane segments (see Muñoz *et al.* 2011). Thus, very little overlap occurs between SP/peptide NK-1 receptor antagonists and non-peptide NK-1 receptor antagonists. This is because NK-1 receptors function as allosteric molecules, where agonists and antagonists stabilize different conformations (Hökfelt *et al.* 2001). Moreover, in cell lines as different as human neuroblastoma, glioma, acute lymphoblastic leukaemia, retinoblastoma, melanoma and pancreatic, larynx, gastric and colon carcinomas, the same NK-1 receptor antagonist elicits growth inhibition (table 2). This implies the existence of a common mechanism for cancer cell proliferation mediated by SP and the NK-1 receptor; moreover, that NK-1 receptor antagonists could inhibit a large number of tumour cell types in which NK-1 receptors are overexpressed (Muñoz *et al.* 2005a, b, 2006, 2010c, e, 2011, 2012a), and that NK-1 receptor antagonists could be candidates for broad-spectrum antineoplastic drugs (Muñoz *et al.* 2010c, 2011).

Many studies have also been carried out *in vivo*. In athymic nude mice NK-1 receptor antagonists elicit tumour regression (Seckl *et al.* 1997; Guha *et al.* 2005; Akazawa *et al.* 2009) and when nude mice, transplanted with human glioma U373 MG xenografts, were treated with NK-1 receptor antagonists (MEN 11,467 or MEN 11,149), the antagonists inhibited tumour growth (Palma *et al.* 2000). It has also been demonstrated that subcutaneous or intravenous administrations of NK-1 receptor antagonists are effective in

preventing tumour growth (Bigioni *et al.* 2005; Palma *et al.* 2000) and that these antagonists diminish tumour volume (more than 80%) (Berger *et al.* 2014; Muñoz *et al.* 2014a). In nude mice, the treatment of MG-63 human osteosarcoma xenografts with fosaprepitant (peri-tumoural subcutaneous injection) showed a significant reduction in tumour volume (Muñoz *et al.* 2014a) and in a similar *in vivo* model (hepatoblastoma xenograft nude mice) it has been reported that the treatment with 80 mg/kg/day of aprepitant for 24 days resulted in a significant reduction of tumour growth, as evidenced by reduced tumour volume and weight, lowered tumour-specific alpha-fetoprotein serum levels, and decreased number of Ki-67 positive cells (Berger *et al.* 2014). Recently, in mice another *in vivo* study has reported that the administration of the NK-1 receptor antagonist Emend IV decreased both tumour volume and cancer cell proliferation (Harford-Wright *et al.* 2014) (table 3). All these experiments demonstrate that, *in vivo*, NK-1 receptor antagonists exert an antitumour action. However, in a murine model of melanoma it has been reported that SP prevents tumour growth and that this action is mediated by T and natural killer cells (Manske and Hanson 2005). The findings reported in this *in vivo* study are not in agreement with those mentioned above, although it should be noted that in this latter study (Manske and Hanson 2005) SP was administered prior to the development of the tumour. It has been suggested that SP could exert a beneficial action in the early stages of DNA damage. However, no experiment has been carried out to study the action of SP in well established tumours. Thus, new experiments should be developed to clarify the issue. It has been reported that aprepitant (doses ranging from 3 to 300 mg/kg/day, 3 days treatment) does not exert an antitumoural effect *in vivo* experiments (Lewis *et al.* 2013a). The lack of results of aprepitant could be due to the methodology (e.g., the animals were only treated with aprepitant for 3 days; the animals were sacrificed 48 h after the administration of the last doses of aprepitant; aprepitant was administered parenterally, not orally, and its intravenously administered prodrug fosaprepitant was not used in the study) and/or to the animal used.

3.2 *Tumour angiogenesis inhibition*

Neovascularization, or neoangiogenesis, is a sequential process, with early endothelial proliferation followed by new vessel formation and increased blood flow, accompanied by the maturation of endogenous neurovascular regulatory systems, occurring late in this process in inflamed tissues (Muñoz *et al.* 2011). The growth of new vessels from a pre-existing vasculature is a common feature of chronic inflammation (early neoangiogenesis is a key step in the transition from acute to persistent inflammation) and wound healing. Moreover, neoangiogenesis, a hallmark of tumour development, has been associated with increased tissue innervation and expression of NK-1 receptors. As mentioned above, it has been reported that in a large majority of tumours, SP and NK-1 receptors are found in the intra- and peritumour blood vessels (Hennig *et al.* 1995), and that SP, an important mediator of neurogenic inflammation through the release of the peptide from peripheral nerve terminals, is involved in the growth of capillary vessels *in vivo* and in the proliferation of cultured endothelial cells *in vitro* (Muñoz *et al.* 2011) (figure 3). Additionally, it has been demonstrated in a pancreatic carcinoma xenograft nude mouse model that SP analogues (SPA) (synonymous with NK-1 receptor antagonists) exert antitumour and antiangiogenic actions (Guha *et al.* 2005), while angiogenesis can be enhanced with the administration of SP. Moreover, in a hepatoblastoma xenograft nude mouse model, it has been recently reported that the drug aprepitant inhibits angiogenesis (Berger *et al.* 2014). The proliferation of endothelial cells by NK-1 receptor agonists increases in a concentration-dependent manner (NK-1 receptor antagonists block the proliferative action of SP), whereas the action of selective NK-2 and NK-3 receptor agonists has no significant effects on the proliferation of endothelial cells (Muñoz *et al.* 2011). These findings indicate that NK-1 receptor agonists, such as SP, can directly stimulate the process of neovascularization, probably through the induction of endothelial cell proliferation (Ziche *et al.* 1990) and that SP-enhanced angiogenesis results from a direct action on microvascular NK-1 receptors. Thus, by means of such receptors, present at high densities in blood vessels, SP may strongly influence vascular structure and function both within and around tumours, by increasing tumoural blood flow and by fostering stromal development (Hennig *et al.* 1995). It has also been reported that in pancreatic cancer, SPA exerts antiproliferative and antitumourigenic effects, although at present these mechanisms (which could be of significant therapeutic value) are not fully understood (Guha *et al.* 2005). In order to clarify them, the following data are believed important: in pancreatic cancer, NK-1 receptors are highly expressed in blood vessels (not only within the tumour mass but also in the peritumour tissue) (Friess *et al.* 2003); nanomolar concentrations of SP both elicit proliferation in pancreatic tumour cells (Muñoz *et al.* 2006) (table 2) and stimulate vessel growth by enhanced endothelial cell proliferation (Ziche *et al.* 1990), and angiogenesis is mimicked by selective NK-1 receptor agonists (Ziche *et al.* 1990). In addition, tumour cell proliferation and neoangiogenesis could be inhibited by NK-1 receptor antagonists, which block the biological actions of SP (Muñoz *et al.* 2011; Berger *et al.* 2014). These data suggest that the NK-1 receptor is involved in the antiproliferative and antioangiogenic actions exerted by SPA and by non-peptide NK-1 receptor antagonists (e.g., aprepitant).

In summary, these data indicate that the SP/NK-1 receptor system controls neoangiogenesis and that may also regulate the growth of the tumoural mass, since NK-1 receptors are overexpressed in tumoural cells and in peritumoural tissues (Hennig *et al.* 1995; Friess *et al.* 2003; Muñoz *et al.* 2011). Thus, by using NK-1 receptor antagonists, the NK-1 receptor target could be used to inhibit both neoangiogenesis and the growth of the tumoural mass (table 3; figure 3).

3.3 *Inhibition of tumour invasion and metastasis*

The value of the surgical removal of tumours has been recognised for hundreds of years. With the successful implementation of operative procedures, complications in cancer treatment have shifted, so that nowadays over 90% of cancer deaths are derived not from the primary tumour but from the development of metastases (Sporn 1996). Accordingly, a major aim in cancer treatment should be to inhibit the spread of tumour cells, thus inhibiting the development of metastases. One model for the development of metastasis is based on the finding that G protein-coupled receptors, also known as serpentine receptors or seven-transmembrane receptors, regulate the migratory activity of tumour cells in a similar way to the recruitment and homing of leucocytes. Furthermore, ligands (e.g., neurotransmitters) to these receptors can induce directed (chemotactic) migration (Entschladen *et al.* 2002; Muñoz *et al.* 2011). This means that the active migration of tumour cells, which is a crucial requirement for metastasis development and cancer progression, could be regulated by signal substances, including neurotransmitters (figure 5). These substances would induce a metastatogenic tumour cell type by directly regulating gene expression and by increasing migratory activity, and this might be prevented by established neurotransmitter antagonists. It has been reported that tumour cell migration (MDA-MB-468 breast and PC-3 prostate carcinoma cells) is induced by noradrenalin, dopamine and SP, and that this process can be inhibited by using, respectively, specific and clinically established adrenoceptor, D_2 receptor or NK-1 receptor antagonists (Lang *et al.* 2004; Muñoz *et al.* 2011) (figure 5). It is also known that SP promotes the migration of pancreatic cancer cell clusters to the dorsal root ganglia of newborns and that SP is involved in pancreatic cancer perineural invasion (Li *et al.* 2013).

Moreover, it has become evident that membrane blebbing is important in cell spreading and cancer cell invasion (Fackler and Grosse 2008; Muñoz *et al.* 2011). In a recent study of human embryonic kidney (HEK) 293 cells, it was shown that aprepitant and L-733,060 (figure 1) block rapid SP-induced changes in cell shape, including blebbing (Meshki *et al.* 2009). It has also been reported that NK-1 receptors are present in the MDA-MB-468 breast cell line; that L-733,060 completely inhibits the SP-mediated increased migratory activity, and that in patients with breast cancer the risk of recurrence or metastasis is reduced four-fold during a 2.5 to 4 year follow-up period when surgery is associated with paravertebral anaesthesia (Exadaktylos *et al.* 2006). This seems to occur because paravertebral anaesthesia blocks the SP-induced migration, invasion and metastasis of the tumour cells. The results suggest that the SP/NK-1 receptor system plays an important role in this process. This hypothesis is quite interesting because the use of paravertebral anaesthesia prior to surgical interventions for cancer could reduce the number of recurrences and metastases, and hence pretreatment with NK-1 receptor antagonists prior to surgery could have synergic effects (Muñoz *et al.* 2010b). In addition, it has been reported that the NK-1 receptor is overexpressed in adenocarcinoma gastric samples and in gastric cancer cell lines and that *in vitro* SP promotes the proliferation, adhesion, migration and invasion of gastric adenocarcinoma cells. In view of these findings, the use of NK-1 receptor antagonists has been proposed for treating gastric carcinoma (Feng *et al.* 2011).

The release of SP plays an important role in neurogenic inflammation. The undecapeptide has been recently shown to increase the permeability of the blood–brain barrier (BBB) following central nervous system insults, making it a possible candidate as a mediator of tumour cell extravasations into the brain to form cerebral metastases (Lewis *et al.* 2013b). It is known that SP promotes BBB breaching by breast cancer cells through changes in microvascular endothelial cell tight junctions (Rodríguez *et al.* 2014). SP, which is secreted from breast cancer cells, induces the migration of these cells across the BBB, leading to the secretion of TNF-α and Ang-2 and promoting BBB impairment and the colonization of the central nervous system by tumour cells (Rodríguez *et al.* 2014). By contrast, it seems that NK-1 receptor antagonists like aprepitant (table 3; figure 1) may prevent breaching of the BBB by cancer cells and their brain colonization (brain metastasis).

These advances open up new perspectives for the specific chemopreventive inhibition of tumour cell invasion and metastasis, since tumour cell migration is a prerequisite for invasion and metastasis and is dependent on the signal substances of the immune and neuroendocrine systems. Thus, not only the neoangiogenesis and growth of the tumoural mass (figure 3), but also the peritumoural infiltration and metastasis (figure 5) could be regulated by the SP/NK-1 receptor system that is overexpressed in tumour cells and in tumoural and peritumoural tissues (including inflammatory cells, fibroblasts, blood vessels, nerves, and ganglia (Hennig *et al.* 1995; Friess *et al.* 2003; Muñoz *et al.* 2011). By using NK-1 receptor antagonists, the NK-1 receptor target could be used to inhibit all the above processes, and some potential clinical applications are already showing promising results: selective antagonists for NK-1 receptors,

such as aprepitant (figure 1), are now available and are widely used in clinical practice for other indications (see below).

3.4 *NK-1 receptor antagonists: synergic effects with chemotherapy*

As observed above, it has been reported that *in vitro* treatment with NK-1 receptor antagonists and MDAs has a synergic effect. Proliferative and antiapoptotic signalling, mediated by the NK-1 receptor, may be involved in producing this effect. MDAs, such as vinblastine, trigger apoptotic signalling through c-jun N-terminal kinases, and the modulation of MAPK pathway elements can enhance or inhibit MDA-induced apoptosis. It has been suggested that the NK-1 receptor antagonist L-733,060 (figure 1) sensitizes cancer cells to the MDA-mediated inhibition of cell viability by reducing antiapoptotic NK-1 receptor signalling, thus enhancing vinblastine-induced cell death (Muñoz *et al.* 2011). A better understanding of the mechanisms underlying this interaction may help us assess the clinical relevance of this novel synergistic combination. Moreover, this combination is synergistic for the growth inhibition of NK-1 receptor-possessing cancer cells, but not for normal cells. These data suggest that the combination of MDAs and NK-1 receptor antagonists could be useful in combinatorial cancer chemotherapy (Kitchens *et al.* 2009; Muñoz *et al.* 2011).

In addition, a synergic effect has been reported for the combination of the NK-1 receptor antagonist L-733,060 (figure 1) with common cytostatic drugs (adriamycin, mitomycin, ifosfamide, cisplatin) in MG-63 human osteosarcoma cells, but not in non-malignant HEK 293 cells (Muñoz *et al.* 2014a). Moreover, pretreatment of HEK 293 cells with a NK-1 receptor antagonist (L-733,060) prior to exposure to cytostatic drugs partially protected these cells from cytostatics (Muñoz *et al.* 2014a). This strategy might be clinically useful for cancer chemotherapy (Kitchens *et al.* 2009).

3.5 *NK-1 receptor antagonists decrease the side effects of radiotherapy and chemotherapy*

The antineoplastic agent cyclophosphamide and X-radiation, respectively, may provoke neurogenic inflammation in the urinary bladder and in the gastrointestinal tract, and this inflammation is mediated by NK-1 receptors. It is also known that NK-1 receptor antagonists reduce plasma protein extravasations caused by antineoplastic drugs (Muñoz *et al.* 2011). Thus, it seems that the neurogenic inflammation induced by cytostatics and radiation therapy is mediated by the release of SP from nerve terminals; after binding to NK-1 receptors located in the blood vessels, SP increases the permeability of these vessels, causing an extravasation of plasma proteins. Accordingly, by blocking the NK-1 receptors with NK-1 receptor antagonists, the triggering of the inflammatory cascade could be aborted and hence the lysis or apoptosis of neutrophils, mediated by inflammatory mediators, would be considerably decreased. These findings are very important because cytostatics and X-radiation produce, first, an inflammation of the mucosa and, second, a breakdown of the mucosal barrier. These sites provide a gateway for germs and elicit systemic infection, which is exacerbated by neutropenia secondary to the use of radiation and cytostatic drugs. In contrast, the use of NK-1 receptor antagonists improves neurogenic inflammation and both direct (induced by the radiation) and indirect (induced by the inflammatory mediators) processes.

In summary, the combined use of NK-1 receptor antagonists and chemotherapy and/or radiation therapy has a dual effect: on the one hand, it exerts a synergistic antitumour action (Kitchens *et al.* 2009) while on the other, it decreases the side effects of chemotherapy and radiation therapy (Alfieri and Cubeddu 2004).

3.6 *Safety and tolerability of the NK-1 receptor antagonists*

NK-1 receptor antagonists do not provoke serious side effects (Seabrook *et al.* 1996; Roila *et al.* 2009; Choi *et al.* 2010; Paul *et al.* 2010; Ständer *et al.* 2010), although headaches, hiccupping, vertigo and drowsiness have been reported after their administration (Roila *et al.* 2009; Ständer *et al.* 2010). Neither does the NK-1 receptor antagonist L-733,060 (figure 1) cause serious adverse cardiovascular effects (Seabrook *et al.* 1996), and aprepitant (figure 1), a selective antagonist of the human NK-1 receptor, has also been shown to be safe. The latter drug, used for the treatment of emesis, is in general well tolerated (with minimal side effects) (Choi *et al.* 2010; Paul *et al.* 2010; Ständer *et al.* 2010).

At present, aprepitant (Emend, MK-869, L-754,030) and fosaprepitant (Ivemend, MK-0517, L-758,298; this is converted to aprepitant by the action of ubiquitous phosphatases) (Saito *et al.* 2013) are the only agents commercially available for the prevention of CINV (chemotherapy-induced nausea and vomiting) and PONV (post-operative nausea and vomiting). It has been reported that a single intravenously dose of fosaprepitant (150 mg) was as safe and effective as the 3-day oral aprepitant regimen (Ruhlmann and Herrstedt 2011). Currently, there are more than 300 NK-1 receptor antagonists and this means that there are more than 300 potential drugs against the treatment of cancer. However, it seems that aprepitant (table 2; figures 1 and 4) is a good candidate for testing its antitumour activity in future human trials, because the drug is currently used in clinical practice for the treatment of emesis and hence the required safety studies for this drug have already been

carried out. In addition, the safety of aprepitant against human fibroblasts has been demonstrated: the IC_{50} for tumour cells is lower than the IC_{50} for fibroblasts and the IC_{50} for non-tumour cells is 90 μM whereas the IC_{100} for tumour cells is 60 μM (Muñoz and Rosso 2010). It has also been shown that the administration of aprepitant (300 mg/day) is well tolerated and no difference in the frequency of adverse events was observed in comparison to placebo (Kramer *et al.* 1998). Thus, in order for NK-1 receptor antagonists to exert an effective antitumour action, it has been suggested that higher doses of aprepitant than those used in CINV should be used and that the number of days on which aprepitant is currently administered should be increased (Muñoz and Coveñas 2013a). These issues should be investigated in-depth in order to demonstrate if an antiemetic could be used as an antitumour drug.

Carcinogenicity studies have been carried out for aprepitant and the carcinogenetic effects of this drug have been related to hepatic CYP metabolism (Muñoz and Coveñas 2013b). The administration of aprepitant (125–2,000 mg/kg/day) to male rats/mice increases thyroid follicular cell adenomas and carcinomas; thyroid parafollicular cell carcinoma; skin fibrosarcomas; and hepatocellular adenomas and/or carcinomas, whereas administration of the drug (125–2000 mg/kg/day) to female rats induces an increase in hepatocellular adenomas and carcinomas and thyroid follicular adenomas. Thyroid and liver tumours are a result of hepatic CYP enzyme induction in rodents, and this is consistent with the results observed in rodents with other compounds that induce hepatic CYP enzymes. However, judging by the concentration of aprepitant that exerts an antitumour effect in *in vitro* experiments, the doses of the drug that could be used in clinical practice would be very low (40-50 mg/kg/day for cancer treatment) in comparison with the doses used in carcinogenicity studies (125-2,000 mg/kg/day for carcinogenesis).

4. Conclusions

NK-1 receptor antagonists (table 3; figure 1) exert three complementary mechanisms: (1) an antiproliferative action, due to the inhibition of tumour cell growth, an action that is specifically exerted through the NK-1 receptors overexpressed by tumour cells, causing tumour cells die by apoptosis (table 2; figure 4); (2) the inhibition of angiogenesis in the tumour mass (figure 3); and (3) blocking the migration of tumour cells (invasion and metastasis) (figure 5). The antitumour activity of NK-1 receptor antagonists appears to be independent of its chemical structures, being associated, rather, with stereochemical features. Moreover, these antagonists inhibit tumour cell growth in a concentration-dependent manner. By contrast, it seems that SP (figure 2) elicits cancer progression after binding to the NK-1 receptor through three mechanisms: (a) as a universal mitogen for tumour cells overexpressing NK-1 receptors (the peptide also exerts an antiapoptotic effect) (table 2); (b) by stimulating the growth of endothelial cells (inducing neoangiogenesis) and up-regulating the growth of the tumour mass (figure 3); and (c) after binding to the NK-1 receptors in tumour cells, by activating the migration of tumour cells, which is a crucial requirement for invasion and metastasis (figure 5). Moreover, SP and the NK-1 receptor are implicated in emotional stress and in chronic inflammation (figures 3 and 5). Both processes are clearly correlated with the increased risk of developing cancer. All these observations suggest that the SP/NK-1 receptor system could play an important role in the genesis and development of cancer and metastasis; that the NK-1 receptor could be a promising target in the prevention and treatment of cancer; and that NK-1 receptor antagonists could improve cancer treatment. Accordingly, NK-1 receptor antagonists could be candidates for a new generation of antitumour drugs. In the twenty-first century, the era of 'molecularly targeted' anticancer therapy and of 'Magic Bullets' for cancer cells, NK-1 receptor antagonists could be considered as new, and highly promising antineoplastic agents, in the form of 'Intelligent Bullets'.

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