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Coexpression of hepatocyte growth factor/scatter factor (HGF/SF) and its receptor cMET predict recurrence of meningiomas

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Abstract

Hepatocyte growth factor/scatter factor (HGF/SF) and its receptor, the cMET tyrosine kinase participate in cancer invasion, angiogenesis and metastasis in a wide variety of neoplastic cells. Meningioma is a bening tumour, however, it has a high rate of recurrence after surgery; the most important factor to predict relapse is the extent of surgical resection, several other potentially predictive factors have been studied with poor results. We examined by immunohistochemistry the expression of HGF/SF and its cMET receptor in a group of patients with benign meningioma with or without recurrence ($n=17$ and $n=25$, respectively), after a minimal follow-up of least 6 years. Expression and coexpression of HGF/SF and cMET were compared with cell proliferation index, vascular density and clinical outcome. Coexpression of HGF/SF and cMET in meningiomas had a significant association with cell proliferation index and with recurrence ($P<0.037$). Determination of HGF and cMET coexpression in meningiomas could be used as a predictor of recurrence.

Keywords: Hepatocyte growth factor; Scatter factor; cMET receptor; Recurrence of meningiomas; Predictive factor

1. Introduction

The hepatocyte growth factor (HGF/SF) is a multifunctional protein secreted by mesenchymatous cells, it has strong mitogenic effect on hepatocytes [1–3]; in epithelial cells it acts as a paracrine activator through a tyrosine kinase receptor (cMET), inducing mitogenesis and cellular motility [4,5]. It is also

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a powerful angiogenic factor and enhances the production of other factors, such as the vascular endothelial growth factor (VEGF) to produce endothelial cell proliferation [6]. It is over-expressed in some malignant neoplasms like thyroid, breast and prostate carcinomas [7–10]. A relationship between tissular contents of HGF/SF and angiogenesis and tumoral invasion has been reported [11], also, HGF/SF and cMET are widely expressed and coexpressed in human gliomas, meningiomas and schwannomas [12].

Most meningiomas are benign, therefore their histologic score is not a useful indicator of recurrence. After surgical treatment 20% of those reported as total resection show recurrence within 10 years and more than 80% will relapse after partial resection [13]. In spite of extensive research to find factors associated to recurrence, the most reliable still remains the extent of resection [14].

We have previously found a relationship between risk of tumour recurrence and HGF/SF contents in meningiomas [15]. Here, we report the presence of HGF/SF and cMET receptor as well as their coexpression and their relation with histological score, cell proliferation, vascular density and risk of recurrence.

2. Materials and methods

2.1. Tissue biopsies

Forty-two surgical specimens from patients with histopathologic diagnosis of meningioma who were surgically treated between January 1995 and December 1997 at the National Institute of Neurology and Neurosurgery in Mexico were collected. At the time of this study (2003) minimal follow up was 6 years.

2.2. Clinical follow-up

Recurrence was defined as the clinical or radiological evidence of tumoral growth. In this study, 17 patients presented recurrence; the remaining 25 patients without evidence of recurrence at follow-up were taken as controls. The extent of surgical resection was measured according to the Simpson scoring system [14]. Tumour location and weight were also recorded. Patients with radiation-related neoplasm according to DeMonte [16] were excluded.

2.3. Histopathologic analysis

A portion of the biopsy was fixed in 10% formaldehyde, 5 μm paraffin embedded slices were obtained histologic type and tumoral degree were determined on hematoxylin–eosin stained specimens according to the WHO 2000 classification [17].

2.4. Vascular density and cell proliferation

Immunohistochemical staining was performed using the avidine–biotin–peroxidase method and counter-stained with hematoxylin–eosine. Tissue samples were incubated for one hour at room temperature with polyclonal mouse or rabbit antibodies against Factor VIII related antigen (or van Willebrand factor) as endothelial cell marker (DAKO Corp. USA) and against proliferating cellular nuclear antigen (PCNA) as a marker of DNA synthesis (DAKO Corp. USA). Quantification of the number of FVIII-positive capillaries (40×) and PCNA-positive neoplastic cell nuclei (40×) in 10 different fields was made independently by two pathologists without previous knowledge of the source of the specimen.

2.5. Hepatocyte growth factor and its receptor

Anti-HGF goat polyclonal antibodies (Sigma USA) and anti-cMET (c-12) rabbit polyclonal antibodies (Santa Cruz, USA) were used for immunohistochemical staining. Samples of regenerating liver tissue were used as positive controls. Intensity of the reaction was semiquantitatively determined by two independent examiners without knowledge of the source of the specimen. Five high-power fields were analyzed in every specimen in areas with high tumoral density. HGF/SF and cMET presence was graded as follows: 1, when 0–33% cells were positive; 2 when 33–66% cells were positive and 3 when 66–100% cells were positive. Interobserver agreement was $>90\%$.

2.6. Statistical analysis

Univariate analysis was used to associate age, gender, histologic type, score, extent of resection,

Table 1
Clinical characteristics of patients with meningioma according to tumour recurrence

Clinical variable	No recurrence ($n=25$)	Recurrence ($n=17$)	P
Female:Male ratio	1.7:1	1.4:1	0.74
Age at diagnosis	52±3 years	44±4 years	0.08
Location	Frontal 26% Sphenoidal 22% Petroclival 13% Other 39%	Frontal 23% Sphenoidal 12% Petroclival 12% Other 53%	0.32
Tumour weight	38±10 g	47±12 g	0.57
Recurrence time		32±5 months	

tumor weight, tumor location, time elapsed and recurrence with cell proliferation index, vascular density, and HGF/SF and cMET results. Mean values were compared with the two-sample indepent *t*-test. Bivariate correlation for parametric values was made with ANOVA and lineal regression. Non-paramtric variables were compared with the Fisher's exact and χ^2 tests analysis when indicated. Multivariate analysis was performed by logistic regression, including all variables associated to recurrence. Variables with $P<0.05$ were considered significative.

3. Results

3.1. Clinical characteristics

Clinical data of patients with meningioma are shown in Table 1. No differences were found between groups with respect to gender, age at diagnosis, tumour location or weight. Mean time (ISD) of recurrence was 32±20 months. Extent of resection was significantly related to recurrence. No differences in recurrence were found between complete and partial resection as stated at the original surgical report ($P<0.3$).

3.2. Histological details

No differences between groups were found according to histological score or type, nor with the cellular proliferation index (Table 2) However, we found a significant relation between vascular density and recurrence (Fig. 1A).

3.3. HGF/SF and cMET expression and co-expression

The expression of HGF/SF and cMET in meningiomas graded 1, 2 and 3 was 60, 36 and 5%, respectively for HGF/SF; and 26, 48 and 26% for cMET. The positive stain was mostly cytoplasmatic and homogeneous, with scarce stromal positive foci. No staining was observed in blood vessels. No significant differences were found when HGF/SF or cMET were independently compared in meningiomas with or without recurrence ($P=0.057$ and $P=0.075$). However, in cases that coexpressed HGF/SF and cMET a significant association with recurrence was found ($P<0.037$) (Table 2 and Figs. 1 and 2), and with cell proliferation but not with vascular density (Fig. 3). A correlation between the expression of HGF/SF and cMET was also found (r-value of 0.562; $P<0.01$); however there was no association between time until recurrence and the isolated expression either of

Table 2
Histological characteristics of meningiomas

Histological variable	No recurrence ($n=25$)	Recurrence ($n=17$)	P
Histological score	1±0.15	1.5±0.26	0.77
Histological type	Meningothelial 52% Transitional 20% Fibroblastic 12% Atypical 8% Metaplastic 4%	Meningothelial 35% Transitional 26% Fibroblastic 18% Atypical 12% Metaplastic 6%	0.2
WHO grade (I/II)	23/2	15/2	0.6
Cellular proliferation index	527±87	747±108	0.1
Vascular density	7±1	12±1	0.001
HGF	0.48±0.16	1.06±0.26	0.057
CMET	1.24±0.24	1.94±0.29	0.075
Coexpression	1.12±0.21	1.88±0.29	0.037

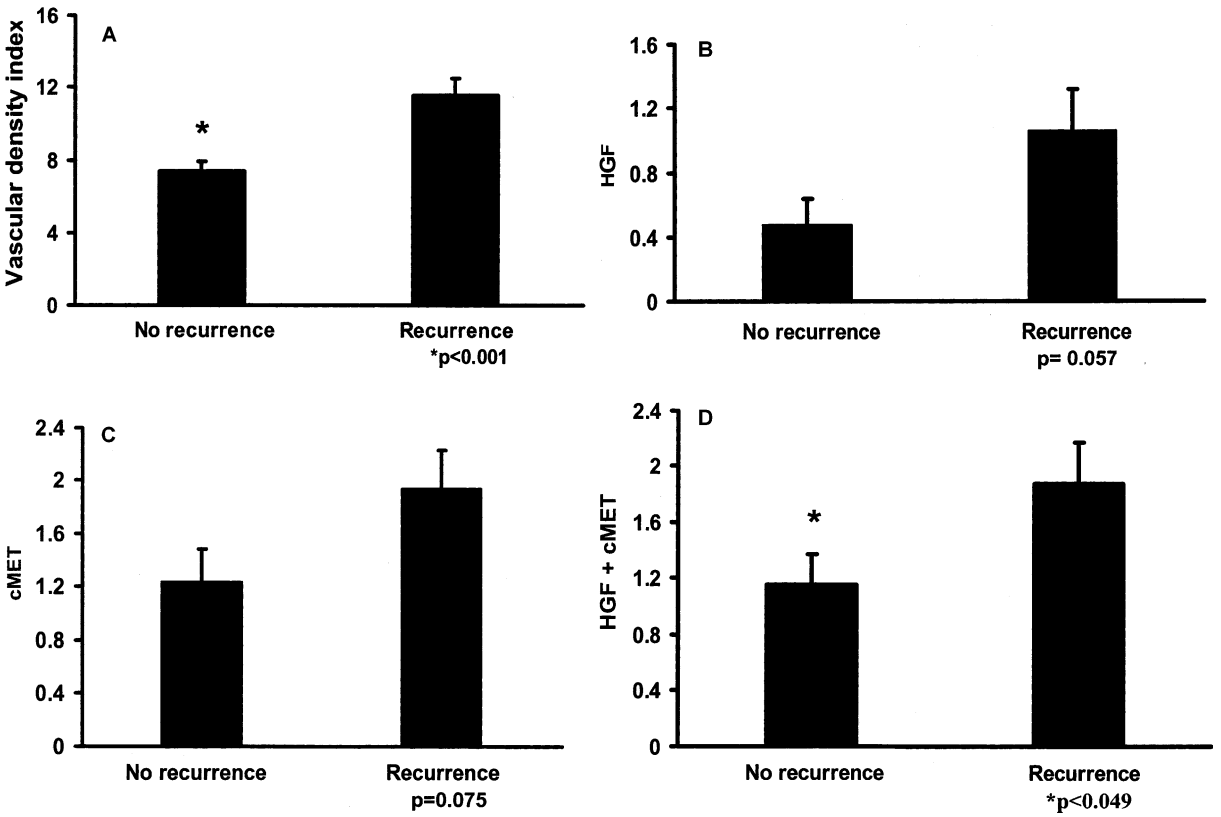

Fig. 1. Vascular density, HGF and c-MET in meningiomas according to their recurrence rate.

HGF/SF or cMET or their coexpression ($P=0.55$, 0.85 and 0.65, respectively).

Three variables had a significant association with recurrence: vascular density, coexpression of HGF/SF and cMET and extent of surgical resection. The arithmetic sum of these 3 variables produces a score that varies from 1 to 12; a recurrence index (RI) of 1 would apply for scores ranging from 1 to 3 points, a RI of 2 for scores from 4 to 7 points, and a RI of 3 for scores from 8 to 12 points. Thus, the risk of recurrence increases along with the increase of this score, maintaining a direct correlation (Table 3 and Fig. 4). Multivariate analysis showed that the variables associated to recurrence were: HGF/SF ($P=0.013$), cell proliferation index ($P=0.027$) and vascular density ($P=0.005$). Histologic grade ($P=0.253$) and cMET ($P=0.208$) were non-significant. However, the number of patients was statistically small for multivariate analysis. Table 4 shows the analysis considering only patients with complete tumour resection, it shows the same associations observed in patients in whom only partial resection had been accomplished.

4. Discussion

This study showed that vascular density and coexpression of HGF/SF and cMET are related to recurrence; this coexpression was a reliable predictor of recurrence even for those meningiomas that had been reported as totally resected at surgery.

Despite its usually benign nature (>91%) [18], long term prognosis of meningioma is tainted by its high recurrence rate even in cases where the surgical report indicates total resection (from 12

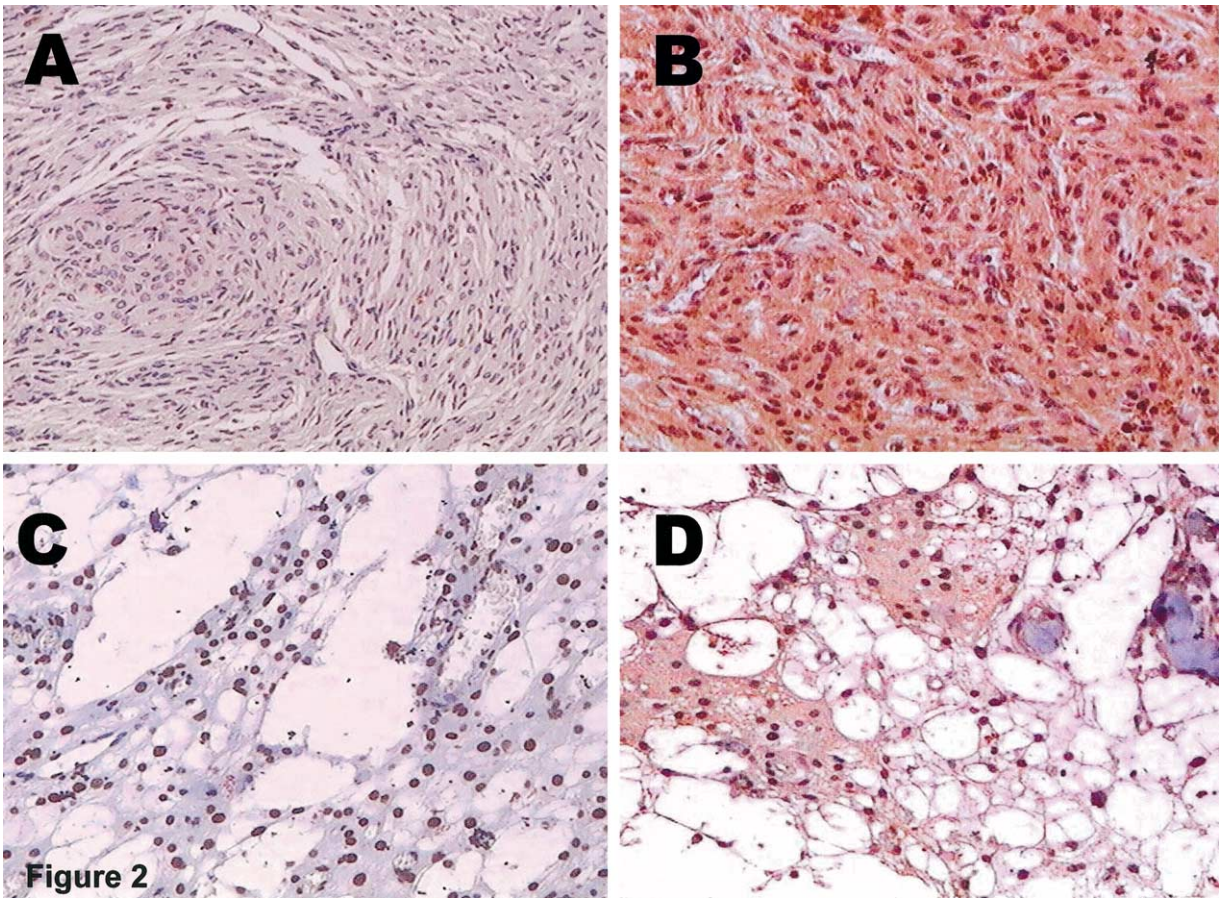

Fig. 2. HGF and cMET. (A) Non-recurrent meningothelial meningioma, it was negative for cMET (10×), (B) Recurrent meningothelial meningioma, positive for cMET (10×), (C) Non-recurrent metaplastic meningioma negative for HGF (20×), (D) Recurrent metaplastic meningioma, positive for HGF (20×).

to 70%) [19]. Histopathological predictors of outcome used in other neoplasias have low accuracy in meningiomas. Extensive research to find quantitative histopathologic prognostic parameters has been conducted [20–26], however, most of these methods have not gained acceptance as practical clinical tools. Measurement of progesterone receptor is weakly related to recurrence [27]. Several growth factors such as, HGF/SF, human growth hormone, VEGF, platelet derived growth factor, and basic fibroblast growth factor, along with their respective receptors intervene in the genesis, progression and growth of meningiomas. HGF/SF has also been related to invasive capability by stimulation of mitosis and cellular proliferation [11]. Expression of human growth hormone and treatment with its antagonist have been evaluated in meningiomas [28]. However, none of the growth factors involved in the pathophysiology of meningiomas, have been studied prospectively to explore a reliable method to predict recurrence risk.

In a previous study [15] we measured the tumour contents of HGF/SF in malignant gliomas and meningiomas and found that patients with meningiomas that had higher contents of HGF/SF had a high risk of recurrence as compared to those with lower HGF/SF contents. In this morphological analysis the presence of HGF/SF alone was not

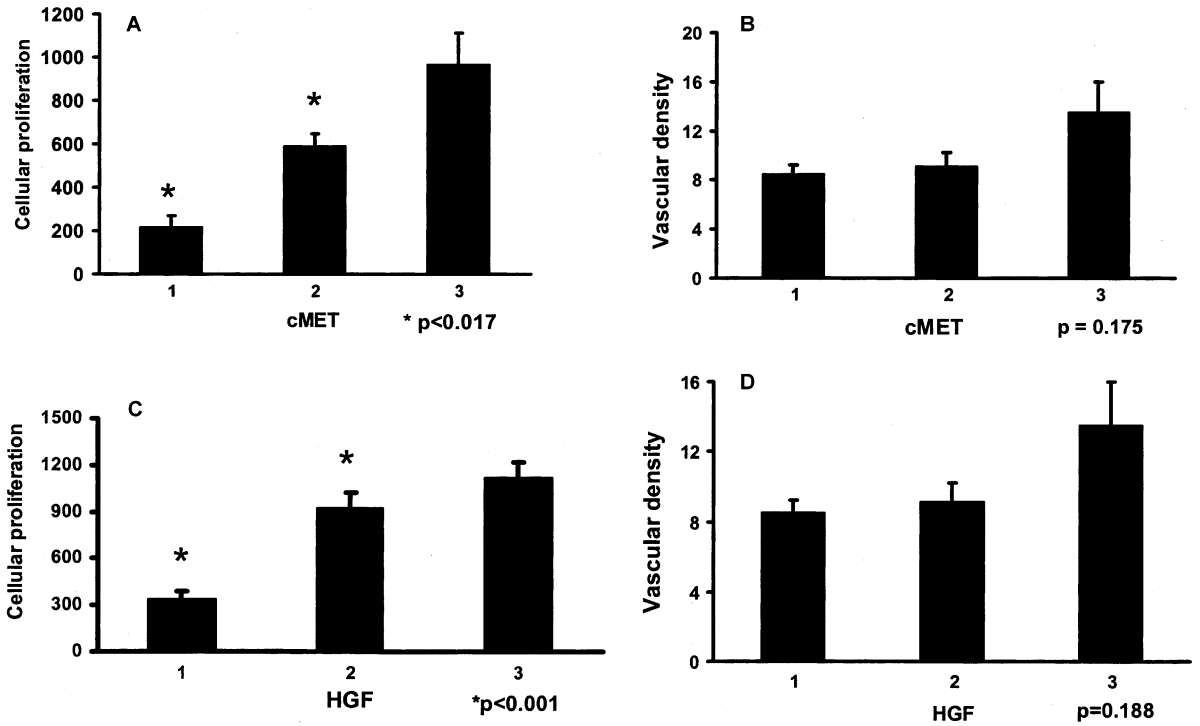

Fig. 3. Immunorreactivity of HGF and cMET in relation with cellular proliferation and vascular density.

significantly higher. However, in contrast with our previous results, the immunohistological method used in this study does not achieve the high sensitivity of ELISA used for quantification of the peptide. The ideal method for practical application of our findings would be to design a reliable quantitative method for determination of HGF/SF and cMET coexpression, which would not depend on expert microscopical evaluation.

Other studies have found that HGF/SF participates in the genesis and progression of neoplasias by stimulating cell motility, proliferation, mitogenesis and angiogenesis [1–9]; in our studies HGF/SF and cMET are related to cellular proliferation [15]. HGF/SF appears to have a fundamental role in the growth promotion of meningiomas. The therapeutic potential of HGF/SF antagonists such as NK4 [10] might yield interesting results in an attempt to prevent recurrence in those tumours with a high content of these peptide.

Our findings support the study of HGF/SF and cMET coexpression and vascular density as predictors

Table 3
Risk of recurrence of meningiomas according to Recurrence Index

Recurrence index	Recurrence risk	*P*
RI 1	1.0	
RI 2	1.5 IC 95% (1.1–2.1)	0.05
RI 3	5.5 IC 95% (1.5–19.3)	0.001

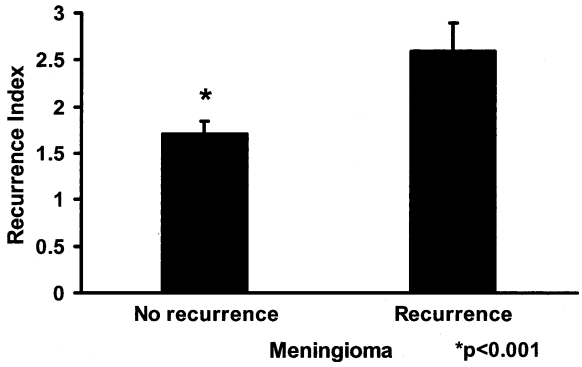

Fig. 4. Recurrence index in patients with meningioma.

Table 4
Recurrence of meningioma in cases reported in surgery as total resection

100% Resection	No recurrence	Recurrences	*P*
Tumour weight (g)	23±6	41±9	0.12
Vascular density	7.5±0.6	10.7±1.3	0.02
Cellular proliferation index	417±79	780±161	0.03
HGF	0.18±0.09	0.6±0.16	0.04
cMET	0.70±0.18	1.2±0.2	0.099
Coexpression	0.88±0.24	1.8±0.32	0.031

for releapse of meningiomas in order to implement additional therapeutic measures, like close follow-up of positive cases or the preventive administration of radiotherapy or chemotherapy.

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