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Review

Cancer progression and substance P

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Summary. The substance P (SP)/neurokinin (NK)-1 receptor system plays an important role in cancer. After binding to the NK-1 receptor, SP induces tumor cell proliferation, migration of tumor cells (invasion, infiltration and metastasis) and angiogenesis. In contrast, NK-1 receptor antagonists inhibit tumor cell proliferation (tumor cells die by apoptosis), block the migratory activity of tumor cells, and exert antiangiogenic properties. The induction of apoptosis offers an appropriate method for cancer treatment. The NK-1 receptor can be considered as a target in cancer treatment. A common mechanism for cancer cell proliferation mediated by SP and the NK-1 receptor occurs and NK-1 receptor antagonists are broad-spectrum antineoplastic drugs. The NK-1 receptor antagonist aprepitant is used in clinical practice and exerts an antitumor action against a large number of different human tumor cells. In the future, such antitumor action should be tested in human clinical trials.

Key words: Angiogenesis, Aprepitant, Metastasis, NK-1 receptor, NK-1 receptor antagonist, Tumor

Introduction

The human genome project, gene therapy, stem cell research and the search for new cytostatic agents are promising lines of research in order to get better perspectives to cure cancer, but currently these lines of research have not been fully successful. None of the current treatments for cancer (surgery, radiation therapy, chemotherapy) have led to better perspectives for cancer patients in the last 20 years (see Muñoz et al., 2010a). For example, it has been reported that for 22 adult malignancies treated between 1990 and 2004, the treatment with cytotoxic chemotherapy showed a minor contribution (2% approximately) to the survival of cancer patients (Morgan et al., 2004) and although primary cutaneous malignant melanoma can be effectively managed with surgical treatment, obtaining high survival rates after a 5-year follow-up, survival dramatically decreases in stages III and IV of this tumor since in both stages no effective treatment exists (Muñoz et al., 2011a). It is known that cytostatic drugs show a low safety profile and severe side effects such as anaemia and leukopenia (neutropenic fever), because these drugs are not specific against tumor cells. Thus, the short-medium-term perspectives are not very promising for oncologic patients and hence it is necessary to explore urgently new research initiatives to improve cancer treatment. This research should be focused on drugs with the same or greater antitumor potential than cytostatic drugs but with fewer side effects and this could only be achieved if the drug was specific against tumor cells. This means that novel molecular targets for blocking tumor growth should urgently be investigated. Accordingly, the following questions arise: Which are the specific drugs against cancer cells? and Which are the molecular targets involved in cancer?

Both questions can be addressed. In cancer research, it is very important to know the genetic mechanisms involved in cancer and the causes of the origin of this disease, but it is also very important to know how the progression of cancer occurs. The answers to the above questions are closely related to cancer progression: many data show the involvement of the substance P (SP)/neurokinin (NK)-1 receptor system in this progression (Muñoz et al., 2010c; see Muñoz and

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Coveñas, 2013a for review) (Tables 1, 2). For example, it is known that: 1) SP is expressed in tumor cells (Fig. 1B,D); 2) The NK-1 receptor is overexpressed in cancer cells (Fig. 1A,C); 3) The NK-1 receptor is involved in the viability of tumor cells, since applying a knockdown method (small interfering RNA gene-silencing), the death of tumor cells by apoptosis was reported; 4) SP, after binding to the NK-1 receptor, induces the proliferation of tumor cells. The peptide acts as a mitogen on cancer cells (see Muñoz and Coveñas, 2013a for review). Accordingly, NK-1 receptor antagonists inhibit tumor cell growth and, in fact, tumor cells die by apoptosis (Muñoz et al., 2007; Muñoz and Coveñas, 2013a) (Table 1, Fig. 2). All these data suggest that the NK-1 receptor is an important target in cancer progression (Muñoz et al., 2011b) and that NK-1 receptor antagonists are a promising therapeutic strategy for the treatment of human cancer, since in comparison with normal cells, tumor cells overexpress NK-1 receptors. Thus, this overexpression suggests a specific treatment against tumor cells by using NK-1 receptor antagonists and hence a considerable decrease in the side effects of the treatment might be observed, in comparison to those found when cytostatic drugs are administered. This is quite important because one of the goals of cancer therapy should be to induce none or the fewest side effects possible. In sum, we review the involvement of the SP/NK-1 receptor system in cancer progression and the use of NK-1 receptor antagonists as antitumor drugs.

It is known that NK-1 receptor antagonists are safe and well tolerated in humans (Muñoz and Coveñas, 2013b) and a few patents regarding the use of NK-1 receptor antagonists as antitumor drugs have also been reported (Muñoz et al., 2012c). In 2005, a Phase II clinical trial was approved to investigate the potential of NK-1 receptor antagonists as antitumor drugs in cancer patients (reference: Eudra CT 2005-001585-13, Spain), but for economical and administrative reasons no such trial has yet been carried out. Thus, novel possibilities for translational research are emerging by studying the involvement of SP in cancer progression.

Involvement of the substance P/neurokinin-1 receptor system in cancer progression: focus on proliferation and migration of tumor cells and on neoangiogenesis

Many studies have demonstrated the involvement of the SP/NK-1 receptor system in cancer progression and currently there are sufficient data to suggest that a common mechanism for cancer cell proliferation mediated by SP and the NK-1 receptor occurs (Muñoz and Coveñas, 2013a) (Tables 1, 2). Both SP and the NK-1 receptor are widely distributed by the whole body. SP is the natural ligand with the highest affinity for the NK-1 receptor. SP is an undecapeptide belonging to the tachykinin family of peptides and is derived from the preprotachykinin A gene, its wide range of biological actions (e.g., inflammation, stress, angiogenesis, emesis, pain, depression, chemotaxis of leukocytes...) being mainly mediated by the NK-1 receptor.

Table 1. Cytotoxicity, mitogenic effect and NK-1 receptor isoforms.

TUMOR	CELL LINE	L-732,138		L-733,060		APREPITANT		SP nM	NK-1 RECEPTOR
		IC_{50} μM	IC_{100} μM	IC_{50} μM	IC_{100} μM	IC_{50} μM	IC_{100} μM		ISOFORMS kDa
Glioma	GAMG	48.1	100	21.3	43	33.1	66.2	50	54, 38, 33
Neuroblastoma	SKN-BE(2)	41	80.5	11.6	21	24.6	48.8	100	54
	IMR-32	45.7	99			19.6	45.1	5	75, 54 ,33
	KELLY	53.2	103.4			27.7	49.5	10	54, 33
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	100	75, 58, 33
Melanoma	MEL-HO	76.3	140.6	27.5	54	29.6	56.5	500	75, 58, 33
	COLO 858	44.6	97.6	8.7	17.5	24.3	52.1	10	75, 58, 33
	COLO 679	64.2	124.7	33.8	67	32.1	58.6	10	75, 58, 33
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	100	75, 58, 46, 33
Larynx carcinoma	HEp-2	38	77.3	21.3	42	22.7	46.5	50	75, 58, 46, 34
Gastric carcinoma	23132-87			14.3	29.6	24.2	52.5	10	75, 58, 46, 34
Colon carcinoma	SW-403			14.5	25.8	30.5	60.5	50	75, 58, 46, 34
Small-cell lung cancer	H-69	51.9	109	18.7	39.2	21.8	45.2	5-100	75, 58, 46, 34
Non-small-cell lung cancer	COR-L23	87	102.3	22	41.5	30	60	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
B Acute lymphoblastic leukemia	SD-1	49.7	103.5	18.4	50	29.4	59.2	5-50	75, 58, 33
Osteosarcoma	MG-63	58.6	100	14.5	30	28.6	80	5-500	46

Results obtained from the cytotoxicity assays (IC50, IC100) regarding the antitumor action of the NK-1 receptor antagonists (L-732,138, L-733,060, aprepitant) on human tumor cell lines. Also indicated is the most mitogenic dose of SP found and the isoforms of the NK-1 receptor detected.

It has been fully demonstrated that cancer cells overexpress NK-1 receptors (Fig. 1A,C) and that after binding to SP, the undecapeptide induces the proliferation of these cells (Muñoz et al., 2011b, 2013a) (Tables 1, 2). NK-1 receptors are generally located in both the plasma membrane and the cytoplasm of cancer cells, but occasionally they have also been located in the nucleus of these cells (González-Moles et al., 2008; Rosso et al., 2008; Muñoz et al., 2012b) (Fig. 1A,C). In humans, the full-length and the truncated subtypes of the NK-1 receptor have been reported: the former mediates a slow growth of cancer cells and the second enhances such growth and stimulates the production of cytokines which upregulate the truncated form (Patel et al., 2005). This form mediates malignancy in tumor cells and 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). It is also known that normal cells express a lower number of NK-1 receptors than tumor cells; that tumor samples from patients with advanced tumor stages exhibit significantly higher NK-1 receptor levels; that astrocytoma/glioma cell lines in culture show a lower number of NK-1 receptors than astrocytoma/glioma primary tumors; that the expression of preprotachykinin A is increased in breast cancer in comparison with that found in normal mammary epithelial cells; that *TAC1R* mRNA is present in human acute lymphoblastic leukemia cell lines, with the highest levels in these cells and the lowest ones in normal cells; that increased mRNA NK-1 receptor expression occurs in malignant tissues, but not in benign ones; that glioblastomas express more NK-1 receptors than astrocytomas, and that the most malignant phenotypes show a higher rate of NK-1 receptor expression and were associated with advanced tumor stages and poorer prognosis (Hennig et

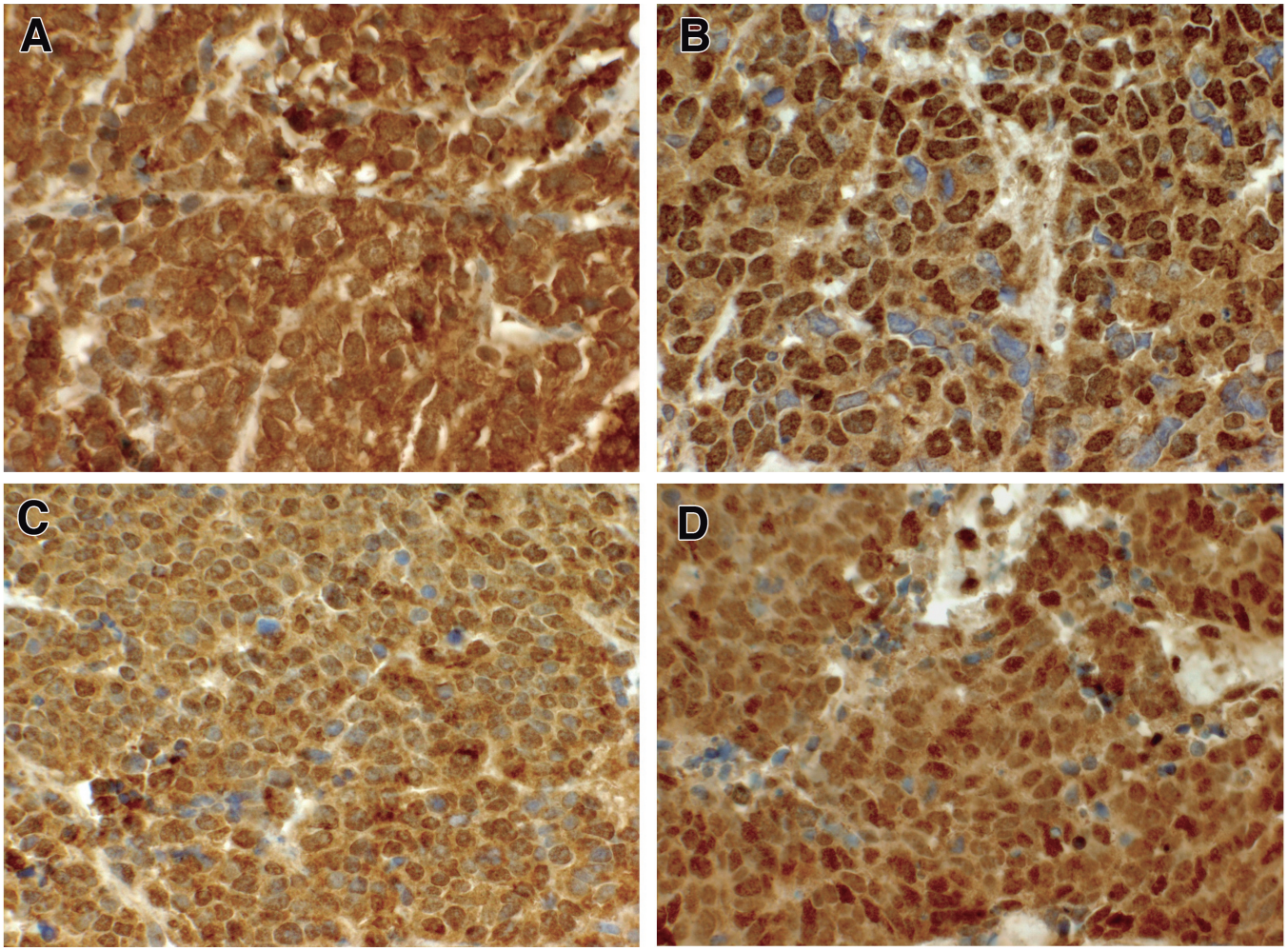

Fig. 1. Presence of NK-1 receptors in non-small-cell **(A)** and small-cell lung **(C)** carcinomas. The immunoreactivity for the NK-1 receptors was found in the cytoplasm and, occasionally, in the nucleus of tumor cells. Presence of SP in non-small-cell **(B)** and small-cell lung **(D)** carcinomas. Immunoreactivity for SP was predominantly located in the nucleus, the nuclear membrane showing a strong staining. In all the cases, the sections were counterstained with hematoxylin. x 40

al., 1995; Luo et al., 1996; Singh et al., 2000; Friess et al., 2003; Muñoz et al., 2010a, 2011b, 2012a). The data suggest that the number of NK-1 receptors could be correlated with the degree of malignancy. In addition, NK-1 receptors play an important role in the viability of tumor cells (Muñoz et al., 2010c, 2012a,b). Cancer cells need such a receptor for their own survival, since it seems that tumor cells depend strongly on the potent mitotic signal mediated by SP and that by means of the overexpression of the NK-1 receptor tumor cells neutralize their own pathways, leading to cell death (Esteban et al., 2006). In addition, SP protects tumor cells from apoptosis (DeFea et al., 2000). Thus, the overexpression of NK-1 receptors visualized by immunohistochemistry could serve to facilitate the identification of tumors for diagnostic or therapeutic purposes (Fig. 1A,C) and suggests the possibility of finding a specific treatment against cancer using NK-1 receptor antagonists. This strategy opens up new approaches for translational research.

SP, after binding to the NK-1 receptor, exerts a mitogenic action in both normal and tumor cells (Muñoz and Coveñas, 2013a) (Table 1). After the activation of the NK-1 receptor by SP, an increase in DNA synthesis occurs in tumor cells, and it seems that SP activates members of the mitogen-activated protein kinase (MAPK) family, including extracellular signal-regulated kinases 1 and 2 (ERK1/2) and p38MAPK (Luo et al., 1996). Once activated, ERK1/2 is translocated into the nucleus, inducing proliferation and protecting the cell from apoptosis (DeFea et al., 2000; Muñoz et al., 2011b). In tumor cells, SP increases the phosphorylation and activity of Akt or protein kinase B, 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) (Fig. 3). 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) (Fig. 3). SP has been found in the cytoplasm and in the nucleus of tumor cells (Fig. 1B,D), suggesting that the undecapeptide could regulate the nuclear activity of cancer cells (Muñoz and Coveñas, 2013a). This finding also implies that the peptide could be secreted by primary tumors. Moreover, it is known that breast cancer cells have high levels of SP but non tumorigenic cells show very low levels of the peptide (Singh et al., 2000). Thus, SP could induce

Table 2. Presence of substance P and NK-1 receptors in tumor cells and samples.

TUMOR/tissues/cells	SUSTANCE P	NK-1 RECEPTOR
Glioma/astrocytoma	Palma et al., 2000	Hennig et al., 1995; Palma et al., 2000; Muñoz et al., 2005b
Melanoma	Khare et al., 1998	Muñoz et al., 2010c
Breast carcinoma	Singh et al., 2000	Hennig et al., 1995; Singh et al., 2000
Thyroid carcinoma		Hennig et al., 1995
Retinoblastoma	Tarkkanen et al., 1983; Muñoz et al., 2005a	Muñoz et al., 2005a
Pancreatic carcinoma		Friess et al., 2003; Muñoz et al., 2006
Neuroblastoma	Nowicki and Miskowiak 2002	Muñoz et al., 2005b
B and T Acute lymphoblastic leukemia	Nowicki et al., 2003	Muñoz et al., 2012a
Lung cancer: Small- and non-small-cells	Muñoz et al., 2012b	Muñoz et al., 2012b
Larynx carcinoma	Esteban et al., 2009	Muñoz et al., 2008; Esteban et al., 2009
Gastric carcinoma		Rosso et al., 2008
Colon carcinoma		Rosso et al., 2008
Oral carcinoma	Brener et al., 2009	Brener et al., 2009
Osteosarcoma		Muñoz et al., 2014

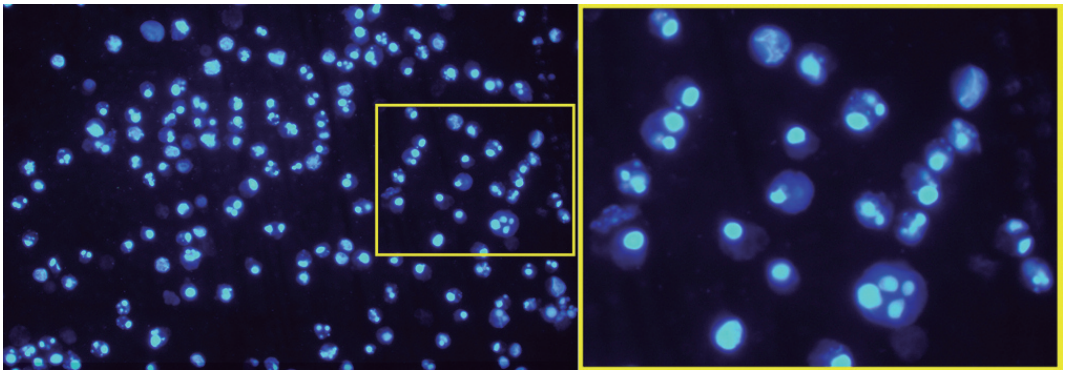

Fig. 2. Culture breast carcinoma cells treated with the NK-1 receptor antagonist aprepitant. Note apoptotic figures: chromatin condensation and nuclear fragmentation are observed (x 40). A high power magnification of the region delimited by the left rectangle is also shown.

mitogenesis and tumor progression via the following mechanisms: 1) autocrine (SP is secreted from tumor). The peptide is also secreted by non-tumor cells (e.g., inflammatory cells); 2) The peptide is released from nerve terminals. A direct interaction between the nervous system and tumor cells occurs; 3) endocrine (SP is released from the tumor mass into the blood vessels); 4) paracrine, since SP exerts a mitogenic action in the endothelial cells favoring neoangiogenesis; and 5) SP reaches the whole body through the bloodstream. This is regulated by the limbic system (Muñoz et al., 2010a, 2011b; Muñoz and Coveñas, 2013a). This is quite important since when there is an increase in the synthesis of SP, as occurs in depression and in chronic inflammatory processes (Cook et al., 1994; Walsh et al., 1995; Keranen et al., 1996a,b; Kramer et al., 1998; O'Connor et al., 2004), the alteration of the SP/NK-1 receptor system could facilitate the development of cancer (Muñoz et al., 2010a, 2011b). In fact, cancer and depression co-occur commonly and chronic and severe depression may be associated with an elevated risk of developing cancer (Spiegel and Giese-Davis, 2003). The elevated risk could be related to the increased level of SP during depression (Kramer et al., 1998), since the increased level of the undecapeptide could accelerate cancer progression. By this mechanism, the link between the emotional stress (e.g., depression) and cancer progression could be partly explained. Moreover, it is known that SP via the NK-1 receptor induces the release of interleukins, taurine and glutamate from tumor cells (Tung and Lee, 1991; Johnson and Johnson, 1993; Gitter et al., 1994); such a release induces an inflammatory process, increasing the levels of SP and hence increasing tumor cell proliferation. Elevated levels of SP and upregulated NK-1 receptor expression have been found in patients with inflammatory bowel disease (O'Connor et al., 2004). In sum, all these data suggest that the use of NK-1 receptor antagonists could exert antiinflammatory and antidepressant effects by blocking the biological actions of SP.

SP induces the migration and spreading of tumor cells (Feng et al., 2011) (Fig. 3). Moreover, SP induces a rapid change in cellular shape (including blebbing). Membrane blebbing is important in cell movement, cell spreading, and cancer cell infiltration (Fackler and Grosse, 2008; Meshki et al., 2009). It is 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). Currently, it is not known how tumor cells cross the blood-brain barrier to form brain metastases. However, SP release is a key component of neurogenic inflammation; the undecapeptide has been recently shown to increase the permeability of the blood-brain barrier following central nervous system insults, making it a possible candidate as a mediator of tumor cell extravasation into the brain to form cerebral metastases (Lewis et al., 2013). In fact, it has been demonstrated that SP promotes blood-brain barrier breaching by breast cancer cells through changes in microvascular endothelial cell tight junctions (Rodríguez et al., 2013). The prevention of metastasis is a major goal in the treatment of cancer, since over 90% of cancer deaths are derived not from the primary tumor but from the development of metastases (Sporn, 1996). All the above data suggest that SP regulates metastasis and hence new strategies using NK-1 receptor antagonists should be developed in the future for the treatment of cancer.

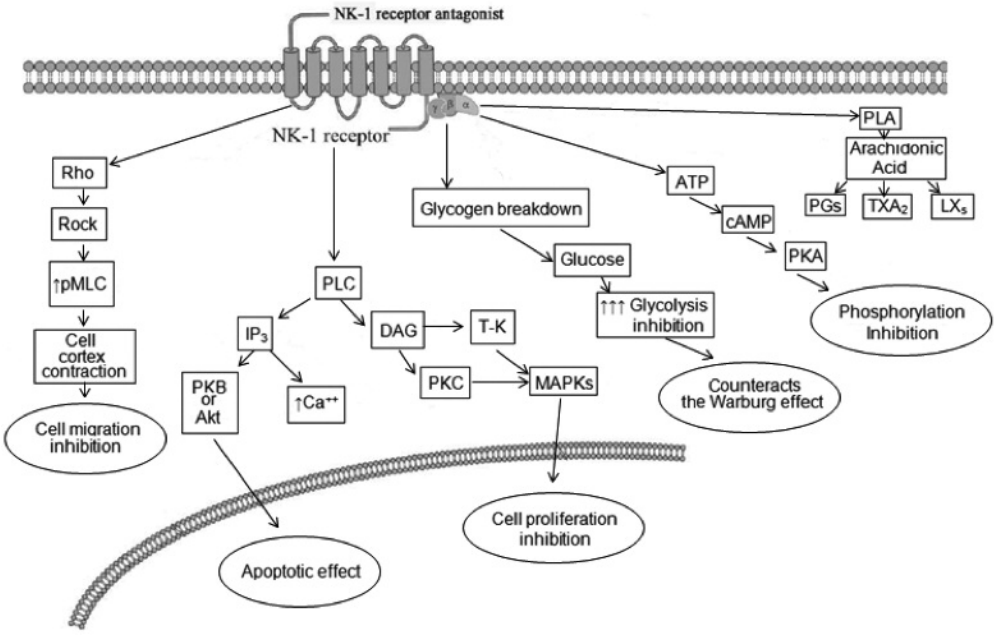

Fig. 3. Model for the signaling pathways downstream of the NK-1 receptor. Activation of this receptor by SP leads to cell proliferation, antiapoptotic effect and cell migration. The pathways indicated are involved in these mechanisms. However, NK-1 receptor antagonists, after binding to the NK-1 receptor, block such pathways and inhibit both tumor cell proliferation and migration, as well as exerting an apoptotic effect on tumor cells. In addition, NK-1 receptor antagonists counteract the Warburg effect. ATP: adenosine triphosphate; cAMP: cyclic adenosine monophosphate; DAG: diacilglicerol; IP3: 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; TK: tyrosine-kinase; TXA2: thromboxane A2.

SP and the NK-1 receptor have been located in intra- and peri-tumoral blood vessels and it is known that during neoangiogenesis, a hallmark of tumor development, both the expression of NK-1 receptors and tissue innervation are increased (Hennig et al., 1995; Muñoz et al., 2011b). Thus, SP facilitates the proliferation of endothelial cells, stimulating vessel growth, and increasing tumoral blood flow (Ziche et al., 1990; Hennig et al., 1995).

The potential use of neurokinin-1 receptor antagonists as antitumor drugs: focus on aprepitant

In the previous section, we have suggested the use of NK-1 receptor antagonists to block the proliferation and the migration of cancer cells, as well as the neoangiogenesis in the tumor mass. One question arises: Which is the most appropriate NK-1 receptor antagonist to be used in a future human clinical trial in order to demonstrate its antitumor, antimetastasis and antiangiogenic action? There are many peptide and non-peptide (the drug aprepitant, L-732,138, L-733,060, CP-99,994, RP-67,580, WIN-51,708...) NK-1 receptor antagonists (Table 1). Peptide NK-1 receptor antagonists suffer from a number of drawbacks, such as poor potency and neurotoxicity. The most appropriate non-peptide NK-1 receptor antagonist is the drug aprepitant (Emend, MK-869, L-754,030) (Table 1, Fig. 4). Aprepitant and its intravenously administered prodrug fosaprepitant (Ivemend, MK-0517, L-758,298) are the only non-peptide NK-1 receptor antagonists currently used in clinical practice. Fosaprepitant is rapidly converted to aprepitant via the action of ubiquitous phosphatases (Saito et al., 2013). Aprepitant and fosaprepitant are used for the treatment of acute and delayed chemotherapy-induced nausea and vomiting and post-operative nausea and vomiting. Chemotherapy induces the release of SP and aprepitant blocks the unwanted actions exerted by SP on the central nervous system (Hargreaves et al., 2011). It is known that a single intravenous dose of fosaprepitant is as effective and safe as oral 3-day aprepitant administration (Muñoz and Coveñas, 2013b). This means that a large part of the required safety and characterization studies for aprepitant have already been carried out (Muñoz and Coveñas, 2013b).

We shall focus our review on non-peptide NK-1 receptor antagonists. The safety of the drug aprepitant has been reported in many human clinical trials (Muñoz and Coveñas, 2013b) and in human fibroblasts, in which the IC_{50} is three times higher than the IC_{50} for tumor cells (Muñoz and Rosso, 2010). Moreover, it is known that the IC50 for non-tumor cells is 90 μM but the IC_{100} for tumor cells is 60 μM approximately (Muñoz and Rosso, 2010). It has also been demonstrated that aprepitant (300 mg/day) was well tolerated and no statistically significant difference in the frequency of adverse events was observed as compared to placebo (Muñoz and Coveñas, 2013b). Aprepitant is associated with minimal side effects and the majority of the adverse events are mild or moderate (Muñoz and Coveñas, 2013b). Aprepitant is mainly metabolized by cytochrome P450, family 3, subfamily A (CYP3A4), in human liver (Sánchez et al., 2004). It is an inhibitor and a substrate of the CYP3A4 metabolic pathway, which in humans is involved in the metabolism of a broad range of drugs. Aprepitant is a mild inducer of CYP2C9 and when used for more than 7 days it may act as an inducer of CYP3A4. Its half-life ranges from 9 to 13 h and it binds to plasma proteins (Ruhlmann and Herrstedt, 2011; Stiff et al., 2013). Moreover, aprepitant has been developed as a nanoparticle formulation to enhance exposure and to minimize food effects (Olver et al., 2007). In humans, the nanoparticle formulation increased 3-4 times the bioavailability of aprepitand (Olver et al., 2007).

Many *in vitro* and *in vivo* studies have reported that NK-1 receptor antagonists exert an antitumor action and

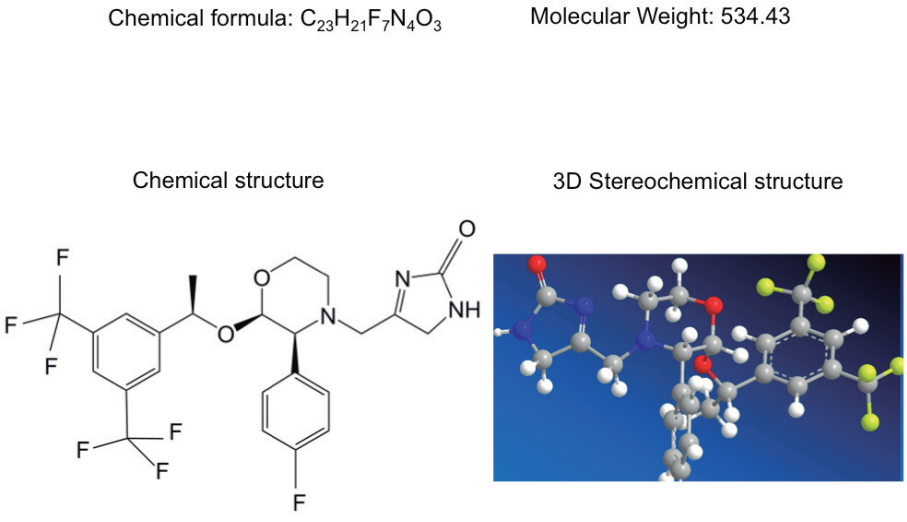

Fig. 4. Chemical formula, chemical structure and 3D stereochemical structure of the drug aprepitant.

inhibit tumor cell growth in a dose-dependent manner (Seckl et al., 1997; Palma et al., 2000; Bigioni et al., 2005; Guha et al., 2005; Manske and Hanson, 2005; Prasad et al., 2007; see Muñoz et al., 2010a, 2011b for review) (Table 1, Fig. 3). After binding to the NK-1 receptors overexpressed in tumor cells, NK-1 receptor antagonists activate the apoptotic machinery and tumor cells die by apoptosis (Muñoz et al., 2010a, 2011b) (Fig. 3). This induction is a highly suitable approach to cancer treatment, although currently the exact mechanisms responsible for inducing apoptosis in tumor cells are unknown. It has been suggested that tumor cells develop strategies to neutralize the multiple pathways leading to cell death, and it has been suggested that one important strategy is the expression of the NK-1 receptor, since in this way tumor cells highly dependent on the SP stimulus, which provides a potent antiapoptotic and mitotic signal (Esteban et al., 2006). This signal could counteract the different death signal pathways activated in tumor cells. By contrast, the absence of the mitotic signal when the NK-1 receptors are blocked with NK-1 receptor antagonists could tilt the balance within the cell to favoring apoptotic/death signals, and hence the cell dies (Esteban et al., 2006) (Fig. 3).

It has been demonstrated that co-administration of a non-peptide NK-1 receptor antagonist and vinblastine was synergistic for the inhibition of the growth of cancer cells expressing NK-1 receptors, but not for non-tumoral cells. It seems that the NK-1 receptor antagonist reduces antiapoptotic NK-1 receptor signaling and hence enhances vinblastine-induced cell death (Muñoz and Coveñas, 2013a). In addition, a synergic effect has been reported for the combination of the NK-1 receptor antagonist L-733,060 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., 2014). 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., 2014). This strategy might be clinically useful for cancer chemotherapy (Kitchens et al., 2009). It has also been reported that cyclophosphamide (an antineoplastic agent) and X-radiation induce neurogenic inflammation, this process being mediated by SP; that placlitaxel (a cytostatic) exerts adverse pulmonary actions, this also being mediated by SP, and that cisplatin impairs renal function. In all three cases an improvement was observed when NK-1 receptor antagonists were administered (Alfieri and Cubeddu, 2000, 2004; Sendo et al., 2004). These data suggest that these antagonists, in addition to exerting an antitumor action, should decrease the side effects produced by chemotherapy and radiation treatments to a considerable extent (Alfieri and Cubeddu, 2000, 2004; Sendo et al., 2004).

It has been reported in astrocytoma cells that SP, after binding to the NK-1 receptor, stimulates glycogen breakdown, producing glucose (Medrano et al., 1994). This is very important because most cancer cells predominantly produce energy by glycolysis followed by lactic acid fermentation; this is known as the Warburg effect (Warburg, 1956). Growing tumor cells have glycolytic rates up to 200 times higher than those of their normal tissues of origin. The release of SP from tumor cells produces glycogen breakdown and the glucose obtained would be used by tumor cells to increase their metabolism (glycolysis) (Medrano et al., 1994). This mechanism could in part explain the Warburg effect. By contrast, after binding to the NK-1 receptors located in cancer cells, NK-1 receptor antagonists would block glycogen breakdown (Medrano et al., 1994). In this way, the antagonists can counteract the Warburg effect because glycolysis is not possible when glucose is absent (Muñoz and Coveñas, 2013a) (Fig. 3).

NK-1 receptor antagonists inhibit the SP-mediated migratory activity of tumor cells and the SP-mediated changes in cellular shape, including blebbing (Lang et al., 2004; Meshki et al., 2009; Feng et al., 2011) (Fig. 3). It has been reported that in patients with breast cancer the risk of recurrence or metastasis is reduced four-fold over a 2.5-4 year follow-up period when surgery is associated with paravertebral anesthesia (Exadaktylos et al., 2006). The SP/NK-1 receptor system could be involved in this process, since it has been suggested that paravertebral anesthesia blocks the SP-induced migration, invasion and metastasis of tumor cells (Muñoz et al., 2010b). This hypothesis is quite interesting, because the use of paravertebral anesthesia prior to cancer surgical intervention could reduce the number of recurrences and metastases in the surgical treatment of tumors, and hence pretreatment with NK-1 receptor antagonists prior to surgical intervention could have synergic effects (Muñoz et al., 2010a). Moreover, the SP/NK-1 receptor system controls angiogenesis, since NK-1 receptor antagonists decrease tumor-associated angiogenesis and block the mitogenesis of endothelial cells mediated by SP (Guha et al., 2005; Muñoz et al., 2010a, 2011b). By contrast, angiogenesis is enhanced after the administration of SP/NK-1 receptor agonists, since these agonists increase the mitogenesis of endothelial cells, stimulating vessel growth (Ziche et al., 1990).

Concluding remarks

The data reported above indicate that the SP/NK-1 receptor system plays an important role in cancer progression, metastasis and neoangiogenesis. SP acts as a universal mitogen for tumor cells overexpressing the NK-1 receptor and NK-1 receptor antagonists also induce a universal effect - apoptosis - on cancer cells. Thus, NK-1 is a promising target in cancer treatment and NK-1 receptor antagonists represent an important opportunity for exploitation as a promising generation of broad-spectrum anticancer drugs. NK-1 receptors could also exert a selective action (contrary to classic cytostatics) against tumor cells, since these cells overexpress the NK-1 receptor. Moreover, because tumor-cell migration is mediated by SP and is a prerequisite for invasion and metastasis and because the

undecapeptide is involved in angiogenesis, NK-1 receptor antagonists could be used to inhibit both processes. It is urgently necessary to test in human clinical trials the antitumor action, the antimetastatic activity and the antiangiogenic action of NK-1 receptor antagonists (e.g., the drug aprepitant). Most clinical trials have focused on the antiemetic action of aprepitant in cancer patients treated with chemotherapy, but the efficacy and safety of aprepitant have not been fully tested as an antitumor drug. Aprepitant is the main NK-1 receptor antagonist candidate for use in the treatment of cancer, since its possible evaluation in human clinical trials could be easier or faster than for less investigated compounds, because many of the required safety and characterization studies have already been completed (Rost et al., 2006; Hargreaves et al., 2011). It seems that by increasing the number of days on which aprepitant is currently administered in clinical practice and using higher doses of the drug than those used in chemotherapy-induced nausea and vomiting, this non-peptide NK-1 receptor antagonist could be effective in cancer. *In vitro* studies have reported that the drug aprepitant exerts an antitumor action against a broad number of different types of human cancer cells (Muñoz and Rosso, 2010) and an *in vivo* study has recently reported that the drug fosaprepitant also exerts an antitumor action against human osteosarcoma xenografts (Muñoz et al., 2014).

In sum, the involvement of the SP/NK-1 receptor system in cancer must be exploited in-depth as a therapeutic strategy. There are sufficient data to suggest that a common mechanism for cancer cell proliferation mediated by SP and the NK-1 receptor occurs and that NK-1 receptor antagonists are broad-spectrum antineoplastic drugs. The ultimate goal will be to demonstrate that aprepitant is able to improve prognosis and to decrease morbidity and mortality in cancer patients by exerting antitumor actions through three mechanisms: 1) an antiproliferative effect (inducing tumor cell death by apoptosis and counteracting the Warburg effect in tumor cells); 2) the inhibition of angiogenesis in the tumor mass; and 3) inhibition of the migration of tumor cells (invasion, infiltration and metastasis).

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