

## 하인두 편평세포암에서 간세포성장인자와 그 수용체인 c-Met의 발현

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### Expression of HGF/c-Met in Hypopharyngeal Squamous Cell Carcinoma

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#### ABSTRACT

Hepatocyte growth factor (HGF), a potent stimulator of hepatocyte growth, stimulates motility, invasiveness, proliferation, and morphogenesis of epithelium, and may be involved in physiologic and pathologic processes such as embryogenesis, wound healing, organ regeneration, inflammation, and tumor invasion. So we examined the role of the HGF/c-Met on invasion and metastasis of hypopharyngeal squamous cell carcinoma (SCC). We performed immunohistochemical stains on 40 normal mucosas and 40 SCCs of hypopharynx with HGF antibody and c-Met antibody. For RT-PCR and Western blot, fresh normal tissues and cancer tissues in hypopharynx obtained from five patients were used. The positive rates of HGF and c-Met expression in hypopharyngeal SCC were 77.5% and 70%, respectively. HGF staining was significantly correlated with lymph node metastasis and pathologic stage ( $p < 0.05$ ). c-Met staining was only significantly correlated with lymph node metastasis ( $p < 0.05$ ). The increased expression of c-Met mRNA (RT-PCR) and protein (Western Blot) were detected in hypopharyngeal cancer tissue. These results suggest that HGF may play an important role in the progression of hypopharyngeal cancer. (Korean J Otolaryngol 2004;47:1135-41)

KEY WORDS : Hepatocyte Growth Factor (HGF) · c-Met · Hypopharyngeal cancer.

가 가  
 가 (tumor and host interaction)  
 , (stromal alterations)가  
 (submucosal spread)가 (fibroblast),  
 (second primary (macrophage), (lymphocyte), (neut-  
 tumor) rophils) (endothelial cells)  
 가 20 , 1)  
 가 Matsumoto 1)  
 가 가  
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 hepatocyte growth factor(HGF) peptide growth  
 factor (hepatocyte)  
 가

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				RT - PCR	Western blotting
				1	5
protease (invasiveness)		(motility) (morphogenesis)		- 70	
	(angiogenesis)			5	
<sup>2,3)</sup> HGF	1984	3	가 3 ,		
	<sup>4-6)</sup> 1989	가	가 1 ,		가
<sup>7)</sup> 1991		scatter	1		
factor가 HGF					
HGF/SF	<sup>8)</sup> HGF		c - Met HGF		
	tyrosine kinase				labelled streptoavidin biotin kit
	proto - oncogene		(LSAB ; DAKO Co., CA. USA)		
c - met gene	<sup>9)</sup> HGF	c -	5 μm		
Met paracrine			xylene		
가	HGF c - Met		ethanol	5	10
가	c - met oncogene		mM citrate buffer(pH 6)	10	microwave
	c - met oncogene	HGF가	3% H <sub>2</sub> O <sub>2</sub> 가	100% methanol	10
가	HGF c - Met		human HGF	human HGF	human HGF
			affinity purified polyclonal goat antibody(R & D systems, Inc, MN, USA)	c - Met	
	HGF c - Met		human HGF receptor(c - Met) polyclonal goat antibody(R & D system)	1 : 100	
c - Met m - RNA	protein	HGF	, 4	universal secondary	
			biotinylated antibody	R.T.U VECTASTAW® ELITE	
			ABC kit(Vector Laboratories, Inc., CA, USA)		
			30		
			DAB(DAKO)	5	
			10 Hematoxylin		Semisynthetic
			crystal mount(DAKO)	mounting	, cover glass
	1994 3	1998 4			
	Hematoxylin - eosin				
		가	RT - PCR	HGF c - Met mRNA	
40				1 ml TRIzol®(GIBCOBRL, Grand Island,	
60.1 ± 8.4( ± )	43	76	NY, USA)	RNA	
38	2			RNA 2 μg	
	AJCC(2002)			Omniscrypt Reverse Transcriptase kit(20511, Qiagen	
(10%),	3 (7.5%),	4		Germany)	{10X Buffer RT 2.0 μl, dNTP
10 (25%),	23 (57.5%)			Mix(5 mM each dNTP) 2.0 μl, Oligo - dT primer(10 μl)	
rate, poor differentiation		well, mode-		2.0 μl, RNase inhibitor(10 units/ μl) 1.0 μl, Omniscrypt	
				Reverse T ranscriptase 2 units, RNase - free water} 20	
				μl	37 60 , 94 5
			cDNA	. PCR Minicycler™(MJ research,	

USA) cDNA Taq DNA polyme-  
 rase 1 unit(Roche Diagnostics Co, Indianapolis, USA)  
 primer  
 human HGF primer human c - Met primer

Log - rank test 가  
 p 0.05

human HGF ;  
 sense : 5 '- ACA TCG TCA CTT CTG GC - 3 '  
 antisense : 5 '- ATC CAT CCT ATG TTT GTT  
 CG - 3 '  
 human c - Met ;  
 sense : 5 '- AGT AGC CTG ATT GTG CAT TT - 3 ;  
 antisense : 5 '- TCT TTC ATG ATG CCC TC - 3 !  
 PCR 96 3 , 96  
 30 , 55 30 , 72 30 30  
 cycles (extension) 72 5

c - Met HGF  
 Taniguchi <sup>17)</sup>  
 30%  
 , 30%  
 HGF 40 31  
 (77.5%) (Table 1),  
 HGF  
 (stromal cell)

Western blotting c - Met  
 phosphate  
 buffered saline(PBS)  
 (100 µg/ml phenylmethylsulfonyl fluoride, 1 µg/ml  
 leupeptin)가 가 RIPA(RadiolImmunoPrecipitation)  
 buffer 1 ml{150 mM NaCl, 1% NP - 40, 50 mM Tris  
 (pH 8.0), 1 mM EDTA, 0.5% Deoxycholate}

HGF  
 (Fig. 1).  
 가 4 cm  
 가 4 cm

Western blot analysis  
 Bio - Rad protein assay(Bio - Rad, Hercules,  
 CA, USA) Well 20 µg

sodium dodesyl sulfate(SDS) -  
 polyacrylamide gel electrophoresis(PAGE)  
 nitrocellulose filter(Amersham, Arlington  
 Heights, IL, USA) 4 c -  
 Met filter 0.1% Tween - 20  
 Tris buffered saline(TBS)  
 peroxidase - conjugated donkey anti - rabbit antibody  
 (Amersham) donkey anti - mouse antibody(Amers-  
 ham) enhanced chemiluminescence  
 detection system(ECL, Amersham) X - ray  
 film

Fisher 's exact test, Mantel -  
 Haenzel test Kaplan - Meier

**Table 1.** Correlation between the expression pattern of HGF and clinicopathologic factors. The positive rates of HGF expression in hypopharyngeal SCC were 77.5%. HGF staining was significantly correlated with lymph node metastasis and pathologic stage (p<0.05)

Variables	Expression of HGF (%)		p-value
	Negative (22.5)	Positive (77.5)	
T	1, 2 (n=11)	4 (36.4) 7 (63.6)	0.227 <sup>1)</sup>
N	3, 4 (n=29)	5 (17.2) 24 (82.8)	0.038 <sup>1)</sup>
	- (n=13)	6 (47) 7 (53)	
M	+ (n=27)	3 (11.1) 24 (88.9)	1.000 <sup>1)</sup>
	- (n=34)	8 (23.5) 26 (76.5)	
Stage	+ (n=6)	1 (16.7) 5 (83.3)	0.016 <sup>1)</sup>
	I, II (n=6)	4 (66.7) 2 (33.3)	
Differentiation	III, IV (n=34)	5 (14.7) 29 (85.3)	0.103 <sup>2)</sup>
	Well (n=11)	1 ( 9.1) 10 (90.9)	
	Moderate (n=16)	4 (25) 12 (75)	
Recurrence	Poor (n=13)	4 (30.7) 9 (69.3)	0.226 <sup>1)</sup>
	Yes (n=13)	1 ( 7.7) 12 (92.3)	
	No (n=27)	8 (29.6) 19 (70.4)	

1) calculated by Fisher's exact test (p<0.05)

2) calculated by Mantel-Henzel test (p<0.05)

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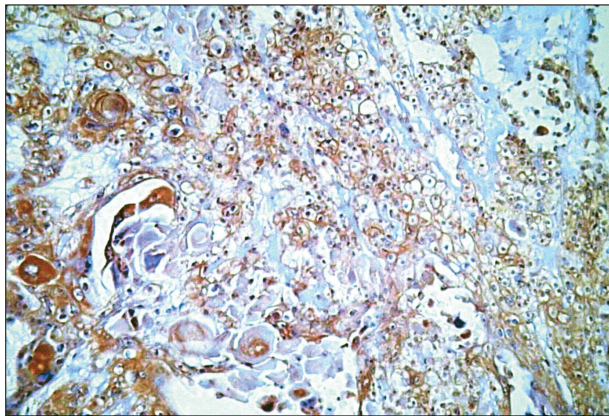
T3 T4 82.8% 가 HGF 가 가  
 가 (Table 1). 가 HGF 7/13 (Fig. 2). 40 28 (70%)  
 (53%) 가 HGF 24/27 (Table 2). c-Met  
 (88.9%) 가 가 (76.5%) T1, T2 7/11 (63.6%) T3, T4 21/29  
 (p<0.05). 가 (83.3%) HGF 가 c-Met 가  
 가 (33.3%) HGF 2/6 (81.5%) 가 6/13 (46.2%) c-Met  
 (85.3%) 가 HGF 29/34 가 가 23/34 (67.6%) c-Met  
 가 (p<0.05). HGF 가 가 (76.5%)가 (33.3%)  
 HGF 12/13 c-Met 가  
 (92.3%) 19/27 (70.4%) c-Met 가  
 c-Met HGF 가 (76.9%)가

(Fig. 2). 40 28 (70%)  
 (Table 2). c-Met  
 T1, T2 7/11 (63.6%) T3, T4 21/29  
 (72.4%) c-Met 가  
 가 가 22/27  
 (81.5%) 가 6/13 (46.2%) c-Met  
 가 가 23/34 (67.6%) c-Met  
 가 5/6(83.3%) c-Met  
 가 (76.5%)가 (33.3%)  
 c-Met 가  
 c-Met c-Met 가  
 c-Met (76.9%)가

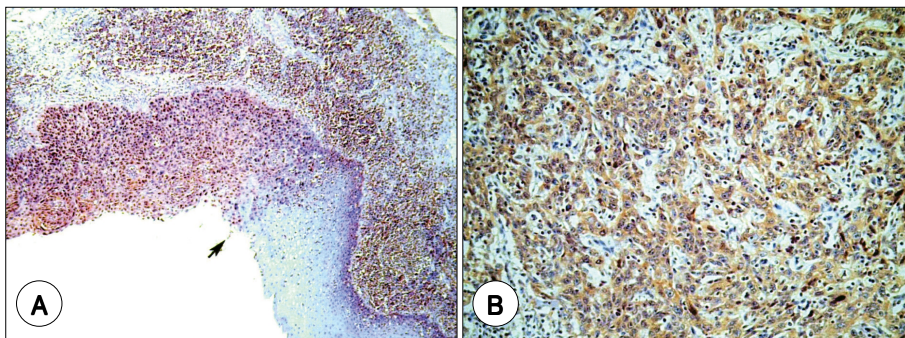
**Table 2.** Correlation between the expression pattern of c-Met and clinicopathologic factors. The positive rates of c-Met expression in hypopharyngeal SCC were 70%. c-Met staining was only significantly corrected with lymph node metastasis (p<0.05)

Variables	Expression of c-Met (%)		p-value
	Negative (30)	Positive (70)	
T	1, 2 (n=11) 3, 4 (n=29)	4 (36.4) 7 (63.6) 8 (27.6) 21 (72.4)	0.704 <sup>1)</sup>
N	- (n=13) + (n=27)	7 (53.8) 6 (46.2) 5 (18.5) 22 (81.5)	0.032 <sup>1)</sup>
M	- (n=34) + (n=6)	11 (32.4) 23 (67.6) 1 (16.7) 5 (83.3)	0.648 <sup>1)</sup>
Pathologic stage	I, II (n=6) III, IV (n=34)	4 (66.7) 2 (33.3) 8 (23.5) 26 (76.5)	0.055 <sup>1)</sup>
Pathologic grades	Well (n=11) Moderate (n=16) Poor (n=13)	3 (27.3) 8 (72.7) 6 (37.5) 10 (62.5) 3 (23.1) 10 (76.9)	0.851 <sup>2)</sup>
Recurrence	Yes (n=13) No (n=27)	3 (23.1) 10 (76.9) 9 (33.3) 18 (66.7)	0.716 <sup>1)</sup>

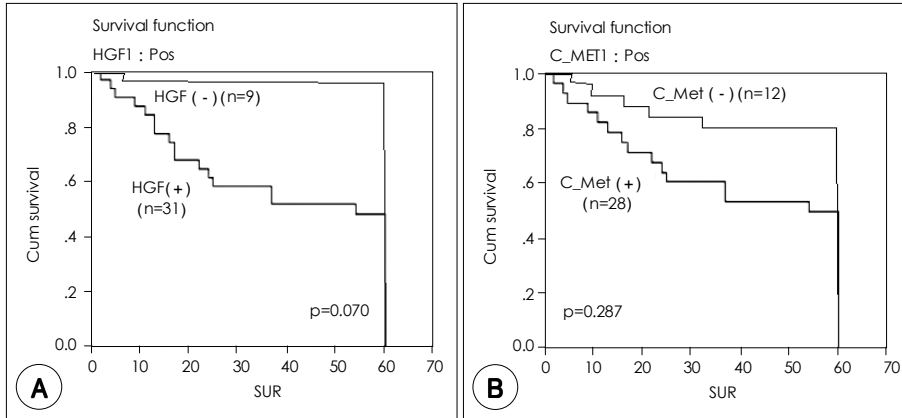
1) calculated by Fisher's exact test (p<0.05)  
 2) calculated by Mantel-Henzel test (p<0.05)



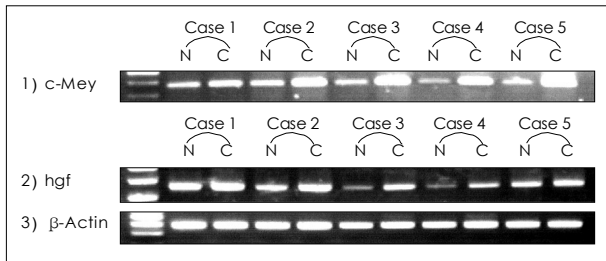
**Fig. 1.** Immunohistochemical localization of HGF protein in hypopharyngeal SCC tissue. HGF was stained mainly in stromal tissue around cancer cells but occasionally in cancer cells and basal layer of normal cells (x 200).



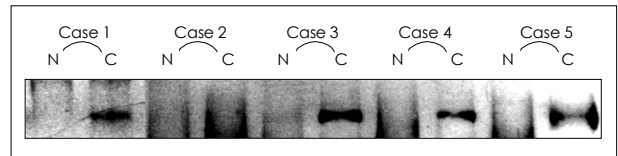
**Fig. 2.** Immunohistochemical expression of c-Met in the human hypopharyngeal carcinoma tissue. There was strong c-Met expression in cytoplasm of cancer cells but not in normal cells. Transition zone was shown in an apparent form (black arrow); magnification x 40 (A). It was shown that the expression was in the cytoplasm of cancer cells diffusely and strongly; magnification x 200 (B).



**Fig. 3.** Survival curves of hypopharyngeal carcinoma patients according to the expression of HGF (A) and c-Met (B).



**Fig. 4.** RT-PCR analysis of HGF and c-Met expression in normal hypopharyngeal mucosa, hypopharyngeal cancer. The increased expression of HGF mRNA and c-Met mRNA were detected in hypopharyngeal cancer tissues.



**Fig. 5.** Western Blotting in hypopharyngeal cancer tissue. The increased expression of c-Met protein were detected in cancer tissues (except Case 2).

(Fig. 5).

(66.7%)  
 40 17 (42.5%)가 HGF  
 10 4 (tube)  
 , 2  
 Kaplan - Meier  
 Log - rank test 가 HGF  
 value 0.07 . c - Met p - HGF  
 (Fig. 3, p>0.05).  
 HGF가  
 RT - PCR Western blotting 가  
 c - Met RT -  
 PCR 3 가 2 가 가  
 2 가 1 HGF가  
 가 HGF가  
 가 HGF  
 c - Met 5 Western blotting HGF c - Met  
 1 4 가



19) HGF가  
 Shimura 20) HGF가  
 paracrine  
 HGF가  
 paracrine HGF  
 HGF가  
 RT - PCR  
 HGF c - Met  
 가  
 Met HGF mRNA  
 가  
 Western blotting 1  
 4 c - Met  
 c - Met  
 HGF c - Met mRNA  
 HGF c - Met  
 가  
 HGF c - Met HGF  
 가  
 HGF c - Met m - RNA  
 가  
 HGF c - Met  
 HGF c - Met  
 HGF  
 가  
 (HGF) · c - Met

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