American Journal of Pathology, Vol. 159, No. 1, July 2001

Short Communication

Induction of c-Met Proto-Oncogene by Epstein-Barr Virus Latent Membrane Protein-1 and the Correlation with Cervical Lymph Node Metastasis of Nasopharyngeal Carcinoma

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Nasopharyngeal carcinoma (NPC) is distinctive in head and neck carcinomas for its close association with Epstein-Barr virus and its highly metastatic nature. Up-regulation of cell motility is essential for enhancement of metastatic potential. The expression of c-Met proto-oncogene, a high-affinity receptor for hepatocyte growth factor/scatter factor, has been reported to correlate with metastatic ability of the tumor cell. We observed close association of c-Met expression with cervical lymph node metastasis (P = 0.0272) in 39 NPC specimens studied immunohistochemically. Epstein-Barr virus-encoding latent membrane protein-1 (LMP-1) is a primary oncogene and is suggested to enhance the metastatic property of NPC. Previously, we reported that LMP-1 enhanced the motility of Madin-Darby canine kidney (MDCK) epithelial cells that was mediated by activation of Ets-1 transcription factor. Therefore, we examined the interrelationships of LMP-1, Ets-1, and c-Met. In immunohistochemical studies, the expression of LMP-1, Ets-1, and c-Met correlated significantly with each other in NPC (LMP-1 *versus* Ets-1, $P < 0.0001$; Ets-1 *versus* c-Met, $P = 0.0012$; LMP-1 *versus* Met, $P = 0.0005$). Transfection of LMP-1-expressing plasmid in MDCK cells induced c-Met protein expression. The c-Met protein was also induced by Ets-1 expression, and induction of c-Met by LMP-1 was suppressed by introducing a dominant-negative form of Ets-1 in LMP-1-expressing MDCK cells. These results suggest that LMP-1 induces c-Met through the activation of Ets-1, which may contribute in part to the highly metastatic potential of NPC. *(Am J Pathol 2001, 159:27–33)*

Nasopharyngeal carcinoma (NPC) frequently shows high metastasis in the early stage of disease.[1] Cervical lymph node metastasis is the most frequent clinical finding of NPC that often prompts patients to seek medical advice.[1] Another distinctive character of NPC in head and neck carcinomas is the consistent association of Epstein-Barr virus (EBV).[2] EBV is considered to be strongly associated with the oncogenesis of NPC.[2] However, the mechanism of NPC metastasis is primarily unknown.

In the sequential steps of metastasis, tumor cell motility is believed to be one of the indispensable capacities, especially for the locomotion of cells.[3,4] Prominent among cell motility-related molecules is the c-Met proto-oncogene product,[5,6] which is the receptor for hepatocyte growth factor/scatter factor.[7,8] c-Met has been shown to stimulate cell motility and invasion,[5,6] and has been reported to be associated with the progression and/or metastasis of a variety of carcinomas.[9–13] However, no study concerning c-Met in NPC has been reported to date to our knowledge.

Latent membrane protein-1 (LMP-1) is an EBV-encoding membrane protein, and one of four EBV gene products (LMP-1, LMP-2A, LMP-2B, and EBV nuclear antigen 1) expressed in NPC tumor cells in latent infection.[14] LMP-1 is considered to be the EBV oncoprotein according to the accumulating studies, such as immortalization of human B lymphocytes[15] and transformation of rodent fibroblasts[16] by LMP-1. In addition to oncogenesis,

Supported by a grant-in-aid for scientific research (no. 10470353) from the Ministry of Education, Science, and Culture of Japan.

Accepted for publication April 11, 2001.

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LMP-1 is suggested to be relevant to the metastatic property of NPC.[17–21] Type II and type III EBV related malignancies as represented on NPC, which express LMP-1, show metastatic phenotypes, whereas type I malignancies, such as Burkitt lymphoma and a subset of stomach carcinoma, which lack LMP-1 expression, are characterized by localized growth.[22] Also, studies, including ours, report that LMP-1-positive NPCs show a more progressive attitude and an increased tendency toward lymph node metastasis than LMP-1-negative NPCs.[18,23] Also, we have recently demonstrated that Madin-Darby canine kidney (MDCK) epithelial cells with LMP-1 expression show enhanced cell motility that is mediated by Ets-1 transcription factor.[17] Therefore, we focused on the contribution of c-Met to up-regulation of cell motility system and LMP-1-mediated metastasis in NPC. In this study, we examined the expressions of c-Met, LMP-1, and Ets-1 in patients with NPC by immunohistochemical analysis, and studied the associations of these proteins with clinical data. Furthermore, we investigated induction of c-Met by LMP-1 in cell line.

Materials and Methods

Patients and Tissues

Thirty-nine tumor specimens from patients with NPC who underwent treatment at National Taiwan University Hospital in 1997 were used. After tissue samples were obtained, they were embedded in optimal cutting temperature (OCT) compound (Miles, Elkhart, IN), frozen immediately, and stored at −80°C.

Immunohistochemical Staining

Five-μm-thick cryostat serial sections were prepared from frozen tissues. A procedure using a standard avidin-biotinylated peroxidase complex method (ABC method[24]) was performed, just as in our previous study.[18] Sections were incubated with antibodies to c-Met (clone C-28, dilution 1:25; Santa Cruz Biotechnology, Santa Cruz, CA), LMP-1 (clone CS1-4, dilution 1:25; DAKO, Copenhagen, Denmark), or Ets-1 (clone C-20, dilution 1:1000; Santa Cruz Biotechnology) at 4°C overnight. They were exposed to a universal secondary antibody (Research Genetics, Huntsville, AL) that contained biotinylated goat anti-mouse immunoglobulin (Ig) G and peroxidase-conjugated streptavidin, followed by 3,3′-diaminobenzidine tetrahydrochloride as a chromogen and hematoxylin as a nuclear counterstain. The specificity of the antibodies to c-Met, LMP-1, and Ets-1 that we used have been previously proven.[9,17,18,25] Thyroid papillary carcinoma was used as a positive control for c-Met[26] and Ets-1,[27] and previously identified LMP-1-positive NPC[18,28] was used as a positive control for LMP-1. The specificity of the staining was also confirmed with each batch of stains by the use of serial sections with nonimmune serum instead of the primary antibody as a negative control.

Evaluation of the Specimens

The specimens were evaluated independently by two of the authors (TH and TY) without previous knowledge of the clinical data, then reviewed by the others. Two examiners each selected two representative fields that contained >200 tumor cells and counted both the immunoreactive cells and the total number of tumor cells. A total of at least 400 cells were evaluated for each staining. The percentages of immunoreactive cells showed a wide range of expressions, from 0 to 100% with one peak <10% for c-Met, LMP-1, and Ets-1. Based on these data, the results for c-Met, LMP-1, and Ets-1 were classified into negative and positive categories: negative, <10% immunoreactive cells; positive, ≧10% immunoreactive cells. Also, the results for c-Met, LMP-1, and Ets-1 were classified into three scores depending on the percentage of immunoreactive cells: expression score 0, <10% immunoreactive cells; expression score 1, 10 to 50% immunoreactive cells; expression score 2, >50% immunoreactive cells, which is consistent with previously published reports.[19,27,29,30] The levels of concordance, which were expressed as the percentage of agreement of the two examiners' scores, were 92.3% (36 of 39 specimens) for c-Met and LMP-1 and 84.6% (33 of 39 specimens) for Ets-1. In the remaining specimens, the score was determined by discussion among the two examiners and the reviewers.

Plasmids and Expression Vectors

The pcDNA3 eukaryotic expression vector (Invitrogen, Carlsbad, CA) was used for the LMP-1 expression constructs.[17,19,20] Ets-1 expression plasmid was constructed by inserting full-length Ets-1 cDNA at the cloning site of pSG5 (Stratagene, La Jolla, CA).[31] The cDNA sequence encoding dominant-negative form of Ets-1 (Ets-DN) was obtained by polymerase chain reaction amplification of human Ets-1 cDNA.[17,31] The cDNA fragment thus obtained was inserted behind the ATG start codon introduced into pCEP4 expression vector (Invitrogen) .[17,31]

Cell Line and Stable Transfection

MDCK cells were obtained from Health Science Resources Bank (Osaka, Japan) and cultured in Dulbecco's modified Eagle's minimal essential medium supplemented with 5% fetal calf serum.[17,32] MDCK cells stably expressing LMP-1 were generated by transfecting pcDNA3 plasmid containing LMP-1 open reading frame, and were selected under 600 μg/ml of G418, as described previously.[17,32] MDCK cells stably expressing Ets-1 were generated by transfecting pSG5 containing Ets-1 cDNA, and selected under 600 μg/ml G418.[31] In addition, MDCK cells expressing LMP-1 were transfected with pCEP4 plasmid containing Ets-DN cDNA fragment, and were then selected under 800 μg/ml of hygromycin B.[17]

Table 1. Association of c-Met, LMP-1, and Ets-1 Expressions with Clinicopathological Factors

Factor	No.	c-Met score* (mean ± SD)	*P* value	LMP-1 score* (mean ± SD)	*P* value	Ets-1 score* (mean ± SD)	
Total patients	39	1.128 ± 0.833		0.949 ± 0.857		0.821 ± 0.790	
Gender							
Male	30	1.233 ± 0.817	0.177	0.967 ± 0.850	0.816	0.867 ± 0.776	0.494
Female	9	0.778 ± 0.833		0.889 ± 0.928		0.667 ± 0.866	
Age							
≧50 years	27	1.222 ± 0.847	0.301	0.963 ± 0.854	0.879	0.815 ± 0.786	0.964
<50 years	12	0.917 ± 0.793		0.917 ± 0.900		0.833 ± 0.835	
Histology (WHO type)							
Squamous cell carcinoma (I)	4	1.750 ± 0.500		0.750 ± 0.957		0.750 ± 0.957	
Nonkeratinizing carcinoma (II)	15	0.800 ± 0.775	0.105	1.000 ± 0.845	0.885	1.133 ± 0.834	0.199
Undifferentiated carcinoma (III)	20	1.250 ± 0.851		0.950 ± 0.887		0.600 ± 0.681	
T classification†							
T1, 2	22	1.182 ± 0.795	0.654	1.000 ± 0.873	0.692	0.909 ± 0.868	0.524
T3, 4	17	1.059 ± 0.899		0.882 ± 0.857		0.706 ± 0.686	
N classification‡							
N0	8	0.500 ± 0.756	0.0272	0.500 ± 0.926	0.110	0.375 ± 0.744	0.0790
N1, 2, 3	31	1.290 ± 0.783		1.065 ± 0.814		0.935 ± 0.772	
M classification§							
M0	38	1.105 ± 0.831	0.307	0.921 ± 0.850	0.248	0.737 ± 0.523	0.155
M1	1	2.0		2.0		2.0	
Stage grouping¶							
Stage I, II	14	1.000 ± 0.784	0.464	0.929 ± 0.917	0.907	0.929 ± 0.829	0.558
Stage III, IV	25	1.200 ± 0.866		0.960 ± 0.841		0.760 ± 0.779	

LMP-1, latent membrane protein-1; WHO, World Health Organization; SD, standard deviation.

*Score comes from the results of immunohistochemistry. Expression score 0, <10% immunoreactive tumor cells; expression score 1, 10 to 50% immunoreactive tumor cells; expression score 2, >50% immunoreactive tumor cells.

†T classification was divided into low T classification category [T1, T2a, T2b, tumor confined to nasopharynx (T1), tumor extends to soft tissue of oropharynx and/or nasal fossa with parapharyngeal extention (T2a), tumor extends to soft tissue of oropharynx and/or nasal fossa without parapharyngeal extention (T2b)] and high T classification category [T3, T4, tumor invades bony structures and/or paranasal sinuses (T3), tumor with intracranial extention and/or involvement of cranial nerves, infratemporal fossa, hypopharynx, or orbit (T4)].

‡N classification was divided into lymph node metastasis negative (N0; no regional lymph node metastasis) and lymph node metastasis positive categories [N1, N2, N3a, N3b; unilateral metastasis in lymph node(s), 6 cm or less in greatest dimension, above supraclavicular fossa (N1), bilateral metastasis in lymph node(s), 6 cm or less in greatest dimension, above supraclavicular fossa (N2), metastasis in lymph node(s) greater than 6-cm in dimension (N3a), metastasis in lymph node(s) in the supraclavicular fossa (N3b)].

§M classification was divided into distant metastasis negative [M0; no distant metastasis) and distant metastasis positive (M1: distant metastasis) categories.

¶Stage grouping was divided into early-stage category [Stage 1 (T1N0M0), Stage IIA (T2aN0M0), Stage IIB (T1N1M0, T2aN1M0, T2bN0M0, T2bN1M0)] and advanced-stage category [Stage III (T1N2M0, T2aN2M0, T2bN2M0, T3N0M0, T3N1M0, T3N2M0), Stage IVA (T4N0M0, T4N1M0, T4N2M0), Stage IVB (anyTN3M0), Stage IVC (AnyTAnyNM1)].

Western Blot Analysis

c-Met protein levels in MDCK cells were analyzed by Western blotting as described previously.[20,32] Ten μg of each cell lysate were solubilized to 7% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred to nitrocellulose membranes (Amersham, Buckinghamshire, UK) by wet blotting. The membranes were incubated with an antibody to c-Met (clone C-28, dilution 1:1000; Santa Cruz Biotechnology) for 2 hours. After incubation with horseradish peroxidase-conjugated anti-rabbit secondary antibody (dilution 1:2000, Amersham), reactive bands were visualized using the enhanced chemiluminescence system (Amersham). In addition, MKN-45 cell lysate was analyzed in parallel as a positive control of c-Met.[33] Expression of 63-kd LMP-1 protein in each cell was also detected with the antibody to LMP-1 (clone CS1-4, dilution 1:1000; DAKO), as previously described.[32] To verify the equal protein loading, the actin level in each lane was examined with the antibody to actin (dilution 1:1000; Biomedical Technologies Inc., Stoughton, MA).

Statistical Analysis

The data were analyzed using Macintosh personal computers (Apple Computer, Cupertino, CA) and Stat View software (Abacus Concepts, Inc., Berkeley, CA). The expressions of c-Met, LMP-1, and Ets-1 in relation to the clinicopathological data were analyzed using Mann-Whitney *U* test, Kruskal-Wallis rank test, or chi-square test. The correlations among c-Met, LMP-1, and Ets-1 were analyzed with chi-square test. *P* values of <0.05 were considered to be statistically significant.

Results

Expressions of c-Met, LMP-1, and Ets-1 Correlated with Cervical Lymph Node Metastasis in Patients with NPC

The expression of c-Met protein in NPC samples was immunohistochemically examined to investigate the association of c-Met protein with metastasis of NPC. The

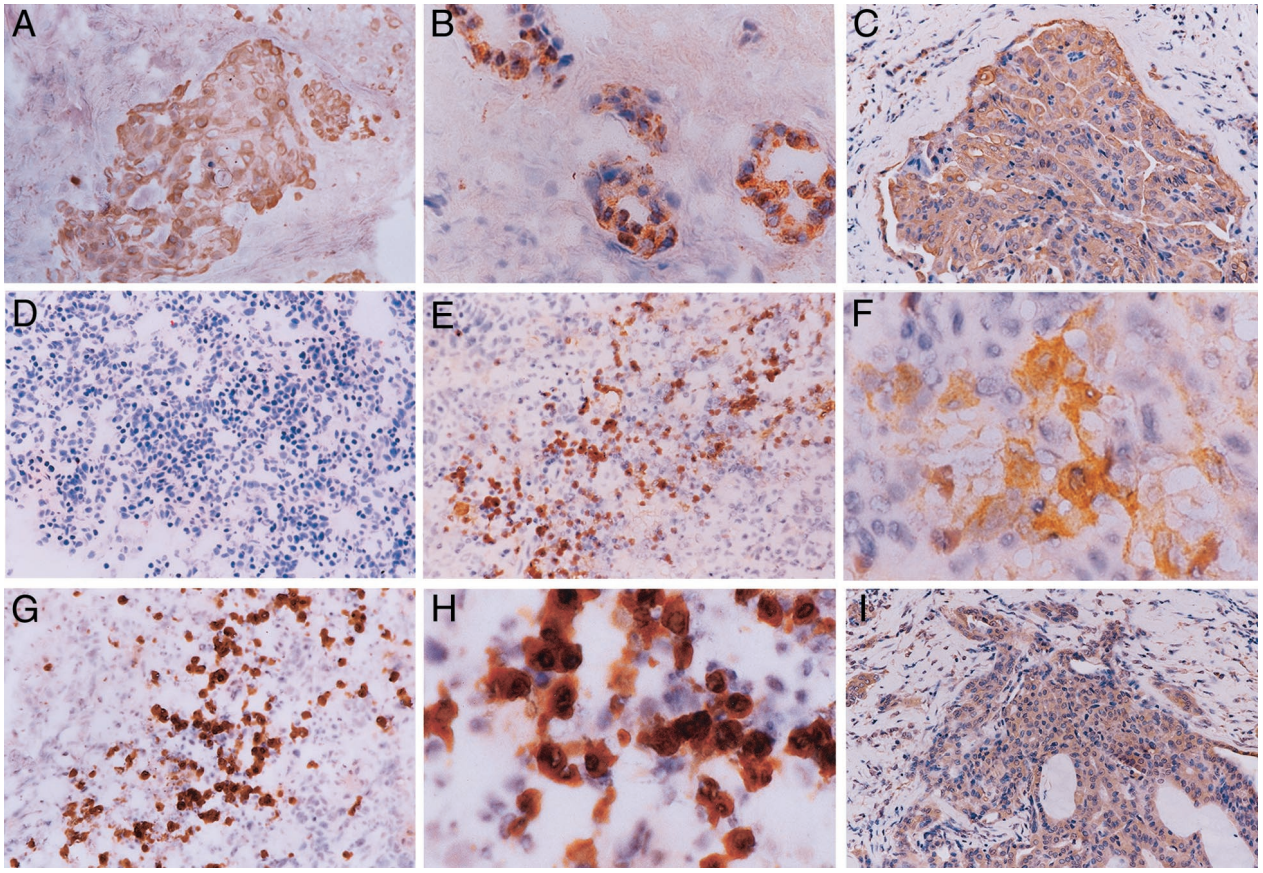

Figure 1. Expressions of c-Met, LMP-1, and Ets-1 by immunohistochemistry in NPC. **A:** Immunostaining for c-Met in tumor cells of NPC. c-Met protein is observed at the membrane and cytoplasm of tumor nests. Nonkeratinizing carcinoma (World Health Organization type II). **B:** Immunostaining for c-Met in microvessels of NPC. c-Met protein is observed in the endothelial cells of microvessels. Nonkeratinizing carcinoma (World Health Organization type II). **C:** Positive control for c-Met. Cytoplasmic immunostaining for c-Met is observed in tumor cells of thyroid papillary carcinoma. **D:** Negative control for c-Met. The sequential section of **A** shows no staining, in which nonimmune serum was used instead of anti-c-Met antibody. **E:** Immunostaining for LMP-1 in tumor cells of NPC. Nonkeratinizing carcinoma (World Health Organization type II). **F:** Immunostaining for LMP-1 in NPC in a larger magnification. LMP-1 protein is detected at the membrane and cytoplasm of tumor cells. Nonkeratinizing carcinoma (World Health Organization type II). **G:** Immunostaining for Ets-1 in tumor cells of NPC. Nonkeratinizing carcinoma (World Health Organization type II). **H:** Immunostaining for Ets-1 in NPC in a larger magnification. Strong immunoreaction for Ets-1 is observed at the nucleus and cytoplasm of tumor cells in NPC. Nonkeratinizing carcinoma (World Health Organization type II). **I:** Positive control for Ets-1. Cytoplasmic immunostaining for Ets-1 is observed in tumor cells of thyroid papillary carcinoma. Original magnifications: ×400 (**A**, **C–E**, **G**, and **I**); ×600 (**B**); ×800 (**F** and **H**).

clinical staging of NPC was performed based on the International Union Against Cancer TNM classification and stage grouping (fifth edition 1997). As summarized in Table 1, each TNM classification and stage grouping was divided into two categories, according to the method used in our previous study.[18] The c-Met protein was immunolocalized on the cell membrane and cytoplasm of the tumor cells (Figure 1A). Some endothelial cells of microvessels were also immunostained for c-Met (Figure 1B). The thyroid carcinoma section that was used as a positive control for c-Met immunostaining showed a similar immunostaining signal to the examined NPC specimens (Figure 1C). The section incubated with nonimmune serum instead of the primary antibody showed no staining (Figure 1D). In 30 cases with lymph node metastasis (N1, N2, N3), the mean c-Met expression score was 1.290 ± 0.783 (the mean ± SD). In eight cases without lymph node metastasis (N0), the score was 0.500 ± 0.756. Thus, the c-Met expression score in lymph node metastasis-positive category was significantly higher than that of the lymph node metastasis-negative category (P = 0.0272) (Figure 2). The LMP-1

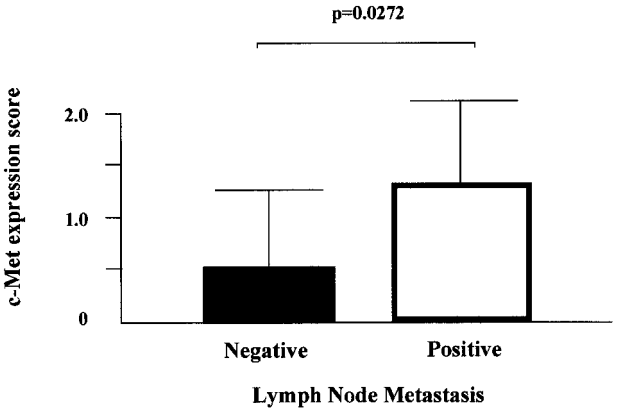

Figure 2. The mean expression scores of c-Met in 39 cases of NPC in relation to lymph node metastasis (columns, mean; bars, SD). The results of immunohistochemistry were classified into three scores as follows depending on the percentage of immunoreactive tumor cells: expression score 0, <10% immunoreactive cells; expression score 1, 10 to 50% immunoreactive cells; expression score 2, >50% immunoreactive cells. The mean expression score in lymph node metastasis-positive category (mean ± SD; 1.290 ± 0.783) was higher than that in lymph node metastasis-negative category (0.500 ± 0.756). Thus, c-Met expression significantly correlated positively with lymph node metastasis by the Mann-Whitney U test (P = 0.0272).

Table 2. Correlations of c-Met, LMP-1, and Ets-1 Expressions with Lymph Node Metastasis in Nasopharyngeal Carcinoma

		c-Met			LMP-1			Ets-1		
	Total	Negative (<10%)	Positive (≧10%)	*P* value	Negative (<10%)	Positive (≧10%)	*P* value	Negative (<10%)	Positive (≧10%)	*P* value
Lymph Node Metastasis										
Negative	8	5 (12.8%)	3 (7.7%)	0.0156	6 (15.4%)	2 (5.1%)	0.0172	6 (15.4%)	2 (5.1%)	0.0284
Positive	31	6 (15.4%)	25 (64.1%)		9 (23.1%)	22 (56.4%)		10 (25.6%)	21 (53.8%)	

LMP-1: latent membrane protein-1. The results for c-Met, LMP-1, and Ets-1 were classified into negative and positive categories depending on the percentage of immunoreactive tumor cells: negative, <10% immunoreactive cells; positive, ≧10% immunoreactive cells.

protein was detected on the membrane and cytoplasm of the tumor cells (Figure 1, E and F), which was consistent with previous reports.[18,26,34] The Ets-1 protein was observed mainly at the nucleus, and also occasionally on the cytoplasm, of the tumor cells (Figure 1, G and H).[17] Infiltrating round cells in the stroma surrounding tumor nests were also occasionally immunostained for Ets-1. The thyroid carcinoma section that was used as a positive control for Ets-1 immunostaining showed a cytoplasmic immunostaining (Figure 1I), as reported previously.[27] The expression scores of LMP-1 and Ets-1 in the lymph node metastasis-positive category (1.065 ± 0.814 for LMP-1 and 0.935 ± 0.772 for Ets-1) were higher than those of the lymph node metastasis-negative category (0.500 ± 0.926 for LMP-1 and 0.375 ± 0.744 for Ets-1), although the differences were not statistically significant. By the negative-positive classification, c-Met, LMP-1, and Ets-1 each significantly correlated with lymph node metastasis, as shown in Table 2 ($P = 0.0156$, 0.0172, and 0.0284, respectively). These results suggest that c-Met, LMP-1, and Ets-1 may contribute to cervical lymph node metastasis of NPC.

Relationship of c-Met with LMP-1 and Ets-1 in NPC

Previously, the contribution of LMP-1 to metastasis of NPC[18] and Ets-1 mediated up-regulation of MDCK cell motility by LMP-1[17] was reported. The relationship of c-Met with LMP-1 and Ets-1 in NPC was examined because the major role of c-Met is considered to be up-regulation of cell motility.[5,6] c-Met expression was negative (score 0) in 11 cases (28.2%) and positive in 28 cases [71.8%; score 1 in 12 cases (30.8%) and score 2 in 16 cases (41.0%)] of a total of 39 cases. LMP-1 expression was negative (score 0) in 15 cases (38.5%), and positive in 24 cases (61.5%) [score 1 in 11 cases (28.2%) and score 2 in 13 cases (33.3%)]. Ets-1 expression was negative (score 0) in 16 cases (41.0%), and positive in 23 cases [59.0%; score 1 in 14 cases (35.9%) and score 2 in 9 cases (23.1%)]. As shown in Table 3, the expressions of LMP-1, Ets-1, and c-Met were significantly correlated to each other by the negative-positive classification (LMP-1 *versus* Ets-1, $P < 0.0001$; Ets-1 *versus* c-Met, $P = 0.0012$;

Table 3. Interrelationships of LMP-1, Ets-1, and c-Met Expressions

Correlation between LMP-1 and Ets-1 expressions

		Ets-1		
		Negative (<10%)	Positive (≧10%)	Total
LMP-1	Negative (<10%)	12 (30.8%)	3 (7.7%)	15 (38.5%)
	Positive (≧10%)	4 (10.3%)	20 (51.3%)	24 (61.5%)
	Total	16 (41.0%)	23 (59.0%)	39 (100%)

Correlation between Ets-1 and c-Met expressions

		c-Met		
		Negative (<10%)	Positive (≧10%)	Total
Ets-1	Negative (<10%)	9 (23.1%)	7 (17.9%)	16 (41.0%)
	Positive (≧10%)	2 (5.1%)	21 (53.8%)	23 (59.0%)
	Total	11 (28.2%)	28 (71.8%)	39 (100%)

Correlation between LMP-1 and c-Met expressions

		c-Met		
		Negative (<10%)	Positive (≧10%)	Total
LMP-1	Negative (<10%)	9 (23.1%)	6 (15.4%)	15 (38.5%)
	Positive (≧10%)	2 (5.1%)	22 (56.4%)	24 (61.5%)
	Total	11 (28.2%)	28 (71.8%)	39 (100%)

The results for c-Met, LMP-1, and Ets-1 were classified into negative and positive categories depending on the percentage of immunoreactive tumor cells: negative, <10% immunoreactive cells; positive, ≧10% immunoreactive cells.
LMP-1, latent membrane protein-1.

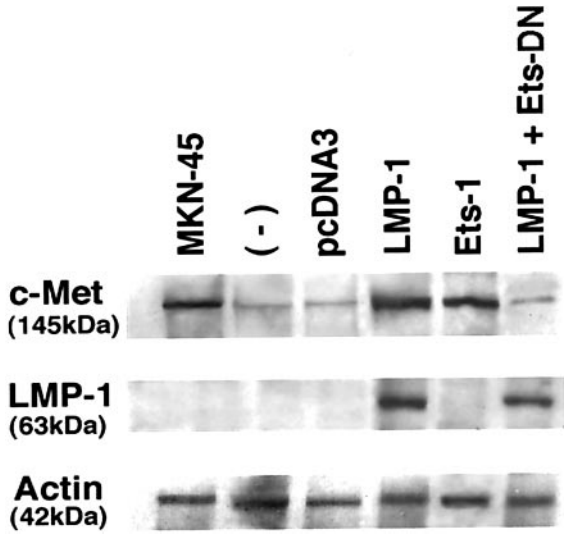

Figure 3. Western blot analysis displaying c-Met protein induction in MDCK epithelial cells. The lysates of MDCK cells transfected with pcDNA3, LMP-1, Ets-1, and LMP-1+ dominant-negative form of Ets-1 (EtsDN) are loaded on corresponding lanes, and examined by Western blotting with antibodies to c-Met, LMP-1, and actin as described in Materials and Methods. c-Met protein was detected as a 145-kd band. **Lane MKN-45**, MKN-45 cell used as a positive control; **Lane (−)**, MDCK cell without any plasmid; **Lane pcDNA3**, MDCK cell transfected with control plasmid; **Lane LMP-1**, LMP-1-transfected MDCK cell; **Lane Ets-1**, Ets-1-transfected MDCK cell; **Lane LMP-1+EtsDN**, LMP-1-transfected cell that was co-transfected with EtsDN. These experiments were repeated several times with similar results; representative blots are shown.

LMP-1 *versus* Met, $P = 0.0005$). By score classification, their expressions were also significantly correlated to each other (LMP-1 *versus* Ets-1, $P = 0.0025$; Ets-1 *versus* c-Met, $P = 0.0246$; LMP-1 *versus* Met, $P = 0.0043$).

Induction of c-Met by LMP-1

The data obtained from immunohistochemistry suggested close association of c-Met with LMP-1 and Ets-1. Therefore, we investigated if LMP-1 could induce c-Met protein by Western blot analysis. As demonstrated in Figure 3, trace amounts of c-Met protein at the molecular weights of 145 kd were detected in the parental untransfected MDCK cells and in the cells transfected with control plasmid. However, c-Met protein was clearly detected in LMP-1-transfected cells, indicating induction of c-Met protein by LMP-1. Transfection of Ets-1 expression in MDCK cells also induced c-Met protein. Previously, we reported induction of Ets-1 by LMP-1 in MDCK cells. Hence we examined whether induction of c-Met by LMP-1 is suppressed by inhibiting Ets-1 signal pathway by transfecting Ets-DN, which lacks a transactivation domain and competitively inhibits Ets-1 function, in LMP-1-expressing MDCK cells. Interestingly, co-transfection of Ets-DN in LMP-1-transfected MDCK cells suppressed induction of c-Met protein. These results suggest that induction of c-Met by LMP-1 is mediated by activation of Ets-1.

Discussion

Up-regulation of cell motility is considered to be an essential factor in the multiple steps of metastasis.[4] Hepatocyte growth factor is a prominent growth factor that enhances cell motility.[5,6] Hepatocyte growth factor is not produced by the tumor but by the stroma surrounding tumor nests.[12,13] The c-Met protein is a hepatocyte growth factor receptor,[7,8] which is expressed on tumor cells.[9–13] Thus, c-Met expression of the tumor plays an important role for tumor cell motility, and as a consequence, influences the metastatic ability of the tumor.[10] As expected, c-Met expression positively correlated with cervical lymph node metastasis in patients with NPC. Therefore, contribution of c-Met expression in the metastatic potential of NPC is suggested.

LMP-1 is of particular interest because it resembles a classical oncogene in its ability to transform rodent fibroblast cell lines.[16] Expression of this viral oncogene in B-cell lines up-regulates CD23, CD40, CD54, bcl-2, and A20 genes.[15,35,36] In epithelical cells, LMP-1 induces expression of the epidermal growth factor receptor and A20 genes.[37] In most cases, effects of LMP-1 are mediated through the activation of nuclear factor-κB and AP-1 by C-terminal activation region-1 and -2.[38,39] We previously reported that LMP-1 induces matrix metalloproteinase-9,[19,20] which destructs the basement membrane,[40] mainly through nuclear factor-κB. Moreover, LMP-1 enhances the motility of MDCK cells through the induction of Ets-1.[17] Hence, the relationship of LMP-1, Ets-1, and c-Met expression with each other in NPC was examined. Immunohistochemical data suggested a close association of LMP-1, Ets-1, and c-Met with each other in NPC specimens.

Although the number of immunohistochemically examined NPC specimens may not be conclusive, the Western blotting data clearly indicated induction of c-Met by LMP-1 in MDCK cells. Ets-1 induction by LMP-1 in MDCK cells was demonstrated previously.[17] In this study, expression of c-Met is also enhanced by induction of Ets-1. Moreover, induction of c-Met by LMP-1 is inhibited by co-transfection of Ets-DN expression plasmid. These results suggest that LMP-1 induces c-Met through the activation of Ets-1.

LMP-1 is reported to be detected in ~60% of invasive NPCs.[18,23,34,41] Interestingly, according to Pathmanathan and colleagues,[42] LMP-1 was detected in all pre-invasive NPCs that quickly developed into invasive NPCs. Thus, LMP-1-mediated enhancement of metastatic potential could be an early event in NPC, although regulation of LMP-1 expression in NPC remains to be elucidated. Our data, suggesting induction of c-Met by LMP-1, further supports the hypothesis that LMP-1 contributes to the highly metastatic nature of NPC.

Acknowledgments

We thank Dr. Hirofumi Nagase (Department of Public Health, School of Medicine, Kanazawa University) for help with statistical analysis of the data; and Professor Shiann-Yann Lee (Department of Otolaryngology, School of Medicine, National Taiwan University) for the conduct of this collaborative work.

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