Overexpression of c-Met in cervical intraepithelial neoplasia

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Summary

Purpose of investigation: The purpose of this study is to evaluate the significance of the c-Met / Hepatocyte Growth Factor Receptor (HGFR) expression in cervical intraepithelial neoplasia (CIN). *Materials and Methods:* Twenty-one patients from two types of cervical intraepithelial neoplasias (LSIL and HSIL), diagnosed in our clinic were studied with c-Met immunohistochemistry. Of the 21 cases, five were diagnosed as LSIL and 16 as HSIL. Normal cervical mucosas from five patients were studied with c-Met as control cases. *Results:* Overexpression of c-Met was found in all five of LSIL specimens. C-Met overexpression was observed in 11 cases of HSIL. No c-Met overexpression was seen in any of the five control cases. *Conclusion:* These results revealed that *c-Met* oncogene overexpression is an important parameter in cervical early oncogenesis and may have a role in malignant transformation of cervical epithelial cells.

Key words: c-Met / HGFR overexpression; Cervical intraepithelial neoplasia; Immunohistochemistry.

Introduction

A variety of growth factors, such as epidermal growth factor (EGF), transforming growth factor-alfa (TGF-a), and transforming growth factor-beta (TGF-b), appear to play a crucial role in human carcinogenesis. The hepatocyte growth factor HGF / receptor system has multifunctional properties, such as: cell proliferation [1], cell movement [2] and morphogenesis [3, 4]. The receptor for HGF is a protein product of a protooncogene c-Met [5, 6] which encodes a transmembrane tyrosine kinase (P190 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]. As cervical preinvasive pathologies seem as a perfect model to analyze carcinogenesis step by step from the disturbance of cell proliferation and/or differentation in squamous epithelium, via low and high grade CIN, also called squamous intraepithelial neoplasia (SIL), to invasive carcinoma [23]. The certain role of human papilloma virus (HPV) in the development of genital intraepithelial neoplasia had been shown with enough evidence [24]. All genital condylomas, and most intraepithelial neoplasia and cervical invasive cancers contain HPV DNAs [23, 25]. HPV types 16, 18, 31, and 33 are most commonly found in cervical, vaginal or vulvar neoplasia, whereas types 6 and 11 are linked to condyloma accuminata or regressive dysplasia [26].

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 mainly produced by mesenchymal cells, although mRNA and protein have sometimes been detected in epithelia [27-30]. However still remains a mystery the expression of HGF and c-Met in squamous cervical intraepithelial lesions.

In this study, the authors examined c-Met expression in CIN.

Materials and Methods

This study included 21 patients diagnosed with CIN in Near East University Hospitals, TRNC during year 2010 and five patients with normal mucosa in 2011. All patients were evaluated and diagnosed by gynecologists according to the American Society of Colposcopy and Cervical Pathologies (ASCCP) guidelines. Five of the specimens were CIN 1, and 16 were CIN 2-3.

Immunohistochemical Analysis

Immunohistochemical evaluation was performed according to the authors' previous studies [31-33]. Formalin-fixed and paraffin-embedded specimens of primary lesions were studied simultaneously. The observers were unaware of the clinical data. Four micrometer sections were deparaffinized in xylene and rehydrated. Antigen retrieval procedure was performed in 50x Tris ethylenediaminetetraacetic acid (EDTA) buffer (pH: 9) in pressure cooker and incubation was done in 20x TBS (Tris buffered Saline Solution) for 15 minutes. Nonspecific protein blockage was performed with peroxidase blocking reagent. All of the immunohistochemical procedures were performed at room temparature. Slides were incubated with polyclonal anti-c-Met antibody (Novocastra, Leica Microsystems; dilution: 1/25). Subsequent procedures were performed using Dako

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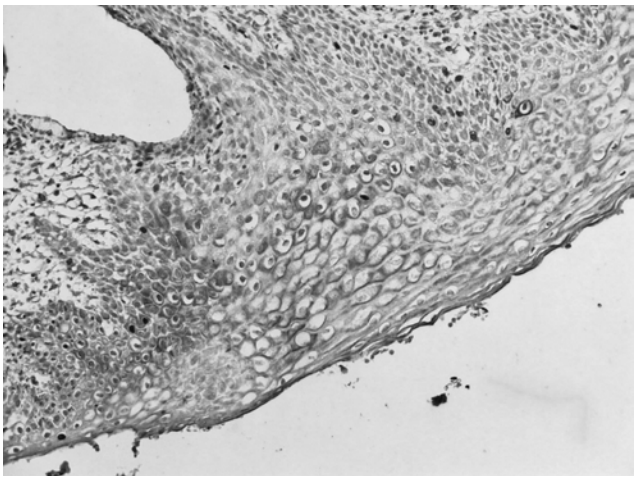

Figure 1. — Immunohistochemical c-met overexpression in CIN – I area (c-Met, x200).

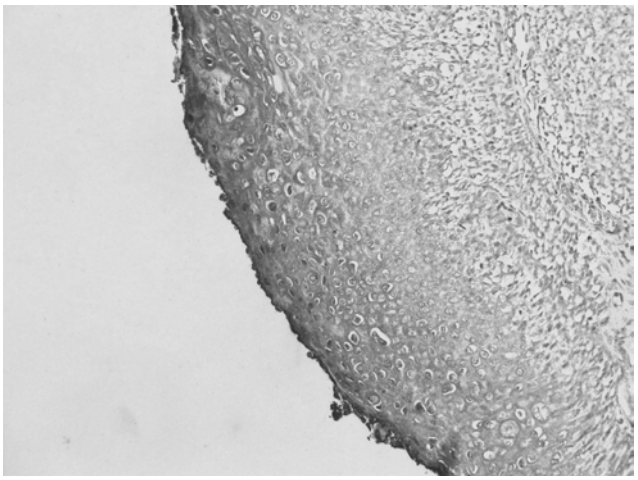

Figure 2. — Immunohistochemical c-met overexpression in CIN – III area (c-Met, x200).

Table 1. — *Results of immunohistochemical analysis*.

Case	Diagnosis	Extensiveness	Intensity	Final score
1	CIN 2	2	2	4
2	CIN 3	2	2	4
3	CIN 3	2	3	6
4	CIN 1	2	2	4
5	CIN 2	1	2	2
6	CIN 2	2	2	4
7	CIN 2	0	0	0
8	CIN 3	2	2	4
9	CIN 1	2	2	4
10	CIN 1	2	2	4
11	CIN 3	2	2	4
12	CIN 1	2	2	4
13	CIN 3	0	0	0
14	CIN 2	2	2	4
15	CIN 2	2	2	4
16	CIN 1	2	3	6
17	CIN 2	2	2	4
18	CIN 3	0	0	0
19	CIN 2	2	2	4
20	CIN 3	1	1	1
21	CIN 3	2	2	4
22	N	1	1	1
23	N	1	1	1
24	N	1	1	1
25	N	0	0	0
26	N	1	1	1

CIN: Cervical intraepithelial neoplasia; N: Normal mucosa.

EnVision Flex (Dako K8000 En Vision Flex Kit, Denmark) 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 haematoxylen, cleaned and mounted. Positive immunostaining was localized to cytoplasmic membrane. Immunoreactivity was evaluated according to the number of the stained cells and the intensity of staining. Positive immunostaining was localized to cytoplasm and membrane. Immunoreactivity was evaluated according to the number of the stained cells and the intensity of staining. An overexpression criterion was defined as discrimination of the dysplastic epithelium and neighboring normal epithelium by staining difference. 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 was accepted as negative; a score greater than 3 was accepted as overexpression.

Statistical analysis

Chi-square was used where appropriate. Differences between groups were tested using the log-rank test; p values of < 0.05 were considered statistically significant. Calculations were done using the SPSS for Windows version 14.0 statistical software.

Results

Results of immunohistochemical analysis are summarized in Table 1. Overexpression of c-Met was found in all 5 of LSIL specimens (100%). C-Met overexpression was observed in 11 of 16 of HSIL cases (69%). Overall c-Met overexpression was found in 16 out of 21 (76%) cervical dysplasia cases (Figures 1 and 2). Staining was membranous and cytoplasmic, distinct in dysplastic tissue. C-Met overexpression was not seen in any of the five control cases.

Discussion

Cervical carcinogenesis and early development of cervical preinvasive lesions are currently being extensively evaluated from several perspectives. Early carcinogenesis of the cervix is directly related to the oncogenic changes caused by HPV [34, 35]. Many genetic changes and growth factors are found to play role in these changes; Epithelial Growth Factor Receptor (EGFR), c-erbB2, Insulin Like Growth Factor (IGF-1 and IGF-2) and Vascular Endothelial Growth Factor (VEGF) are some well-known ones [36-44]. Apart from their oncogenic role, some of them are found to be related to patients' survival and prognosis and are being used for therapeutic aims [45-47].

Hepatocyte Growth Factor Receptor (HGFR) which is a protein product of a proto-oncogene c-met encoding a trans-membrane tyrosine kinase, has structural and functional features of other growth factor receptors [8, 9, 48]. Its auto phosphorylation by ligand binding stimulates its intrinsic tyrosine kinase activity with resultant changes in cellular morphology, motility, and growth. Ligand of HGFR and HGF/SF is implicated in the mode of stromal invasion or aggressiveness of human cervical squamous carcinoma cell lines [49]. It is well known that transfection of cells that are c-Met negative with the HGF/SF receptor gene results in an increased motile and invasive nature [48-51], demonstrating the potential of this oncogene in enhancing cellular properties that are central to the initiation and development of metastasis. It is therefore possible that preinvasive status and malignant cells with aberrant expression of the c-Met protein are prone to have more aggressive and severe cell behaviors.

The step-by-step progression model of cervical preinvasive diseases to invasive cancer that takes place in CIN continuum, remains and states an important histopathological concept to evaluate a perfect carcinogenesis model. Years of epidemiologic and preventive research focused on the HPV cervical cancer model have revolutionized the knowledge and understanding of cervical precancer [52]. HPV infection serves as a broad transition and discrimination state between normal tissue and cancer. This malignant transformation zone gives a big opportunity to evaluate and understand all histologic, hormonal, genetic, and growth factor changes that takes place. CIN 3, particularly full thickness carcinoma in situ, shares the same HPV-type spectrum and cofactor profile as invasive cancer with the same aneuploid DNA content and genetic instability. CIN 2 demonstrates greater heterogeneity in biology and definition [53]. CIN 1 is no longer considered as not representing precancer because it usually reflects HPV infection only. Persistent HPV infection with oncogenic HPV types is strongly linked to precancer which then may progress to high grade SIL (CIN 2-3) and invasive cancer in some proportion of the cases. HGF and its receptor c-Met expression level under the effect of HPV persistent infection may be one of the parameters that regulates the CIN prognosis and progress to more severe degree. As found in this study, c-Met seems to have a dominant role in this transition. Tissue studies of hysterectomy or conisation patients with CIN diagnosis may evaluate the HGF ligand and receptor expression levels in neighboring normal and pathologic cervical tissue and possible difference will play an important role in future diagnostic and therapeutic plans for these patients.

Conclusion

CIN cases display immunohistochemical c-met overexpression. The authors believe that c-Met may play a role in transformation of cervical squamous cell dysplasia.

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