Expression of Metastasis-associated Gene-1 Is Associated With Bone Invasion and Tumor Stage in Human Pituitary Adenomas

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Abstract. *Background: Metastasis associated gene-1 (MTA1), was initially discovered in aggressive human cancer cell lines and has been subsequently associated with the invasiveness and metastatic potential of cancer cells. Materials and Methods: In the present study, we evaluated the expression levels of MTA1 in a cohort of human pituitary tumors (n=95) and examined the relationship between MTA1 expression and the pathological, clinical and aggressiveness of these tumors. Results: MTA1 was expressed at significantly higher levels in large tumors and in those with higher tumor grade. It was also observed that tumors that had invaded the suprasellar bones and tumors that destructed the sella had significantly higher levels than those without bone involvement (p<005). Although there did not appear to exist any relationship between MTA1 and cystic lesions in the tumors, endocrine-active tumors, namely those secreting prolactin, growth hormone, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) had significantly lower MTA1 transcript levels than inactive tumors. Conclusion: MTA1 is associated with the aggressive nature of pituitary tumors and may be a potential therapeutic target in this tumor type.*

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Metastasis-associated gene-1 (*MTA1*), was first discovered almost two decades ago in highly metastatic rat mammary cancer cells (1). Human MTA1, a protein of 83 kDa in size, was subsequently identified from an aggressive human melanoma cell (2) and was found to share 96% homology. Although the function of the MTA1 protein is not entirely known, it is believed that it forms part of the nucleosome remodelling deacetylase protein complex with other proteins in regulation of nucleosome and histone deacetylase activities (3-5). MTA1 is also widely involved in oestrogen receptor transactivation (6) and metalloproteinase-9 (MMP-9) expression (7). MTA1 and ADP-ribosalyation factor-1 also form an autoregulatory mechanism for gene expression during the regulation of oncogenesis (8).

MTA1 has been shown to increase lung metastasis of breast cancer by increasing the transcription of Signal Transducer and Activator of Transcription-3 (STAT3) transcription (9). MTA1 can also induce epithelila-mesenchymal transition (EMT) in various cancer cells including breast cancer and colorectal cancer cells (10-12). MTA1 is also a powerful stimulus of the Wnt signalling pathway and regulates the stability of the tumor suppressor p53 (13), the stability of Hypoxia Inducing Factor-1 alpha and also interacts with endophilins (14).

MTA1 has been shown to regulate the expression of hyaluronan-mediated motility receptor and the really interesting new gene finger protein RNF144 in human breast cancer, *via* which it can influence the migration and invasion of cancer cells (15, 16). Other molecules regulated by MTA1 include transglutaminase-2 (or tissue transglutaminase), vascular endothelial growth factor (VEGF), maspin, Breast Cancer Susceptibility Gene-1 (BRCA1), Paired Box 5 (PAX5) and the SIX Homeobox 3 (SIX3).

Despite the important role of MTA1 in tumour progression, it is not known whether MTA1 is involved in the progressive growth of pituitary adenoma. Pituitary adenoma is a benign tumour reasonably well-observed in the central nervous system. Despite being described as benign, it often takes an aggressive development route, which in many ways resembles a malignant growth pattern, namely invasion of the surrounding structures such as the cavernous sinus, hypothalamus, and sphenoid sinus. The aggressive growth of pituitary adenomas is referred to as invasive adenoma (17). Invasive pituitary adenomas are characterized by rapid growth, large size, poor treatment efficacy, and a high recurrence rate. Clinically, there are also some small tumors with obvious invasive behaviour and apoplexy. It is unclear what mechanisms determine this invasive growth pattern, although pituitary tumor-transforming gene, proliferation-related factors, such as Ki-67, proliferation cell nuclear antigen and angiogenic factors, such as vascular endothelial growth factor have been implicated in this pattern (18, 19). Recently, we also reported on a pivotal relationship between the Mammalian Target of Rapamycin (mTOR) and its regulators regulatory-associated-protein of mTOR (RAPTOR) and RAPTOR-independent companion of MTOR in the aggressive growth of pituitary adenomas (20).

In the present study, we investigated the expression of *MTA1* gene transcript in a cohort of human pituitary adenomas and describe how MTA1 expression is linked to tumour staging and the invasion of surrounding bony tissues.

Table I. *Clinical and pathological information of patients.*

Group		n
Gender	Male	51
	Female	44
Age	<45 years	45
	>45 years	50
Tumor size	<1 cm	2
	1-2cm	17
	2-3 cm	34
	>3 cm	42
Intratumoral haemorrhage	No	77
	Yes	18
Suprasella invasion	No	59
	Yes	36
Invasion	No	68
	Yes	27
Knosp grade	0-2	70
	3-4	25
Endocrine tumor	No	59
	PRL	5
	GH	8
	FSH	2
	ACTH	6
	TSH	4
	LH	2
	Mixture	9

ACTH: Adrenocorticotropic hormone; FSH: follicle stimulating hormone; GH: growth hormone; LH: luteinizing hormone; PRL: prolactin; TSH: thyroid stimulating hormone.

Materials and Methods

Clinical and pathological demographics of the patients. Patients' clinical history, diagnostic images and endocrine tests were routinely recorded. The present study included a total of 95 patients with pituitary adenomas who underwent trans-sphenoidal or craniotomy surgical resection from January 2012 to December 2012 at the Department of Neurosurgery, Beijing Tian Tan Hospital. Age, gender and hormonal functioning were reviewed. The diagnosis was confirmed by postoperative pathology. Immunohistochemical examination was used to determine the endocrine type. Preoperative magnetic resonance imaging (MRI) was performed to determine image characteristics: tumor size, cystic lesion, intratumoural haemorrhage and invasion type. Tumor size was measured as the greatest diameter of tumour obtained on the gadolinium-enhanced T1-weighted image. Intratumoral haemorrhage and cystic lesion was defined by preoperative MRI and were confirmed intraoperatively. The invasion type was determined based on the invasion site of the tumour and included cavernous sinus invasion, sphenoid sinus invasion and suprasellar invasion. The criterion for suprasellar invasion was tumor growth toward the suprasellar region with invasion in the third ventricle and/or lateral ventricle. The criterion for sphenoid sinus invasion was tumour growth downward to the sphenoid sinus cavity or tumour growth into the clivas. The cavernous sinus invasion was defined as the tumour extending lateral to the lateral tangent of the intra- and supracavernous internal carotid artery or beyond that (grade 3 or 4). With reference to the classification by Knosp *et al.* (21) and the method proposed by Vieira *et al.*, (22, 23), Hardy's classification includes four tumor stages, in which tumour size and invasion were both taken into account, these were used in the present study (24). Full details are shown given in Table I.

Collection of pituitary adenomas. Pituitary adenoma samples were freshly-collected immediately after microsurgical resection at the Department of Neurosurgery, Beijing Tian Tan Hospital, supported by ethics approval by the Local Research Ethics Committee with patients' consent. The tissues were immediately frozen and stored in liquid nitrogen until use.

Tissue processing and generation of genetic materials for genetic-based analyses. Frozen tumor tissues were frozen sectioned at 10 μm. A small number of sections were used for histological evaluation and the rest were combined and then homogenized in an RNA extraction solution. Total RNA was extracted using a Triagent™ based on manufacturers' instructions (Sigma-Aldrich, Poole, Dorset, England) and quantified using a spectrophotometer. Equal amount of total RNA was used to generate complementary DNA, cDNA, using a reverse transcription kit from Promega (Southampton, Hampshore, England). The quality of cDNA samples was verified using Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a housekeeping control.

Quantitative gene transcript analyses. This was based on the Amplifluortm Technology in quantitative gene transcript analysis

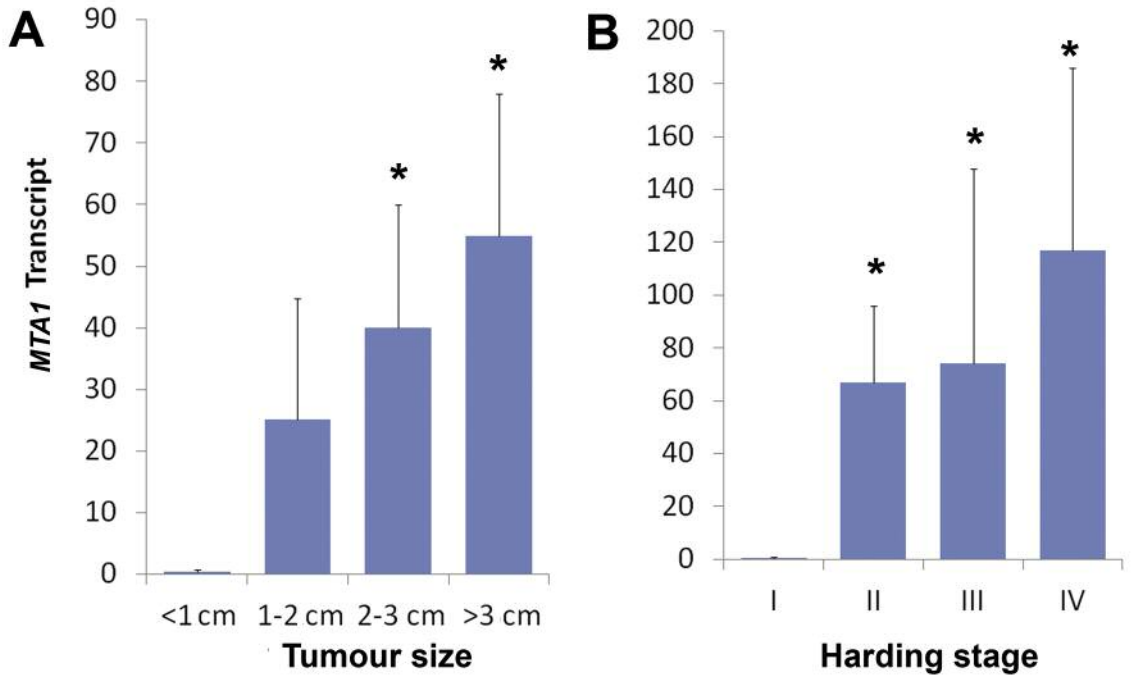

Figure 1. *Expression of MTA1 in relation with tumor size (A) and Hardy tumor staging (B). *p<0.05 Statistically significant difference compared to tumors <1 cm (A) and stage I (B).*

(25-27). Briefly, pairs of specific primers were designed to amplify a region of human *MTA1* (primers: MTA1ZR ACTGAACCTGACC GTACAGAAGGGGAAATAGAAGAGGA; MTA1F GAGGAA CAGCTCCCGATG). To one primer of the primer sets was added a Z sequence which is complementary to the Amplifluor probe (6-carboxyfluorescein n (FAM) tagged, synthesised by Biosearch Technologies, Inc., Novato, CA, USA). Each reaction was comprised of a forward primer, a reserve Z primer at one tenth of the concentration, the Amplifluor probe, cDNA from tumors and a custom master mix (Life Technologies, Paisley, UK). An internal standard was included as control. Quantitative polymerase chain reaction (PCR) analysis was conducted using the Stepone Plus instrument (ABI, Paisley, Scotland, UK). *GAPDH* transcript was also simultaneously quantified and used as a housekeeping control (primers: GAPDHF CTGAGTACGTCGTGGAGTC; GAPDHZR ACTGAACCTGAC CGTACACAGAGATGATGACCCTTTTG).

Statistical analysis. The SigmaPlot (Version 11) statistical package (Systat Software Inc., San Jones, California, USA) was used. Student's *t*-test and ANOVA tests were used for normalized data and Mann-Whitney *U*-test and Kruskall–Wallis test for non-normalized data.

Results

MTA1 expression levels and tumor staging. Using the tumor size as an indicator, it was found that large tumors had significantly higher levels of *MTA1* transcript than small tumors, namely, tumors greater than 3 cm and 2-3 cm had markedly higher levels of *MTA1* than tumors smaller than 1 cm (p=0.002 and 0.005, respectively) (Figure 1A). Hardy's tumour staging mainly considers the size of tumors and tumour invasion of the surrounding tissues. As shown in Figure 1B, high-grade tumors had significantly higher levels of *MTA1* (p=0.025, 0.0084 and 0.025 for grade II, III and IV vs. grade I, respectively). We also analysed *MTA1* expression against the Knosp classification of our cohort. An interesting observation was the absence of a significant correlation between staging and *MTA1* level (data not shown).

Invasive growth pattern and levels of MTA1 expression. Based on MRI imaging, we divided the pituitary adenomas into those with suprasellar invasion and those without. As shown in Figure 2A, invasive tumors had a significantly higher expression of *MTA1* (p=0.027). Likewise, we grouped the tumors into those with sella destruction and those without. In a similar fashion to invasion, tumors associated with sella destruction had significantly higher levels of *MTA1* transcript than those without (Figure 2B).

Endocrine functions of pituitary tumors and MTA1 expression. In comparison to the levels of *MTA1* in inactive tumors, tumors secreting prolactin (PRL) (p=0.003), growth hormone (GH) (p=0.027), follicle stimulating hormone (FSH) (p=0.0051), luteinizing hormone (LH) (p=0.015) and with

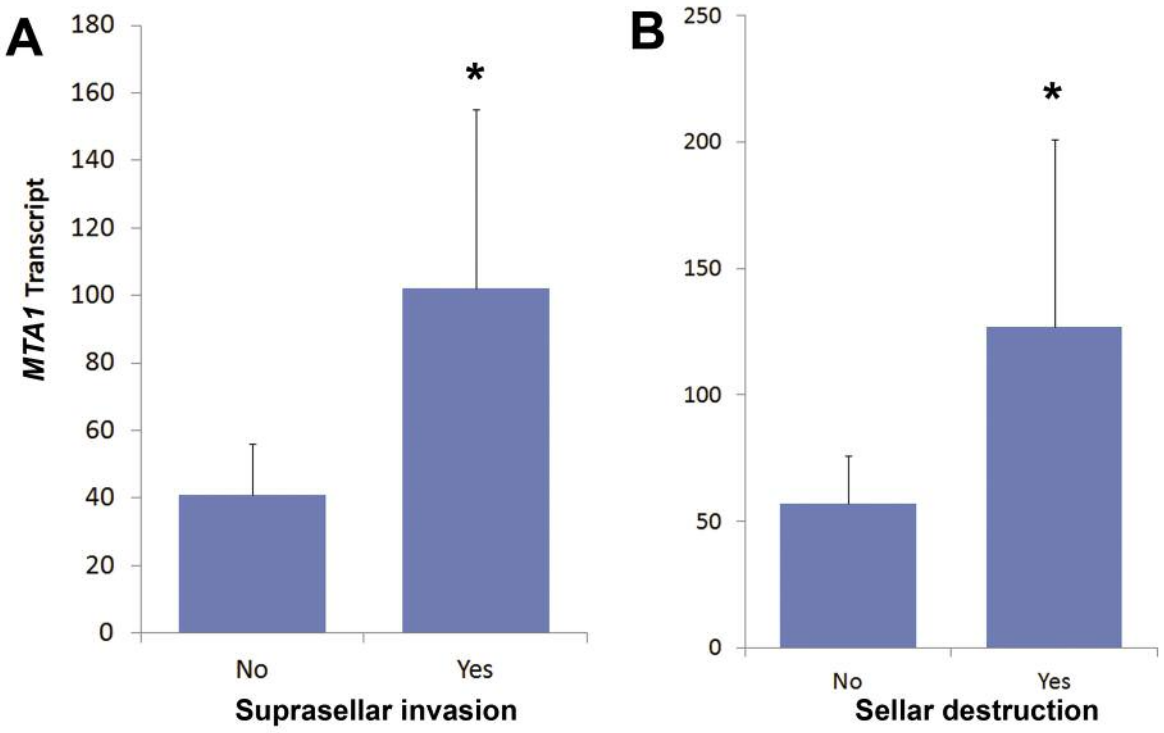

Figure 2. *MTA1 transcript levels in tumors with invasive growth pattern (A) and with sella destruction (B). *Statistically significant difference at p<0.05.*

mixed secretion (p=0.0043) had significantly lower levels (Figure 3). Although tumors secreting adrenocorticotropic hormone (ACTH) and thyroid stimulating hormone (TSH) had higher levels of *MTA1*, there was little difference between these and the inactive tumors.

Discussion

Pituitary tumors often develop an aggressive pattern by invading the surrounding tissues, namely the *sella fossa*, from which, more aggressive tumors go on to destroy the bony tissues. This is reflected in the current cohort, in that 28.4% (27/95) of tumors had an invasive growth pattern. These tumors demonstrated high levels of *MTA1*. Similarly, tumors of 20 out of the 95 patients with fossa destruction also showed markedly higher levels of *MTA1* transcript. This is an interesting finding, which indicates that the level of *MTA1* is associated with the aggressive growth of pituitary tumors. This association is perhaps to be expected for *MTA1*, the overexpression of which has been repeatedly shown to be related to an increase in cell migration and MMP9 expression, traits linked to tumour spread and tissue destruction.

Owing to its indicated role in cancer aggression, MTA1 has been investigated in a large number of clinical cancer types and found to have a strong clinical relevance to disease progression. The current study, to our knowledge for the first time, shows that the same is true for pituitary adenomas. Overall, MTA1 appears to be a general indicator of the progressive growth of solid tumors.

Given the expression pattern of MTA1 in solid tumors, MTA1 presents a good target for cancer therapy. It has been indeed shown recently that resveratrol and its naturally occurring analogue pterostilbene are potential inhibitors of MTA1 and inhibited the growth and spread of prostate cancer in *in vivo* models (28). MTA1 can be regulated by the nuclear factor kappa-light-chain-enhancer of activated B (NF-κB). thus a combined use of HER2 inhibitors and NF-κB inhibitors may be of interest in cancer therapies(29).

The relationship between MTA1 and the endocrine functions of pituitary tumors is interesting. While it is not clear how this relationship occurs, it has been reported that MTA1 is also highly expressed in other types of neuroendocrine tumors, namely ileal neuroendocrine neoplasms (30). In that study, it was found that an increase of MTA1 occurred together with the overexpression of HER2, indicating that this may be a HER2-related event for this tumour type. Another report showed overexpression of MTA1 protein in pancreatic endocrine tumors, a pattern

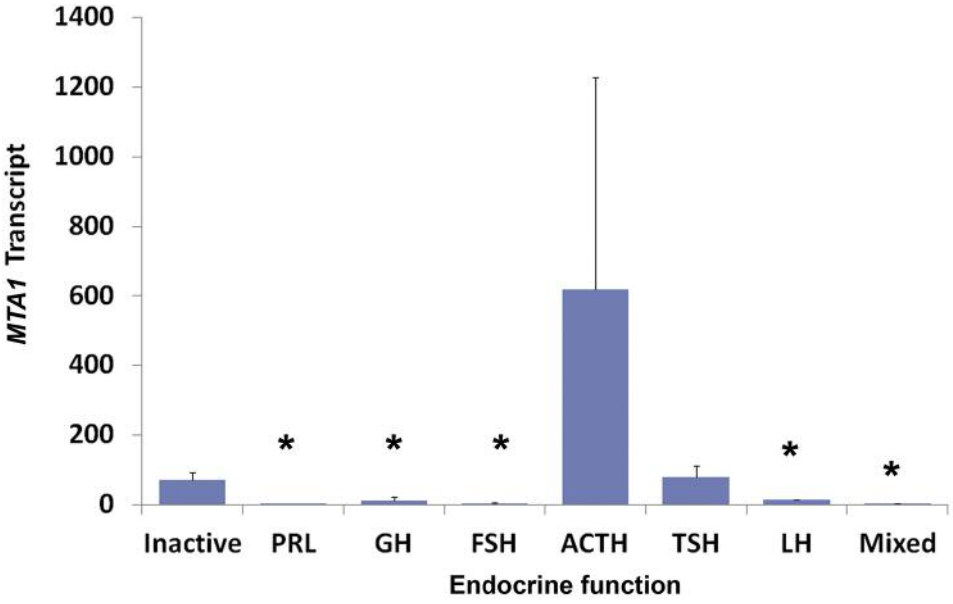

Figure 3. *MTA1 expression and endocrine function of pituitary adenomas. *Statistically significant difference at p<0.05 vs. inactive tumor.*

associated with the aggressive nature of tumors, although no association was indicated with endocrine function (31). A future study with a larger cohort would be useful in clarifying this association. Certainly, cell and animal models will provide further information about how MTA1 regulates endocrine functions of pituitary tumors.

In conclusion, pituitary adenomas aberrantly express *MTA1* and the levels of its expression correlate with the aggressive pattern of the tumors.

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