

Expression of Galanin in Melanocytic Tumors

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Abstract. *Introduction.* Galanin is a neuropeptide with wide-ranging effects, especially within the endocrine and the nervous systems. Galanin and its receptors are present in human skin. Galanin is expressed in different neural, endocrine and neuroendocrine tumors and, on the other hand, several neuropeptides, especially α -MSH, seem to play a role in the pathogenesis of melanoma.

Objective. To investigate the expression of galanin in cutaneous melanomas and melanocytic nevi and correlate it with α -MSH expression and several prognostic factors for melanoma.

Patients and methods. We performed an observational, retrospective study of the immunohistochemical expression of galanin and α -MSH in samples of cutaneous melanomas diagnosed in the last 5 years in a tertiary care hospital. Different types of melanocytic nevi were also analyzed.

Results. A total of 130 pigmented lesions were studied: 38 primary cutaneous melanomas, 6 cutaneous metastases of melanoma and 86 melanocytic nevi. Immunostaining with galanin and α -MSH was significantly higher in melanomas than in melanocytic nevi ($p < 0.001$), although spindle cell and blue nevi showed significant expression of α -MSH. More than 50% of nodular melanomas and 90% of superficial spreading melanomas were positive for galanin and α -MSH, and the latter also showed the highest percentage of positive cells for galanin (mean $35,09 \pm 28,16$) as well as for α -MSH (mean $67.64\% \pm 35,38$). A positive correlation of 71% was found for immunostaining of both neuropeptides in melanomas. No significant correlation was observed between galanin expression and age, gender, location of the lesions, Breslow index, Clark level and mitotic index.

Conclusion: Our study shows the expression of galanin in cutaneous melanoma and its significant correlation with α -MSH immunostaining.

Key words: galanin, α -MSH, melanoma, melanocytic nevus.

ESTUDIO SOBRE LA EXPRESIÓN DE GALANINA EN TUMORES MELANOCITARIOS

Resumen. *Introducción.* La galanina es un neuropéptido que controla numerosas funciones en el sistema nervioso y endocrino y que está presente en la piel. Diferentes tumores neurales, endocrinos y neuroendocrinos expresan galanina y, por otro lado, varios neuropéptidos, especialmente la α -MSH, se han involucrado en la patogénesis del melanoma.

Objetivo. Estudiar la expresión de galanina en melanomas y nevi melanocíticos cutáneos, comparándola con la de α -MSH, y relacionándola con variables clínicas e histológicas con valor pronóstico en el melanoma.

Métodos. Estudio observacional, retrospectivo de la expresión de galanina y α -MSH mediante inmunohistoquímica en una muestra significativa de secciones histológicas de los melanomas cutáneos diagnosticados

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en el hospital San Jorge de Huesca en los últimos 5 años, y un número similar de distintos tipos de nevi melanocíticos.

Resultados. Se estudiaron un total de 130 lesiones pigmentadas: 38 melanomas cutáneos primarios, 6 metástasis cutáneas de melanoma y 86 nevi melanocíticos. El inmunomarcaje con galanina y α -MSH fue significativamente mayor en melanomas que en nevi ($p < 0,001$), aunque dentro de los nevi destacan la expresión de α -MSH en los azules y fusocelulares. Más del 50% de los melanomas nodulares y del 90% de los de extensión superficial fueron positivos para galanina y α -MSH, y además estos últimos fueron los que mostraron un mayor porcentaje de células positivas tanto para galanina (media= 35,09 \pm 28,16%) como para α -MSH (media=67,64% \pm 35,38%), siendo la correlación entre ambos en melanomas del 71%. No se encontró asociación estadísticamente significativa entre la expresión de galanina y las variables edad, sexo, localización, índice de Breslow, nivel de Clark y proliferación celular.

Conclusión. Nuestro estudio demuestra la presencia de galanina en secciones histológicas de melanoma cutáneo, y esta inmunoreactividad se relaciona significativamente con la de α -MSH.

Palabras clave: galanina, α -MSH, melanoma, nevus melanocítico.

Introduction

The majority of cells in our skin interact with the nervous, endocrine, and immune systems. These interactions, mediated by neuropeptides, neurotransmitters, neurotrophins, and neurohormones, influence a large number of physiologic and pathologic processes in the skin; this is what has come to be known as “neurocutaneous biology.”

Neuropeptides are endogenous substances synthesized in neurons and/or cells involved in neural processes; however, it has been demonstrated that many non-neural cells are also able to produce them. In the skin, these include keratinocytes, melanocytes, lymphocytes, macrophages, Merkel cells, and Langerhans cells. The best studied neuropeptides in the skin are substance P, neurokinin A, neurotensin, calcitonin gene-related peptide, neuropeptide Y, somatostatin, β -endorphin, enkephalin, atrial natriuretic peptide, melanocyte stimulating hormone (MSH), and adrenocorticotrophin (ACTH).¹ Most of these neuropeptides are able to mediate cutaneous neurogenic inflammation via induction of vasodilation, plasma extravasation, and increasing cytokine levels and expression of cell adhesion molecules. They are also involved in the immunosuppression induced by UV radiation, both locally and systemically.²

In terms of the role of neuropeptides in melanoma, it has been demonstrated that melanomas display immunoreactivity for ACTH, α -MSH, β -endorphin,³ gastrin-releasing peptide,⁴ and growth hormone⁵; however, α -MSH and its related melanocortin peptides appear to be the most strongly implicated in the biology of melanoma. α -MSH is the most potent naturally occurring melanotropic peptide and its effects are mediated by binding to the melanocortin receptor (MCR), which is expressed by numerous cells. It has multiple functions, including the modulation of a wide range of inflammatory stimuli, such

as proinflammatory cytokines, cell adhesion molecules, and proinflammatory transcription factors. All this endows it with a cytoprotective role in cutaneous cells against external insults such as UV radiation.⁶ Regarding melanoma, while some studies attribute a potential role to α -MSH in the delay of metastasis through the reduction of cell migration and invasion, others find that it is capable of reducing the ability of the immune system to detect tumor cells, thereby promoting tumor dissemination.⁷

Galanin is a neuropeptide containing 29 or 30 amino acids (in humans) and is widely distributed in the central and peripheral nervous system, as well as in the endocrine system. Both its endogenous and exogenous effects are mediated by 3 subtypes of receptors: GALR1, GALR2, and GALR3. This ubiquitous neuropeptide controls numerous processes (Table 1), and given also that its sequence is conserved between species,⁸ it is likely to function as an important intercellular and intracellular messenger.⁹ Few studies have addressed the role of this neuropeptide in the skin. In experimental animals it has been shown that galanin inhibits the cutaneous inflammatory response by modulating the vascular response.¹⁰ Furthermore, its presence has been demonstrated in some cutaneous structures, supporting its role in the physiology of the skin.¹¹

In addition, galanin is implicated in some malignant tumors. The expression of galanin and its receptors is common in gliomas, pheochromocytomas, pituitary tumors, and in particular, neuroblastic tumors.¹² This coexpression supports a role for galanin in the pathogenesis of these tumors via autocrine and paracrine mechanisms. On the other hand, it has been observed that galanin has antiproliferative activity and functions as an inducer of apoptosis in colon carcinoma and neuroblastoma cells¹³; in fact it has proved useful in the treatment of colon cancer, in combination with octreotide and serotonin.¹⁴

Table 1. Summary of the Effects and Known Biological Actions of Galanin^{9,12}

Biological Effects
<p><i>Endocrine System</i></p> <ul style="list-style-type: none"> • Induces release: <ul style="list-style-type: none"> – Gonadotrophins – Prolactin – Growth hormone – Luteinizing hormone and somatostatin • Inhibits glucose-induced insulin release • Gastric motility and secretion <p><i>Central Nervous System</i></p> <ul style="list-style-type: none"> • Alters the release of various neurotransmitters (acetylcholine) • Learning and memory • Epileptic activity • Antinociceptive effect • Spinal reflexes • Feeding • Neural regeneration
Actions That Increase Expression of Galanin
<ul style="list-style-type: none"> • Administration of estrogens • Neuronal activation • Denervation or neural damage • Development
Future Therapeutic Applications of Galanin or its Agonists
<ul style="list-style-type: none"> • Alzheimer disease. • Chronic pain • Depression • Eating disorders • Cancer

No studies have addressed the expression of galanin in melanomas, despite the fact that a review of the literature reveals that the human galanin receptor has been characterized pharmacologically in vitro in cells from the human Bowes melanoma cell line, which constitutes the most important tool for the study of the receptor.¹⁵ Thus, we hypothesized that galanin could be expressed in benign and/or malignant melanocytic tumors, and that the expression of the neuropeptide could be related to that of other neuropeptides commonly found in melanoma, specifically α -MSH.

The aim of the present study was therefore to analyze the expression of galanin in cutaneous melanomas and melanocytic nevi and compare it with the expression of α -MSH in those tumors. In addition, we assessed the relationship between expression of galanin and certain clinical and histological variables with prognostic value in malignant melanoma.

Material and Methods

Study Design

A retrospective, cross-sectional, observational study was undertaken to analyze immunoreactivity for the neuropeptide galanin in histological sections of benign and malignant melanocytic tumors.

Study Subjects

An analysis was performed of cutaneous melanomas and melanocytic nevi diagnosed in the last 5 years in Hospital General San Jorge, Huesca, Spain. Histological samples of these patients were stored in the hospital's pathology department. Noncutaneous melanomas were excluded from the study.

A total of 72 melanomas were available. To calculate the sample size required we used the percentage of melanomas shown in other studies to be immunopositive for α -MSH, namely 53%.³ The size of our sample, with an accuracy of 10% and assuming an alpha risk of 5%, was 39 melanomas, and an equal number of melanocytic nevi were included. Thus, in the case of the melanomas, samples of each of the different clinical types were selected at random: lentigo maligna melanoma, nodular melanoma, superficial spreading melanoma, and acral lentiginous melanoma (around 10 of each type). Since only 2 acral lentiginous melanomas were available, the sample was made up with more of other clinical types, along with 2 melanomas on nevi. In the case of melanocytic nevi, a similar number of cases to that included in the melanomas was elected at random to include each of the most common types: intradermal, junctional, compound, dysplastic, blue, spindle-cell, and congenital nevi. Finally, a small group of cutaneous melanoma metastases was included in the study.

The variables collected for the patients included in the study were those that, according to the American Joint Committee on Cancer Melanoma Staging Committee, are statistically significant prognostic factors for survival in patients with melanoma: Breslow depth, Clark level, and ulceration as histological variables in melanomas, and age at diagnosis, sex, and site of lesion as clinical variables were assessed in all the melanocytic lesions studied.

Immunohistochemistry

The immunohistochemical study was undertaken using 5 μ m sections of formaldehyde-fixed paraffin-embedded tissue, as described previously.³ The sections were analyzed using an automated immunostaining system (TechMate 500, Biotech

Table 2. Summary of the Cases Studied and the Associated Clinical Variables^a

Type of Lesion	No.	Sex		Age, y	Site of Lesion ^b				
		Men No. (%)	Women No. (%)	Mean (SD)	Head/ Neck	Trunk	Arms	Legs	Hands/ Feet
Samples studied	130	44 (33.80%)	86 (66.20%)	45.89 (21.08)					
Melanomas	38	21 (55.26%)	17 (44.74%)	65.66 (16.30)	17	10	4	5	2
Lentigo maligna	12				12	0	0	0	0
Nodular	11				3	5	2	1	0
Superficial spreading	11				1	4	2	4	0
Acral lentiginous	2				0	0	0	0	2
Melanoma on nevus	2				1	1	0	0	0
Skin metastases of melanoma	6	2 (33.33%)	4 (66.67%)	60 (15.48)					
Melanocytic nevi	86	21 (24.40%)	65 (75.60%)	36.17 (16.15)	14	47	8	10	2
Intradermal	11	0	11		6	4	0	0	0
Compound	12	2	10		1	9	1	0	0
Junctional	10	1	9		1	8	0	0	1
Blue	14	4	10		4	2	4	3	0
Dysplastic	15	7	8		1	13	0	1	0
Spindle-cell	14	2	12		0	5	3	5	1
Congenital	10	5	5		1	6	0	1	0

^aData are shown as number of cases unless otherwise indicated.

^bThe site of the lesion could not be determined in 5 melanocytic nevi.

Solutions, Dako, Glostrup, Denmark) with a detection kit (CheMate, code K4001, Dako) according to the manufacturer's instructions and using 3-amino-9-ethyl carbazole as the chromogen. In all cases, antigen recovery was performed using a pressure cooker, as described previously.¹⁶ The following antibodies were used: anti-human galanin (Oxford Biotechnology Ltd, Oxford, UK), anti- α -MSH (Bachem UK Ltd, Saffron Walden, UK), and anti-Mib1 (Ki-67) (DAKO, Carpinteria, USA). Representative sections were examined alongside positive^{7,11} and negative controls.

Assessment of immunostaining was performed by counting the number of cells expressing the relevant antigen (cytoplasmic or nuclear staining for cytoplasmic and nuclear markers, respectively) irrespective of staining intensity in 10 large microscopic fields. Following counting, the percentage of positive cells was calculated.

Statistical Analysis

The distribution of frequencies was calculated for qualitative variables and the mean and 2 SD for quantitative variables (except Breslow depth).

Given that the sample size was greater than 60, we assumed that it was normally distributed. Therefore, to compare the means, we used the Student *t* test and one-way analysis of variance (ANOVA). The association between qualitative variables was assessed by χ^2 test. Correlations

between 2 continuous quantitative variables were analyzed using the Pearson correlation coefficient. Statistical significance was established at $P < .05$. Statistical analyses were performed using SPSS version 13.0.

Results

A total of 130 lesions were studied, of which 38 were primary cutaneous melanomas, 6 cutaneous melanoma metastases, and 86 melanocytic nevi. The 38 melanomas were distributed as follows: 12 lentigo maligna melanomas, 11 nodular melanomas, 11 superficial spreading melanomas, 2 acral lentiginous melanomas, and 2 melanomas on nevi. Of the melanocytic nevi, 11 were intradermal, 12 compound, 10 junctional, 14 blue, 15 dysplastic, 14 spindle cell, and 10 congenital (Table 2).

The mean age of the patients was 46 years (range, 8-92 years) and was significantly higher in the group of patients with melanoma (65 years) than in those with melanocytic nevi (36 years) ($P < .001$). In terms of sex distribution, the group included 44 men (33.8%) and 86 women (66.2%). This difference was due to the predominance of women in the group of patients with nevi (21 men and 65 women) but not in those with melanoma or metastases (23 men and 21 women); in the group of patients with melanocytic nevi there was a clear predominance of women, except in the groups with congenital and dysplastic nevi (Table 2).

Immunostaining With Antibodies Against Galanin and α -MSH

Some degree of immunostaining for galanin was observed in 23.8% of the samples studied (n=31), while 47.7% of samples (n=62) were immunopositive for α -MSH. The immunostaining was cytoplasmic in both cases, sometimes appearing granular and sometimes more diffuse (Figure 1).

Only 13% of melanocytic nevi were positive for galanin and in most cases the staining was very limited, around 1% of cells and almost always in nests of cells close to the dermal-epidermal junction (Table 3). However, 45.58% of melanocytic nevi were positive for α -MSH, especially junctional nevi (60%), spindle-cell nevi (87.71%), and blue nevi (90.91%) (Table 3). In addition, however, it was notable that the mean percentage of cells that were immunopositive for α -MSH was also considerable in both

the blue nevi (20.18% [12.25%]) and spindle-cell nevi (27.79% [35.99%]).

In the melanomas, 90.9% of superficial spreading melanomas were positive for galanin and 100% were positive for α -MSH. The majority of nodular melanomas (54.55%) were also positive for galanin and 63.64% were positive for α -MSH. None of the lentigo maligna melanomas was positive for galanin and only 16.67% were positive for α -MSH. Our case series only contained 2 acral lentiginous melanomas, neither of which was positive for either of the neuropeptides. Two melanomas on nevi were included, one of which had 5% of cells positive for galanin and both had a high level of immunostaining for α -MSH (95% of cells); interestingly, only the cells of the melanoma and not those of the nevus were positive (Figure 2).

Superficial spreading melanomas were not only most often positive for galanin but also displayed the greatest

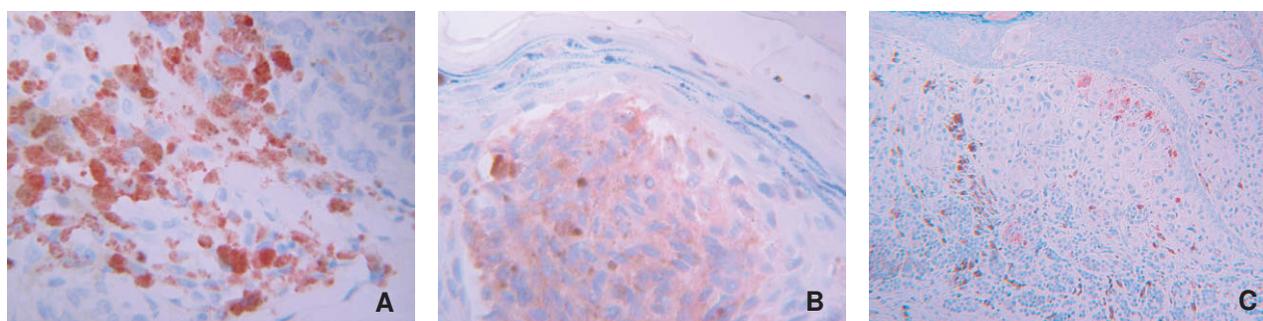


Figure 1. Details of immunostaining for galanin (A), α -melanocyte stimulating hormone (B), and ki-67 (C) in melanoma cells. Note the difference between the cytoplasmic labeling in the first 2 and the nuclear staining in the third. Galanin (A) displays a granular staining pattern in some cells while in others it is more homogeneous.

Table 3. Summary of Immunostaining With Galanin and α -MSH in the Melanocytic Lesions Studied

Type of Lesion	No. Galanin+ Cells (%)	No. α -MSH+ Cells (%)	Mean % of Galanin+ Cells (SD), Range	Mean % of α -MSH+ Cells (SD), Range
Melanomas	17 (44.74%)	22 (57.89%)	15.82 (26.22), 0.90	31.66 (39.47), 0.97
Lentigo maligna melanoma	0 (0)	2 (16.67%)	0	3.33 (88), 0.30
Nodular melanoma	6 (54.55%)	7 (63.64%)	19.09 (30.73), 0.90	20.82 (30.14), 0.90
Superficial spreading melanoma	10 (90.91%)	11 (100%)	35.09 (28.16), 0.80	67.64 (35.38), 2.97
Acral lentiginous melanoma	0 (0%)	0 (0%)	0	0
Melanoma on nevus	1 (50%)	2 (100%)	2.50 (3.54), 0.5	95.00 (0), 95.95
Cutaneous metastasis of melanoma	2 (33.33%)	2 (33.33%)	1.17 (2.04), 0.5	1.17 (2.04), 0.5
Melanocytic nevi	12 (13.95%)	38 (45.78%)		
Intradermal	1 (9.09%)	3 (29.27%)	0.09 (0.30), 0.1	0.27 (0.47), 0.1
Compound	2 (16.67%)	3 (25%)	0.17 (0.39), 0.1	0.25 (0.45), 0.1
Junctional	3 (30%)	6 (60%)	6.60 (18.83), 0.60	8.70 (18.33), 0.60
Blue	3 (21.43%)	10 (90.91%)	1.79 (5.35), 0.20	20.18 (12.25), 0.40
Dysplastic	2 (13.33%)	3 (20%)	0.13 (0.35), 0.1	7.33 (15.34), 0.40
Spindle-cell	1 (7.14%)	12 (85.71%)	0.36 (1.37), 0.5	27.79 (35.99), 0.95
Congenital	0 (0%)	1 (10%)	0	0.50 (1.58), 0.5

Abbreviation: MSH, melanocyte stimulating hormone.

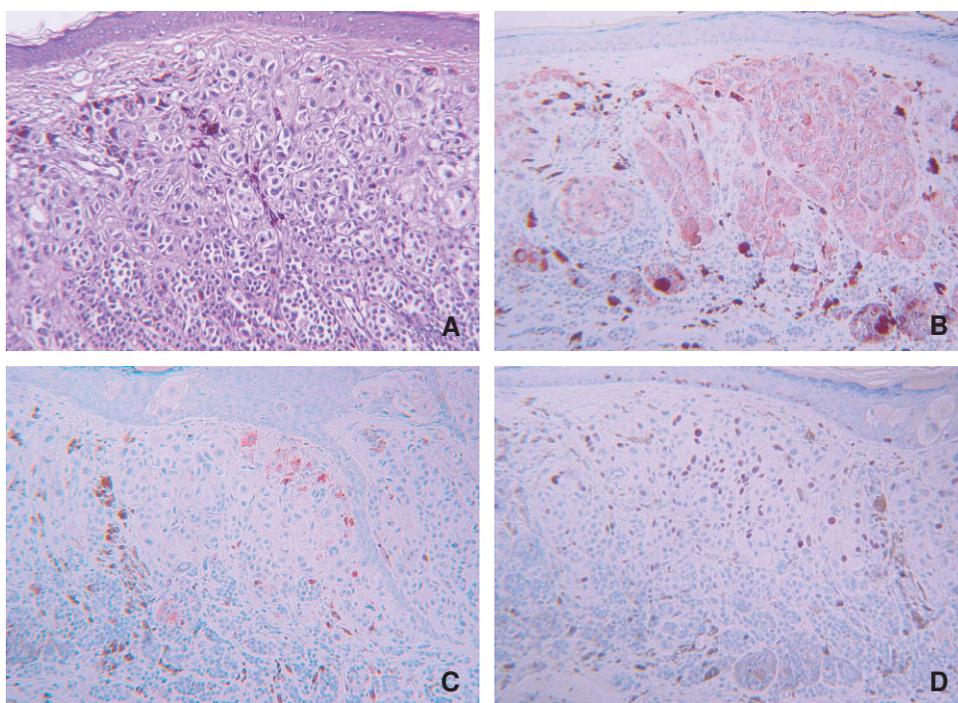


Figure 2. (A) Histological image of a melanoma on a nevus. (B) Ninety-five percent of the melanoma cells expressed α -melanocyte stimulating hormone (in red) while the nevus cells (in blue) did not express the neuropeptide. (C) Few cells were positive for galanin (red). (D) Immunoreactivity for ki-67 was also seen in the melanoma but not the nevus component.

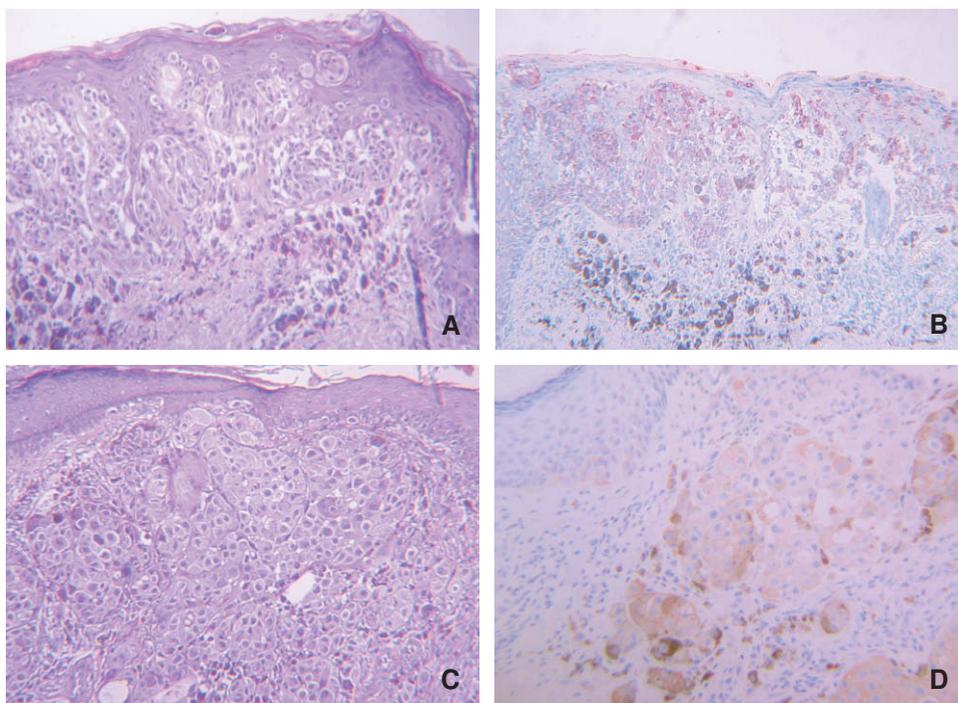


Figure 3. (A) Histological appearance of a superficial spreading melanoma. (B) Intense immunostaining for galanin. (C) Histological appearance of a nodular melanoma. (D) Most of the tumor cells are positive for galanin, both in the superficial and deep components of the tumor.

percentage of cells positive for this neuropeptide (35.09% [28.16%]) followed by nodular melanomas (19.09% [30.73%]) (Figure 3). These same types of melanoma exhibited notable immunostaining for α -MSH: mean percentage of positive cells, 67.64% (35.38%) in superficial spreading melanomas and 20.82% (31.14%) in nodular

melanomas (Table 3). It should be noted that in some cases the percentage of cells positive for galanin was as high as 90% of the tumor cells and included both superficial and deep elements of the tumor (Figure 4). Figure 5 shows the mean percentages of positive cells in the different types of melanoma for galanin and α -MSH, as well as for ki-67.

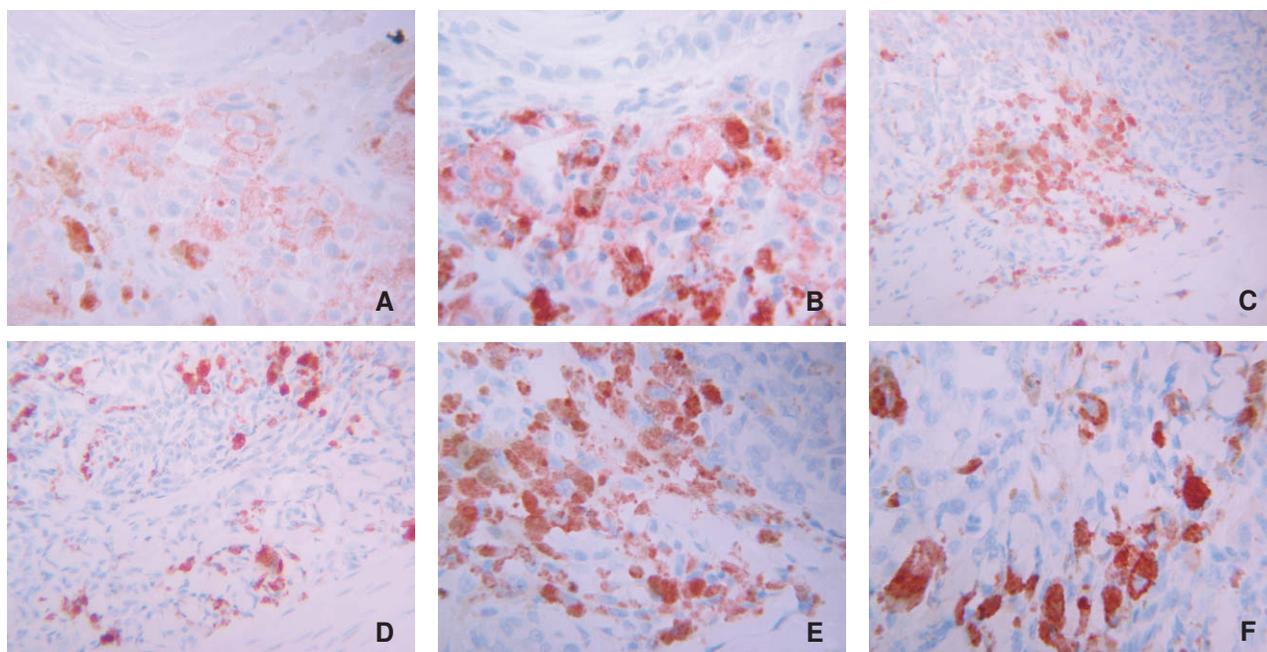


Figure 4. Comparison of immunostaining for galanin (A, C, and E) and α -melanocyte stimulating hormone (B, D, and F), revealing very similar expression in a nodular melanoma. Both more superficial (A and B) and deeper (C and D) cells in the tumor express both neuropeptides. (E and F) Detailed view of the characteristics of the immunostaining.

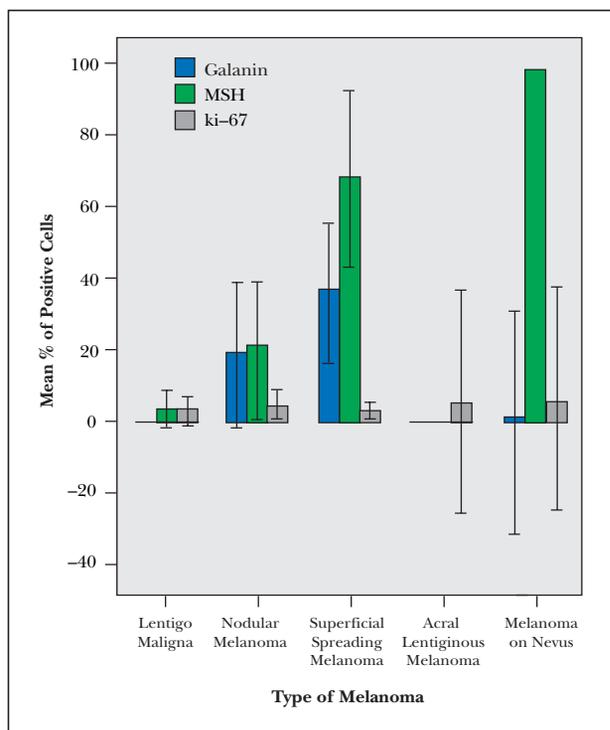


Figure 5. Graph showing the mean percentage of cells with positive immunostaining for galanin, α -melanocyte stimulating hormone (MSH), and ki-67 in melanomas. The error bars for acral lentiginous melanoma and melanoma on nevus are very large, since only 2 cases were included in each of those groups. Error bars show 95% confidence interval.

The differences between the means were statistically significant for both galanin (ANOVA, $P=.012$) and α -MSH (ANOVA, $P<.001$).

The immunostaining for galanin and α -MSH in the samples studied, especially the melanomas, displayed similarities (Figure 4), but in the majority of cases the percentage of positive cells was greater for α -MSH than for galanin. This impression based on histology was confirmed with the Pearson correlation coefficient; thus, the degree of correlation between the 2 variables was 71% of the maximum possible in the melanomas and only 34% in the melanocytic nevi.

Figure 6 shows the mean percentage of cells positive for galanin and α -MSH in the different types of lesion studied. The percentage of galanin-positive cells was significantly higher in the melanomas than in the cutaneous metastases or melanocytic nevi (ANOVA, $P=.003$). Thus, if we distribute the cases into 2 groups—melanomas and melanocytic nevi—an association can be observed between melanomas and immunostaining with galanin ($\chi^2=13.69$, $P<.001$), with an odds ratio (OR) of 4.69 (95% confidence interval [CI], 1.85-12.05), in other words, melanomas are 4.69 times more likely than melanocytic nevi to be immunopositive for galanin. However, no such association was observed for α -MSH ($\chi^2=0.57$, $P=.45$), a finding which is explained by the high level and frequency of expression of this neuropeptide in blue nevi and spindle-cell nevi.

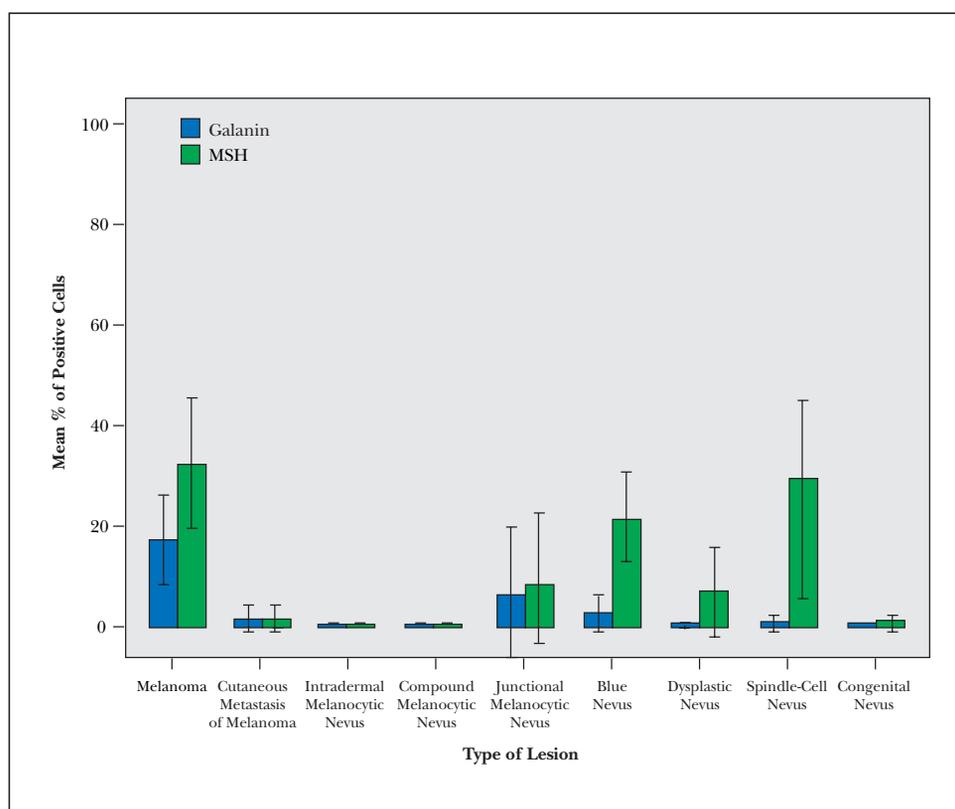


Figure 6. Graph showing mean percentages of cells with positive immunostaining for galanin and α -melanocyte stimulating hormone (MSH) in melanocytic lesions. Error bars show 95% confidence interval.

Relationship Between Clinical Variables and the Expression of Galanin and α -MSH

No sex differences were observed in the mean percentage of cells positive for galanin (Student *t* test, $P=.60$) or α -MSH ($P=.64$). We also found no differences in the mean age of patients with positive or negative immunostaining for galanin (Student *t* test, $P=.1$) or α -MSH (Student *t* test, $P=.96$) (Table 4).

In terms of the site of the lesion, we observed greater immunolabeling for both galanin and α -MSH in those lesions located on the lower limbs (Table 4). However, in multivariate analysis to predict positive immunostaining for galanin or α -MSH in melanomas including the variables site of lesion and type of melanoma, only type of melanoma was associated with the expression of the neuropeptides.

Relationship Between Expression of Galanin and α -MSH and the Variables Breslow Depth and Clark Level

We assessed the relationship between immunostaining with galanin and α -MSH in melanomas and the depth of the

lesion (Table 4). No significant differences were observed in the mean percentage of cells positive for galanin according to Breslow depth (Student *t* test, $P=.48$). However, melanomas with a Breslow depth less than or equal to 1.5 mm displayed a greater percentage of cells positive for α -MSH (52.18% [40.44%]) than those with a Breslow depth greater than 1.5 mm (29.17% [36.11%]), although this difference was not statistically significant. We did not detect a significant relationship between expression of galanin or α -MSH and Clark level (ANOVA, $P=.77$ for galanin and $P=.97$ for α -MSH).

Relationship Between Galanin Expression and Cell Proliferation Assessed Using Anti-Mib1 (ki-67) Antibodies

In our case series, immunostaining for ki-67 was generally very limited, with a mean number of positive cells of 5% (4.15%) in melanomas with a Breslow depth of less than or equal to 1.5 mm and 6% (5.77%) in those with a Breslow depth of greater than 1.5 mm (Student *t* test, $P=.64$). In contrast, ki-67 expression displayed a weak correlation with both galanin (Pearson correlation coefficient, $r=0.27$) and α -MSH (Pearson correlation coefficient, $r=0.31$), as can be seen in Figure 5.

Table 4. Summary of the Relationship Between Galanin and α -MSH Immunostaining and Different Variables^a

Variable	Mean % Galanin+ Cells	Mean % α -MSH+ Cells	Statistical Test
Sex			Student <i>t</i> test
Men	6.52% (16.32%)	17.64% (28.50%)	
Women	4.91% (16.68%)	15.10% (29.17%)	
	<i>P</i> =.60	<i>P</i> =.64	
Age, y			Student <i>t</i> test
Immunopositive	51.32 (18.92)	45.85 (21.73)	
Immunonegative	44.19 (21.53)	46.03 (21.06)	
	<i>P</i> =.1	<i>P</i> =.96	
Site			
Head/neck	4.10% (14.95%)	12.77% (26.28)	
Trunk	5.09% (15.5%)	11.60% (25.85%)	
Arms	5.33% (11.38%)	29.17% (29.29%)	
Legs	14.04% (28.93%)	44.46% (41.01%)	
Hands/feet	0	2.00% (4.00%)	
Breslow depth			Student <i>t</i> test
<1.5 mm	19.18% (21.68%)	52.18% (40.44%)	
>1.5 mm	20.42% (34.61%)	29.17% (36.11%)	
	<i>P</i> =.48	<i>P</i> =.11	
Clark level			ANOVA
In situ	16.11% (26.67%)	33.89% (45.33%)	
I and II	8.33% (7.64%)	35% (44.44%)	
III and IV	20.63% (31.19%)	37.88% (39.04%)	
	<i>P</i> =.77	<i>P</i> =.97	

Abbreviations: ANOVA, analysis of variance; MSH, melanocyte stimulating hormone.

^aData are shown as mean (SD).

Discussion

Our study demonstrates that some cutaneous melanomas, specifically superficial spreading and nodular melanomas, express galanin to a greater or lesser degree, and that expression of galanin is strongly correlated with α -MSH expression in these tumors. However, our results display a greater specificity for galanin, since some melanocytic nevi exhibit significant expression of α -MSH, especially blue nevi and spindle-cell nevi.

The close relationship of melanomas with the immune system is clear, but in addition, some neuropeptides may also play a significant role in their pathogenesis, a possibility which appears logical given the embryonic origin of melanocytes, namely the neural crest. Galanin has been detected along with its messenger RNA in melanoma cell lines.¹² However, no data has been published on the expression of galanin in melanocytic tumors.

According to our results, the expression of galanin in melanocytic lesions depends on the type of lesion and is significantly higher in melanomas than in nevi. If we compare this expression with that described by other authors in different types of tumor, we find that staining is always cytoplasmic,¹² as was the case in our study. Furthermore, we did not find histological differences between superficial spreading melanomas and nodular melanomas on the basis of the presence or absence of galanin expression, an

observation that coincides with the results obtained in other types of tumor.¹⁷

Neuroblastic tumors display a more constant immunostaining for galanin; tumors such as pheochromocytomas display around 60% positivity (62% in suprarenal pheochromocytomas, 40% in jugulotympanic paragangliomas, and 15% in carotid paragangliomas).^{12,18} Thus, the percentage of superficial spreading (90%) and nodular melanomas (55%) that express galanin can be considered very high.

Perel et al¹⁸ observed expression of galanin in all neuroblastic tumors studied; however, the expression was much lower in metastases,¹⁹ as was also observed in our case series, where very little immunostaining for galanin was observed in cutaneous metastases.

In our study, we found no relationship between the expression of galanin and the rate of cell proliferation based on immunostaining with ki-67, a finding which is consistent with in vitro observations in human Bowes melanoma cells, in which galanin has been reported to have no effect on the rate of cell proliferation.^{12,20}

According to our results, galanin shows no relationship with any of the clinical and histological prognostic markers for melanoma studied. Berger et al²¹ also found no association in the case of peripheral neuroblastic tumors. Nevertheless, some studies attribute some positive prognostic value to the presence of galanin in pituitary adenomas.²² This is

consistent with the observation that galanin has antiproliferative activity and functions as an inducer of apoptosis in colon cancer cells and neuroblastoma cells.¹³

The results of our study reveal a significant correlation between the expression of galanin and α -MSH in melanomas, apparent both in histological images and the statistical analysis. Furthermore, the percentage of melanomas positive for α -MSH in our study (57%) was similar to that observed by other authors.³ However, we also detected significant immunostaining with α -MSH in blue nevi and spindle-cell nevi, an observation which has not been described previously.

According to the results of this study, immunostaining for galanin and α -MSH in melanomas depends on the type of tumor and is significantly higher in superficial spreading and nodular melanomas. Some other neuropeptides, such as gastrin-releasing peptide, have also been found to be upregulated only in certain types of melanoma, specifically nodular melanomas.⁴ In the case of α -MSH, Nagahama et al³ reported variations in the immunostaining of different types of melanoma (superficial spreading [2/9], nodular [4/5], and acral lentiginous melanomas [3/11]; the authors did not assess lentigo maligna melanoma); their findings differed from the results observed in our case series in that those authors observed low expression of α -MSH in superficial spreading melanoma. Interestingly, we found that in melanomas on nevi α -MSH was expressed in almost all of the melanoma cells but not in the nevus cells, an observation which has not been reported previously.

No studies have been published to date indicating a relationship between galanin and α -MSH. However, it appears that the expression of galanin in pituitary adenomas is linked to expression of ACTH,^{12,22} a neuropeptide that like α -MSH is derived from proopiomelanocortin, and both are present in melanomas.³ The role of α -MSH in melanomas is still not clear.²³ In our study, melanomas with a Breslow depth less than or equal to 1.5 mm contained a higher percentage of α -MSH-positive cells than those with a Breslow depth of more than 1.5 mm, and that could support some possibility of a favorable prognosis associated with this neuropeptide, as suggested by other authors.²³

It is difficult to establish the significance of the presence of galanin in a variable percentage of cells in certain histological types of melanoma, particularly when the significance of these different subtypes of melanoma is still not fully accepted.²⁴ Galanin has been shown to play a role in the cutaneous inflammatory response of experimental animals,²⁵ to possess antiproliferative activity and to act as an inducer of apoptosis in other tumors,¹³ and to be tightly linked to the hormonal system, for instance through estrogen-mediated control of galanin secretion.⁹ Inflammation, apoptosis, and hormones, especially estrogens, have been linked in one way or another to melanoma and may be one of the links between galanin and this tumor.

Finally, it should be taken into account that this study has certain limitations, such as the low number of acral lentiginous melanomas and melanomas on nevi. In addition, the number of melanomas, although corresponding to a representative sample of those diagnosed in our hospital in the last 5 years, was only 38. Consequently, prospective studies are required involving a larger number of this type of tumors to confirm our findings.

In conclusion, our study shows for the first time that galanin is present in histological sections of cutaneous melanoma. This forms a basis for future studies to investigate the role of this neuropeptide in melanomas, or at least some types of melanoma. Future studies will be required to confirm our findings in larger case series, assess the expression of galanin receptors in different types of melanoma, and to establish whether this neuropeptide acts on melanoma cells in an autocrine or paracrine fashion. Analysis of the relationship between galanin expression, apoptosis, and inflammation may help to elucidate the role of this ubiquitous neuropeptide in cutaneous melanoma.

Conflicts of Interest

The authors declare no conflicts of interest.

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