C-Met expression pattern in uterine leiomyoma

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Summary

Aim: Growth factors take place in the formation and growth of uterine leiomyomas (LMs). Transforming growth factor beta (TGF-β), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), and insulin-like growth factor (IGF) contribute to the pathophysiology of LMs when they bind with a specific membrane receptor and transmit a signal into the cell. Little is known about hepatocyte growth factor (HGF) and its receptor system c-Met in formation and growth of uterine LMs. The aim of this study was to evaluate the c-Met receptor expression on human myometrium and uterine LMs. *Materials and Methods:* The study was performed on human myometrium and uterine LMs. Expression of c-Met receptor was evaluated by immunohistochemical analysis. *Results:* Overexpression of c-Met was found in all LM cases and in none of normal myometrium samples c-Met overexpression was seen. *Conclusion:* HGF and c-Met receptor complex seem to have role in development of uterine LMs.

Key words: Hepatocyte growth factor; c-Met receptor; Uterine leiomyoma.

Introduction

Uterine leiomyomas (LMs) are benign monoclonal tumors arising from the smooth muscle cells of the myometrium. The pathogenesis of LMs is multifactorial. Genetic predisposition, steroid hormones, and growth factors which are important in fibrotic processes and angiogenesis take place in the formation and growth of uterine leiomyomas [1]. Transforming growth factor beta (TGF-β), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), and insulin-like growth factor (IGF), which are involved in fibrotic processes and angiogenesis, may also contribute to the pathophysiology of LMs [2-3]. Growth factors may foster LM growth through local paracrine and/or autocrine mechanisms [4].

The hepatocyte growth factor (HGF)/ receptor system has multifunctional properties, such as cell proliferation, cell movement, and morphogenesis [5-6]. The receptor for HGF is a protein product of a proto-oncogene c-Met which encodes a transmembrane tyrosine kinase (P^{190} c-Met) with structural and functional features of a growth factor receptor [7-9]. Autophosphorylation of this receptor by ligand binding stimulates its intrinsic tyrosine kinase activity with resultant changes in cellular morphology, motility, and growth. Overexpression of this oncogene was shown in different human solid tumors such as hepatomas, carcinomas of colon, rectum, stomach, pancreas, thyroid, kidney, ovary, endometrium, bladder, breast, and prostate [10-22].

Aberrant expression of gene products of several growth factors and/or their receptors, such as HGF and its receptor c-Met, may be associated with genetic alterations in epithelial cells which, in turn, may be linked to carcinogenesis process. HGF is thought to be produced principally by mesenchymal cells, although mRNA and protein have sometimes been detected in epithelia [23-26].

The role of some peptide growth factors in uterine LM is accepted but little is known about HGF and its receptor system c-met. For this reason the authors decided to evaluate c-Met receptor expression by immunohistochemical analysis.

Materials and Methods

This study included 20 patients diagnosed with uterine LM and four patients with normal myometrium in Near East University Hospital, Nicosia, North Cyprus, and in Florence Nightingale Hospital, İstanbul, Turkey during years 2010 and 2011.

Immunohistochemical Analysis

Immunohistochemical evaluation was done according to the authors' previous studies [27-29]. Formalin fixed and paraffin embedded specimens of primary lesions were studied simultaneously. Four micrometer sections were deparaffinized in xylene and rehydrated. Antigen retrieval procedure was performed in x50 tris/EDTA buffer (pH: 9) in pressure cooker and incubation was done in x20 tris-buffered saline (TBS) solution for 15 minutes. Non-specific protein blockage was performed with peroxidase blocking reagent. All of the immunohistochemical procedures were done at room temperature. Slides were incubated with polyclonal anti-c-Met antibody (dilution: 1/25). Subsequent procedures were performed using a specific immunoperoxidase staining kit. The antibody was visualized by freshly prepared solutions of 0.04% 3',3'- diaminobenzidine tetrahydrochloride and 0.03% hydrogen peroxide and the sections were counterstained with haematoxylin, cleaned, and mounted. Im-

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Figure 1. — Extensive and intense immunoreactivity in epithelial component of prostate tissue (arrows) (c-Met, x200).

munoreactivity was evaluated according to the number of the stained cells and the intensity of staining. Positive immunostaining was localized to cytoplasm and membrane. Prostate tissue was used as positive control. Extensive and intense immunoreactivity was observed in epithelial component (Figure 1). Immunoreactivity was evaluated according to the number of the stained cells and the intensity of staining. Extensiveness of staining was scored as 0 = 0%, 1 = 1-30%, 2 = over 30%. Intensity was scored as 1 (mild), 2 (moderate), and 3 (intense). A numerical value is gained by product of these two scores. A final score between 0-3 is accepted as negative, a score greater than 3 was accepted as overexpression.

Table 1. — *Results of immunohistochemical analysis.*

Case	Diagnosis	Extensiveness	Intensity	Final score
1	LM	2	2	4
2	LM	2	2	4
3	LM	2	3	6
4	LM	2	3	6
5	LM	2	2	4
6	LM	2	2	4
7	LM	2	2	4
8	LM	2	2	4
9	LM	2	2	4
10	LM	2	2	4
11	LM	2	2	4
12	LM	2	2	4
13	LM	2	2	4
14	LM	2	3	6
15	LM	2	2	4
16	LM	2	2	4
17	LM	2	3	6
18	LM	2	2	4
19	LM	2	2	4
20	LM	2	3	6
21	M	1	1	1
22	M	1	1	1
23	M	1	1	1
24	M	1	1	1

LM = Leiomyoma
M = Normal myometrium.

Results

The indication for operation for all patients was abnormal uterine bleeding. Mean age at the time of surgery was 40.6 ± 6.5 years (range 28 - 52). None of the patients were postmenopausal. All of the patients were evaluated before surgery to exclude any other gynecological pathology. The ones that were using oral contraceptives were operated after three months from cessation of the pills. None of the patients were on any other hormonal medications.

Results of immunohistochemical analysis are summarized in Table 1. Extensiveness and intensity scores were calculated as previously defined for all samples. Product of the extensiveness and intensity score yielded a final score for every patient. When final staining score was evaluated, there were no significant difference in this score with respect to patient age and size of the LM (Figure 2).

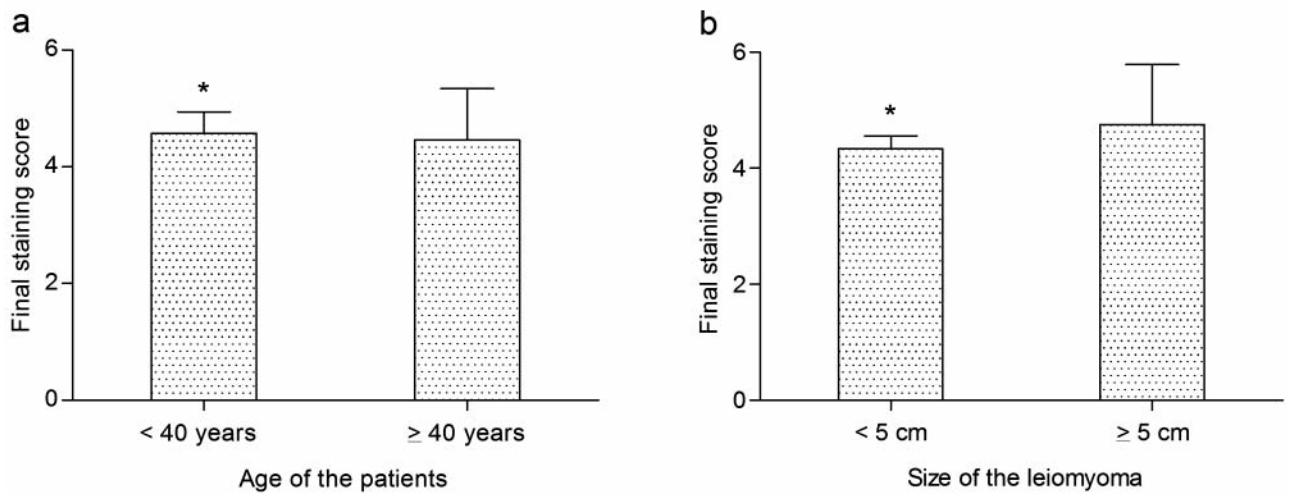

Figure 2. — a: Evaluation of final staining score according to patient age. b: Evaluation of final staining score according to leiomyoma size, *p >0.05.

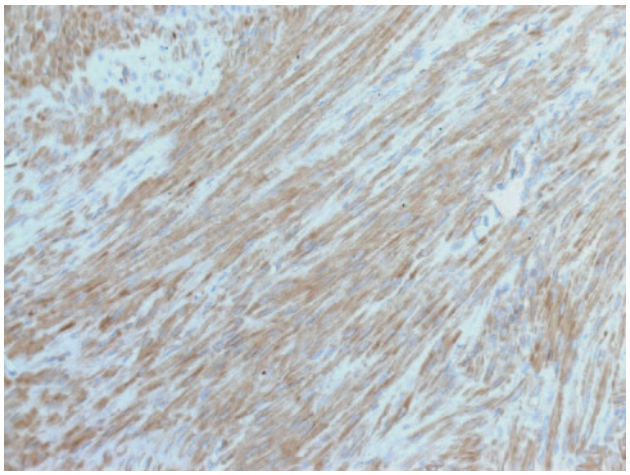

Figure 3. — c-Met overexpression in leiomyoma (c-Met, x200).

The final staining score between 0-3 was accepted as negative, a score greater than 3 was accepted as overexpression, as previously described. The mean, median, and standard deviation of the final staining score among LMs were 4.5, 4.0, and 0.9, respectively. Overexpression of c-Met was found in all LM cases (100%). Figure 3, demonstrates overexpression of c-Met in uterine LM. However, none of the normal myometrium samples were found to have c-Met overexpression (0%). Besides, all of the normal myometrium samples had a final staining score of 1.

Discussion

Growth factors are polypeptides or proteins that are secreted by a number of cell types and have a wide range of biologic effects. They are essential elements in controlling the proliferation rate of cells, and overexpression of either the growth factor or its receptor may contribute to tumorigenesis [30]. Several growth factors and their receptors have been examined for their role in pathogenesis of uterine LM. Today it is thought that the role of growth factors in the development of uterine LM appears to be more important than estrogens, but estrogens stimulate growth factors and their receptors synthesis [31].

It has been described in many studies that myometrium and LMs contain many peptide growth factors, mainly EGF, aFGF, bFGF, IGF-I, PDGF, and TGF-β. [32-34].

HGFR, which is a protein product of a proto-oncogene c-Met encoding a transmembrane tyrosine kinase, has structural and functional features of other growth factor receptors [10]. It is known that, endogenous HGF is important for inducing self-repair responses in numerous organs. c-Met is transcriptionally induced by hypoxia and inflammatory cytokines or pro-angiogenic factors that are abundant in the reactive stroma of full-blown tumours. Hence, c-Met activation is a late event that aggravates the intrinsic malignant properties of already transformed cells by conveying proliferative, anti-apoptotic, and promigratory signals [35]. To the authors' knowledge; although c-Met overexpression has been demonstrated in many human solid tumors, there is no study examining c-Met expression in uterine LM.

It is apparent from this study that normal myometrium has no c-Met overexpression detected by immunohistochemical analysis. It has been demonstrated that uterine LM has c-Met overexpression, but it is still not known which factors induce HGF/ c-Met complex. Sozen *et al.* stated that steroid hormones (estrogen, progesterone, and glucocorticoid) stimulates VEGF and FGF secretion and activation [36]. Steroid hormones can also be the promoter of HGF/ c-Met complex.

Measuring HGF content and c-Met receptor expression by both immunological and polymerase chain reaction (PCR) techniques both myometrium and LMs can give the exact results. The authors only analysed Met overexpression by immunohistochemical analysis. This is the limitation of this study.

In conclusion, HGF and c-Met receptor complex seem to have role in development of uterine smooth muscle tumors and especially LMs.

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