Acta Histochemica xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Increased nuclear localization of substance P in human gastric tumor cells

Miguel Muñoz [a,*], Marisa Rosso [a], Andrés Carranza [a], Rafael Coveñas [b]

[a] Virgen del Rocío University Hospital, Research Laboratory on Neuropeptides (IBIS), Sevilla, Spain
[b] Institute of Neurosciences of Castilla y León (INCYL), Laboratory of Neuroanatomy of the Peptidergic Systems (Lab. 14), University of Salamanca, Salamanca, Spain

ARTICLE INFO

Article history:
Received 21 November 2016
Received in revised form 8 March 2017
Accepted 8 March 2017
Available online xxx

Keywords:
Inmunohistochemistry
Nucleus
Stomach
Tachykinin

ABSTRACT

Gastric cancer (GC) is an aggressive disease that remains the fourth most common type of cancer and is the second leading cause of cancer-related death worldwide. Treatment of advanced or metastatic GC has seen little progress and median overall survival in this group remains <1 year. It is urgent to investigate new mechanisms to understand GC progression. It is known that substance P (SP), after binding to the neurokinin-1 (NK-1) receptor, elicits GC proliferation; that GC cells and samples express NK-1 receptors; that NK-1 receptor antagonists, in a concentration dependent manner, inhibit the proliferation of GC cells and that these cells die by apoptosis. However, the presence of SP in GC and normal gastric cells is unknown. In order to know more on the involvement of the SP/NK-1 receptor system in GC, we studied in thirty human GC and normal gastric samples the immunolocalization of SP after using an immunohistochemical technique. SP was observed in the cytoplasm and in the nucleus of GC and normal gastric cells. The nuclear expression of SP was higher in GC cells than in normal cells. No significant difference was observed when the cytoplasmatic expression of SP in normal and GC cells was compared. The findings suggest that SP plays an important role in both nuclear function and GC.

1. Introduction

Gastric cancer (GC), which it is often diagnosed at an advanced stage, is the fourth most common type of cancer and the second leading cause of cancer-related death worldwide (Ferro et al., 2014). The treatment of this aggressive disease is surgical resection with adjuvant chemotherapy or chemoradiation. This procedure has led to improved survival (Carcas, 2014; Sasako et al., 2011), but the treatment of advanced or metastatic GC has unfortunately little progress and, in this case, the median overall survival is <1 year (Cervantes et al., 2013). This means that new strategies are required to improve the survival of GC patients.

Many works have demonstrated the involvement of the substance P (SP)/neurokinin (NK)-1 receptor system in cancer. SP peptide is widely distributed throughout the body and organic fluids and, via the NK-1 receptor, induces the proliferation and migration of tumor cells, metastasis and angiogenesis (Muñoz and Coveñas, 2014; Muñoz et al., 2015). Regarding GC, it is known that the SP/NK-1 receptor system is involved in the proliferation, adhesion, migration and invasion of MKN45 GC cells (Feng et al., 2011); that human GC cells and tissues express different isoforms of the NK-1 receptor (Feng et al., 2011; Rosso et al., 2008), and that the administration of high doses of SP favors gastric carcinogenesis induced by *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine, this effect being associated with the stimulation of the proliferation of antral epithelial cells (Tatsuta et al., 1995). Moreover, in human GC (in which NK-1 receptors and nerves containing SP have been observed), it has been suggested that the number of SP-positive nerves would be related to GC progression (Feng et al., 2011). The above studies and many others have demonstrated that the SP/NK-1 receptor system is overexpressed in tumor cells (Muñoz and Coveñas, 2014; Muñoz et al., 2015) and this suggests that these cells, via the NK-1 receptor, need the signal mediated by SP. This is a crucial point, since for example it is known that SP exerts an antiapoptotic effect by activating Akt (Koon et al., 2007). By contrast, it has been reported in many human cancer cell lines that the treatment with NK-1 receptor antagonists inhibits the proliferation of tumor cells and, induces the death of tumor cells by apoptosis, as well as inhibits the angiogenesis and the migration of tumor cells (Muñoz and Coveñas, 2014; Muñoz et al., 2015). In this sense, in human GC cell lines it has been reported that NK-1 receptor antagonists (e.g., aprepitant, L-733,060), after binding to the NK-1 receptor, blocked the mitogenic action of SP, inhibited cell

* Corresponding author at: Hospital Infantil Universitario Virgen del Rocío, Unida de Cuidados Intensivos Pediátricos, Av. Manuel Siurot s/n, 41013, Sevilla, Spain.
E-mail address: mmunoz@cica.es (M. Muñoz).

http://dx.doi.org/10.1016/j.acthis.2017.03.003
0065-1281/

Table 1
Gastric tumor samples. SP immunostaining.

Case	Sex	Age	Grade Well (WD), moderate (MD), poor (PD)	Immunostaning extension		Cytoplasm			Nucleus		
						Intensity degree			Intensity degree		
				Cytoplasm	Nucleus	1	2	3	1	2	3
1	F	70	WD	60	70	20	30	10	5	10	55
2	M	56	WD	70	60	40	20	10	20	20	20
3	M	66	WD	40	80	20	20			40	40
4	M	73	WD	10	70	10					70
5	M	61	WD	10	70	10				30	40
6	M	70	WD	60	70	10	40	10	5	5	60
7	M	48	WD	70	90	10	60			10	80
8	M	65	WD	40	70	10	30			30	40
9	F	70	WD	60	70	10	40	10	0	10	60
10	M	83	WD	40	70	20	20			20	50
11	F	72	MD	80	50	20	20	40	20	20	10
12	F	56	MD	70	30	20	40	10	20	5	5
13	M	44	MD	80	90		80				90
14	M	81	MD	70	70	10	50	10	10	50	10
15	M	55	MD	70	10			70	10		
16	M	46	MD	70	10	70			10		
17	M	58	MD	80	90	80					90
18	M	69	MD	20	70	10	10			30	40
19	F	79	MD	40	30	30	10		25	5	
20	F	70	MD	70	5		20	50	5		
21	M	61	PD	70	80		35	35	20	50	10
22	F	76	PD	60	50	10	40	10	10	30	10
23	F	77	PD	70	70		20	50		40	30
24	M	81	PD	70	70		20	50		40	30
25	M	80	PD	40	90	40				10	80
26	F	38	PD	70	70	20	30	20	10	30	30
27	F	53	PD	80	80	30	30	20	20	30	30
28	M	69	PD	50	80	50				20	60
29	M	73	PD	80	80	10	30	40	10	10	60
30	F	61	PD	60	40		35	25		15	25

proliferation and induced the death of these cells apoptosis pathway (Muñoz and Rosso, 2010; Muñoz et al., 2010b; Rosso et al., 2008).

All the data reported above suggest that the SP/NK-1 receptor system is crucial for the survival of tumor cells (e.g., GC cell). In sum, cancer cells need the SP stimulus and the expression of the NK-1 receptor. The undecapeptide can be released from nerve terminals and/or can circulate through the bloodstream. However, the presence of SP in GC cells is currently known. In order to know more about the involvement of the SP/NK-1 receptor system in human GC, the aim of this study was to carry out an immunohistochemical study and to demonstrate the expression, localization and distribution of SP in human normal and GC samples.

2. Material and methods

In this study, informed consent, protocols and procedures were approved by the local ethic committee (Virgen del Rocío University Hospital, Seville, Spain). Guidelines of the ethics and legal recommendations of Spanish and European laws were followed (date: 13 July 2006, Act N°7/06) as well as the study was performed in accordance with the Declaration of Helsinki.

Human primary gastric adenocarcinomas and normal stomach samples were obtained, after surgical intervention, from the same patients (n = 30) (Department of Pathology, Hospital 'Virgen del Rocío', Seville, Spain). Informed consent or substitute for it was obtained from all patients for being included in the study: 19 men (63.3%) and 11 women (36.6%) were included. Age at diagnosis ranged between 38 and 83 years old, with an average age of 65.36 years. According to the degree of differentiation, GC was classified as well (10 cases), moderate (10 cases) and poor (10 cases) differentiated (Table 1).

Formalin-fixed and paraffin-embedded tumors (gastric adenocarcinomas) and normal stomachs were cut at 5 μm and dried overnight at 37 °C. Sections were deparaffinized with xylene, hydrated through a series of solutions containing decreasing concentrations of ethanol, and immersed in water. After pressure cooker antigen retrieval in citrate buffer (pH 6.0), slides were cooled at room temperature for 10 min. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 30 min at room temperature. After washing with 0.05 M Tris, sections were incubated with 10% non-immune pig serum for 30 min at room temperature. Later, they were incubated overnight at 4 °C with 1/1000 diluted anti-SP primary antibody (S-1542, Sigma- Aldrich San Louis Missouri, USA). By dot blot, this antibody reacted with SP. Cross-reactivity was observed with SP (7–11) and SP (6–11) fragments and a weak cross-reactivity with SP (1–7) and neurokinin A. The antibody anti-SP used here did not show cross-reactivity with neurokinin B, neuropeptide Y, vasoactive intestinal peptide, calcitonin gene-related peptide, calcitonin and somatostatin. Sections were then washed in 0.05 M Tris at room temperature. The next step was the addition of Envision System-HRP (Dako Diagnósticos S.A., Barcelona, Spain) reagents during 30 min at room temperature. Slides were rinsed with 0.05 M Tris, and the immunoreactivity was visualized with 3, 3′-diaminobenzidine chromogen solution (DAB+; Dako). Cell nuclei were lightly counterstained with hematoxylin. Finally, as previously published (Muñoz et al., 2012) and in order to determine the specificity of the immunostaining, primary lung cancer was used as positive control. As negative control, the primary antibody was omitted, being replaced by non-immune serum (Fig. 1G, H). In both cases, the results obtained confirmed the specificity of the SP antibody used here.

Slides were evaluated by two independent investigators. In each slide, 10 representative microscopic high-power fields were stud-

Please cite this article in press as: Muñoz, M., et al., Increased nuclear localization of substance P in human gastric tumor cells. Acta Histochemica (2017), http://dx.doi.org/10.1016/j.acthis.2017.03.003