

The modified Immunomax of HTLV-1 tax protein can label HTLV-1-related cases in T-cell lymphomas

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(32) TBE MODIFIED IMMUNOMAX OF HTLV-I TAX PROTEIN CAN LABEL HTLV-I-RELATED CASES IN T-CELL LYMPHOMAS

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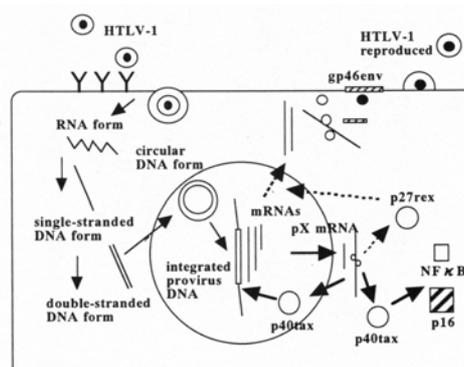
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Introduction

Adult T-cell leukemia/lymphoma (ATLL) is the representative neoplastic disease related to HTLV-1 infection¹. In the recent years molecular mechanism of HTLV-1 oncogenesis has been clarified^{2,3}. Figure 1 indicates the synthesis of HTLV-1-related proteins in the HTLV-1 life-cycle⁴. One of the HTLV-1 pX proteins, p40tax: is thought to play the most important role in the oncogenesis of ATLL², trans-activating the host cell genes and interacting with cytoplasmic proteins such as p16³ and NF- κ B. On the other hand, p27rex modulates the activation of the proviral DNA to reproduce HTLV-1². In the processes of the HTLV-1 reproduction, glycoprotein (gp) 46 (gp46env) is synthesized to constitute the envelope of HTLV-1.

Figure 1. Synthesis of HTLV-1-related proteins in the life cycle of HTLV-1

Based on the pX mRNA from the integrated proviral DNA, p40tax (Tax), p27rex (Rex) and p21 are synthesized. Tax exists in cytoplasm to interact with several proteins such as p16 and NF- κ B and in nucleus to reproduce itself and to trans-activate several host cell genes. Rex modulates activation of the proviral DNA to reproduce HTLV-1. Glycoprotein 46env (gp46env) and its precursor appear in the processes of the HTLV-1 reproduction and finally constitute the envelope of HTLV-1.



We must to characterize HTLV-1-related lymphomas including ATLL in malignant lymphomas (MLs) in a HTLV-1 endemic area, although some histopathological features of ATLL helped to differentiate typical pleomorphic cases from the others⁵. At first, we developed in-situ-hybridization (ISH) of mRNAs of the HTLV-1 proviral DNA, preparing highly biotinylated probes by means of polymerase chain reaction (PCR)^{6,7}. Next, we tried to detect gp46env by means of Elite-ABC of monoclonal antibody (MoAb) 6C2, which was supplied commercially to detect the gp46env even on paraffin sections. As shown in table 1, MoAb 6C2 labeled lymphoma cells in 78 out of 112 T-cell ML and reacted the cell membrane of lymphoma cells in 44% case of the typical ATLL type and in 25% cases of low-grade T-cell MLs. And we have been investigating immunohistochemistry of HTLV-1 p40tax and the others HTLV-1-related proteins, introducing antigen-retrieval treatments. As shown in table 1, the antigen-retrieval Elite ABC of MoAb Lt-4 could label lymphoma cells in 54 cases (63%) of 86 T-cell MLs, in 43 cases (67%) of 64 T-Pleomorphic lymphoma and anaplastic large cell lymphomas. But nuclear stain of Lt-4 was seen only in 17 (31%) of 86 T-MLs and 8 (29%) of 28 ATLL type. Comparison of the ISH of mRNA of HTLV-1 pX with the Elite ABC of gp46env (6C2) or the antigen-retrieval Elite-ABC of p40tax (Lt-4) in T-cell MLs is shown in Table 2. Twenty six cases (69%) of HTLV-1 pX-mRNA ISH-negative 38 T-MLs showed positive lymphoma cells in the Elite ABC of 6C2, suggesting that the detection sensitivity of the ISH was not enough to detect the gag-pol mRNA (the full-sized mRNA of HTLV-1 proviral DNA) and the env mRNA. HTLV-1 mRNAs other than the pX mRNA. Nineteen cases (54%) of 35 pX-mRNA

Table 1. HTLV-1 gp46env (MoAb 6C2, Elite ABC) and p40tax (MoAb Lt-4, antigen-retrieval Elite ABC) in T-cell malignant lymphomas in HTLV-1-endemic area, Kagoshima, in Japan

Subtype	Number of gp46env-positive cases		Number of p40tax-positive cases	
	Positive/Examined	Cell-membrane-positive cases /positive cases	Positive/Examined	Nucleus-positive cases /Positive
Total of T-cell lymphomas	78/112 (70%)	35/78 (45%)	54/86 (63%)	17/54 (31%)
High grade cases	65/86 (76%)	31/65 (48%)	44/66 (67%)	12/44 (27%)
T-lymphoblastic	2/7 (29%)	0/2 (0%)	1/2 (50%)	0/1 (0%)
T-Pleomorphic and ALC	63/79 (80%)	31/63 (49%)	43/64 (67%)	12/43 (28%)
ATLL type	34/39 (87%)	15/34 (44%)	21/28 (75%)	8/21 (38%)
Clear cell type	15/19 (79%)	7/15 (46%)	11/19 (58%)	2/11 (18%)
Others	14/21 (67%)	9/14 (64%)	11/17 (65%)	2/11 (18%)
Low grade cases	16/31 (52%)	4/16 (25%)	10/20 (50%)	5/10 (50%)
Chronic lymphocytic leukemia	1/2 (50%)	0/1 (0%)	0/1 (0%)	-
T-zone lymphoma	8/14 (57%)	2/8 (25%)	4/7 (57%)	2/4 (50%)
Lymphoepithelioid cell type	2/4 (50%)	1/2 (50%)	3/3 (100%)	1/3 (33%)
AILD type	5/11 (45%)	1/5 (20%)	3/9 (33%)	2/3 (66%)

Table 2. Detection of pX Tax/Rex mRNA, glycoprotein 46 env (gp46env) and pX Tax protein (p40tax) in T-cell malignant lymphomas in a HTLV-1-endemic area, Kagoshima, in Japan

pX Tax/Rex mRNA (in-situ-hybridization)	Numbers of T-cell malignant lymphomas						
	Glycoprotein 46env (gp46env) (Elite-ABC)			pX Tax protein (p40tax) Antigen-retrieval Elite ABC			
	Not-detected	Detected	Detected on cell-membrane	Not-detected	Detected	Detected in nuclei	
Total	not-detected	12	26	15 (58%)	16	19	5 (26%)
	detected	15	35	20 (57%)	12	28	9 (32%)
ATLL	not-detected	0	1	0 (0%)	0	0	0 (0%)
	detected	3	20	14 (70%)	6	17	6 (35%)

(%): Percent of cases of which lymphoma cells showed gp46env-positive stain on cell membrane or p40tax-positive stain on nuclei.

ISH-negative T-MLs showed positive lymphoma cells in the antigen-retrieval Elite ABC of Lt-4 suggesting that the sensitivity detecting activation of proviral DNA is much higher in the antigen-retrieval Elite ABC of p40tax than in the ISH of pX-mRNA. But Lt-4 labeled only 17 (74%) of 23 typical ATLL type and showed nuclear stain only in 6 cases (35%) of them. It is necessary to make higher detection sensitivity of the histochemistry labeling HTLV-1-related Mb.

Introducing and improving the ImmunoMax method reported Merz and Feiler et al^{19,10} we succeeded to establish the modified ImmunoMax 1,000 times more sensitive than the Elite ABC. The detail of the routine and the quantitative procedures of the modified ImmunoMax are reported¹¹. Here, we reported the way to see which one of the three MoAbs (Lt.4 WATM-1 and WATM-3) against HTLV-1 p40tax is the best in the modified ImmunoMax to label HTLV-1-related T-MLs.

Materials and Methods

Two lymph nodes of HTLV-1-associated non-neoplastic lymphadenopathy (tNNLA)¹² and one lymph node of ATLL (T-cell pleomorphic lymphoma, large cell type) were employed for quantitative analysis of antigens labeled by MoAbs. For the routine staining of the modified ImmunoMax of MoAbs, lymph node and skin lesions of 15 T-cell MLs were employed. The T-cell MLs comprised 8 T-cell pleomorphic lymphomas (T-Pleo.), 2 immunoblastic lymphadenopathy (IBL)-like T-MLs and 5 anaplastic large cell lymphomas (ALCs).

HTLV-1 infection was examined by means of polymerase chain reaction (PCR) of the primers of SK43 and 44 for HTLV-1 proviral DNA pX region. DNA was extracted from the sections, digesting dewaxed tissue in proteinase K solution at 37 °C for overnight and destroying the activity of proteinase K by incubating in boiling water for 3 min. The supernate (5 µl) was used as the DNA template for the PCR. It was examined by the PCR of the primers of GH20 and 21 for human β -globin (HBG) gene whether the template DNA included an enough amount of DNA for PCR¹¹. The PCR was performed in the

protocol comprising pre-denature at 94 °C for 5 min., 30 cycles of denature at 94 °C for 30 sec., annealing at 50 °C for 30 sec. for SK43-44 or at 55 °C for 30 sec. for GH20-21, and extension at 72 °C for 30 sec., and post-extension at 72 °C for 5 min.

MoAbs employed in this study were mouse MoAb Lt-4, rat MoAbs WATM-1 and WATM-3 against HTLV-1 pX p40tax¹³. Reaction dilution of these MoAbs was determined, by testing the serially diluted MoAb solutions on MT-2 cell block sections by the following modified ImmunoMax. The three MoAbs were diluted at 1:1,000 by BSA-PBS solution including rabbit serum (7:1).

Detailed procedures of the quantitative and the routine methods of the modified ImmunoMax¹¹ are indicated in Table 3. Dewaxed sections were processed by antigen-retrieval treatment heating sections in 4M urea solution at more than 100 °C for 5 min. after boiling by means of a high pressure cookpot¹⁴. In the quantitative modified ImmunoMax, the following processes were performed by a capillary method (MicroProbe, Fisher Scientific), controlling reaction time and temperature. After incubating sections in 3% H₂O₂ PBS for 20 min. to destroy activity of endogenous peroxidase, blocking of non-specific protein binding, reaction of MoAb and endogenous biotin masking (DAKO Biotin Blocking System), DAKO catalyzed signal amplification system (CSA) was performed. Coloration of the reaction products was performed in DAB-H₂O₂ Peroxidase reaction for 5 min. Nuclei were stained by methyl green.

Table 3. Procedure of the modified ImmunoMax

- 1, Dewax
- 2, Antigen retrieval
- 3, Destroying endogenous peroxidase activity
 - a) Incubate in 3% H₂O₂ PBS For 20 min. at room temperature
 - b) Wash 3 times by water and Wash (#1)
- 4, Blocking non-specific binding of primary antibody to proteins
 - a) Incubate in 2% casein PBS including horse serum (80JLLI for 1 ml) for mouse primary antibody for 30 min. at room temperature (% 1)
 - b) Remove excess the solution and no wash
- 5, Primary antibody reaction. Dilute primary antibody in BSA-PBS including rabbit serum (7:1) incubate (&1 or &2) and Wash(#1)
- 6, Endogenous biotin masking PAKO Endogenous Biotin Blocking Kit
 - a) Incubate in 0.1% avidin Tris including 0.03M NaCl for 15 min. at room temperature (%1) and Wash (#1)
 - b) Incubate in 0.01% biotin Tris including 0.03M NaCl for 15 min. at room temperature (%1) and Wash (#1)
- 7, Secondary antibody reaction for 30 min. to 1 hour at room temperature (%2) and Wash (#1)
- 8, Streptavidin-peroxidase complex reaction for 15 min. at room temperature (%2) and Wash (#1)
- 9, Catalyze reaction of biotinylated tyramide for 15 min. at room temperature and Wash (#2)
- 10, Streptavidin-peroxidase complex reaction for 15 min. at room temperature (%2) and Wash (#2)
- 11, Development of DAB-H₂O₂ Peroxidase reaction for 5 min. at room temperature and wash in water several times
- 12, Nuclear counter stain by methyl-green
- 13, Dehydration and mount in plastic medium

Quantitative procedure should be performed by capillary method such as MicroProbe. In the routine procedure, demarcate the area where should be stained by DAKO pen in order to avoid dryness of the section in the reaction just after the wash by water in the step 3b.

Tris: 0.1% Tween 20 or 0.1% Triton X100 Tris

Primary antibody reaction:

For routine procedure, Incubate (&1): Incubate sections overnight at 4 °C

For quantitative procedure by MicroProbe.

Incubate (&2): Incubate sections for 30 min. at 60°C or for 1 hour at the room temperature.

Wash:

For routine procedure,

Wash (#1): Wash in Tris for 5 min. 3 times at 35 ~40 °C

Wash (#2): Wash in Tris for 5 min. 3 times at room temperature

For quantitative procedure,

Wash (#1).. 3 cycles of rinse in Tris 3 times and hold in Tris for 1 min. at 40 or 60 °C

Wash (#2): 3 cycles of rinse in Tris 3 times and hold in Tris for 1 min. at room temperature

For quantitative procedure

%1: For 10 min. at 60 °C or as indicated.

%2: For 5 min. at 60 °C or as indicated.

Quantitative evaluation of the modified ImmunoMax stain was performed on color slide photos. Color image of a unit area was transferred to the computer image by a slide scanner. The computer image was divided into R (red)-G (green)-B (blue) images under channel division function of Adobe Photoshop. On the R image nuclei (cells) were counted. On the B image DAB-brown colored stain was evaluated in area and mean density under density slice function of NIH-Image. The estimated antigen labeled by MoAb was product of the area and the mean density per one cell according to an arbitrary unit.

Result

In two cases of HANNLA lymphocytes labeled by these MoAbs located in the developing paracortex. Figure 2 shows amount of antigens labeled by the three MoAbs per one cell in two cases of HANNLA and one case of ATLL. HANNLA 1 was the lesion with developing paracortex and HANNLA 2 was the lesion with developed paracortex¹⁰. Antigens labeled by Lt-4 and WATM-1 increased gradually in the order of HANNLA 1, HANNLA 2 and ATLL. Antigen labeled by WATM-3 showed different tendency from those of Lt-4 and WATM-1. The amount of antigen labeled by WATM-1 was the smallest in each of the three lesions.

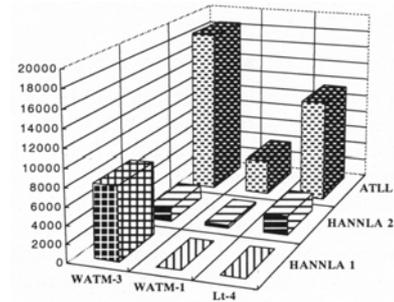


Figure 2. Immunoquantitative evaluation of the quantitative modified ImmunoMax of the monoclonal antibodies, Lt-4, WATM-1 and WATM-3 against HTLV-1 p40tax in 2 cases of HTLV-1-associated non-neoplastic lymphadenopathy (HANNLA) and ATLL

Table 4. Relationship between HTLV-1 infection and the modified ImmunoMax of HTLV-1 p40tax in 15 cases of T-cell malignant lymphomas

Case	PCR analysis of HTLV-1 infection		The modified ImmunoMax of HTLV-1 pX Tax protein (p40tax)	
	HBG	HTLV-1 pX	Lt-4	WATM-1
1, T-Pleo. S	amplified	amplified	+/-	+/-
4, T-Pleo. M/L	amplified	amplified	+/-	+/-
8, T-Pleo. L	amplified	amplified	+	+
9, IBL-like T-ML	Not	amplified	+	+
14, ALC	amplified	amplified	++	+
3, T-Pleo. M/L	amplified	amplified	++	++
10, IBL-like T-ML	Not	amplified	++	++
11, ALC	amplified	amplified	++(N++)	-
6, T-Pleo. L	amplified	amplified	++(N++)	+/-
7, T-Pleo. L	amplified	amplified	++(N++)	+/-
12, ALC	Not	amplified	++(N++)	+
13, ALC	amplified	amplified	++(N++)	+
15, ALC	amplified	amplified	++(N++)	+
5, T-Pleo. L	amplified	amplified	++(N++)	++
2, T-Pleo. M/L	amplified	Not	++(N++)	-

HBG: Human β -globin gene

amplified: Amplified DNA band was seen at the expected length.

Not: Amplified DNA band was not seen at the expected length.

Not in HBG column: By 2 times of PCR employing the same primers and 1:1,000 diluted product of the first PCR, a small amount of DNA extracted from the paraffin section was recognized.

The evaluation of the stain of the modified ImmunoMax

-: No stain.

+/-: Many weakly positive lymphoma cells.

+: Many positive lymphoma cells.

++: Many strongly positive lymphoma cells.

(N++): Many strongly positive nuclei of lymphoma cells

Table 4 shows the result of PCR analysis of HTLV-1 infection and the modified ImmunoMax of Lt-4 and WATM-1 in 15 cases of T-cell MLs. There were three cases showing no bands at the expected length in the electrophoresis of the PCR of HBG and revealing the amplified DNA in that of the PCR of HTLV-1 pX region. In spite of a small amount of the extracted DNA recognized by means of 2 times PCR of HBG

analyzing 1:1,000 diluted product of the first PCR, proviral DNA pX region detected by the PCR indicated that these T-cell MLs were also HTLV-1-related T-cell MLs. In 7 of the 14 T-MLs with HTLV-1 infection proved by PCR analysis, Lt-4 stained strongly cytoplasm and nuclei of lymphoma cells and nuclei of squamous cell. But WATM-1 did not stain nuclei of the squamous cells and showed various positive figures of lymphoma cells from almost none to strongly positive stain. In the other 7 of the 14 T-MLs the reaction of Lt-4 with lymphoma cells was parallel to that of WATM-1. In one case (the case 2) of T-MLs without HTLV-1 infection, Lt-4 reacted strongly with cytoplasm and nuclei of the lymphoma cells but WATM-1 did not.

Figure 3 shows the modified ImmunoMax of Lt-4 and WATM-1 in the case 5, T-cell Pleomorphic lymphoma large cell type. (Table 4). Lt-4 stained lymphoma cells and nuclei of epidermal squamous cells (Fig. 3a). WATM-1 stained dominantly cytoplasm of the lymphoma cells. Spotty stain of WATM-1 was seen in nuclei of a few lymphoma cells (Fig. 3b).

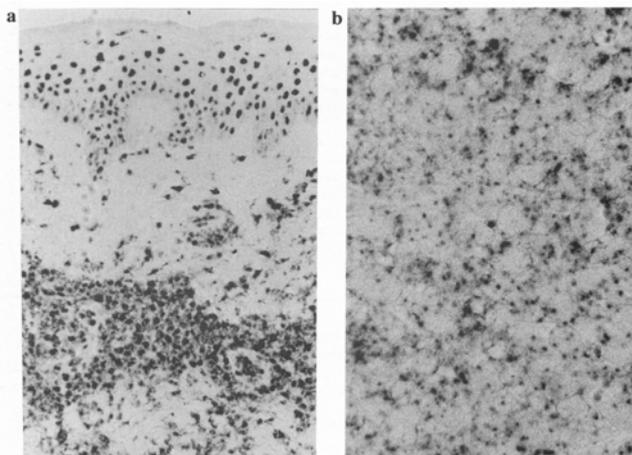


Figure 3. The modified ImmunoMax of anti-p40tax MoAbs, Lt-4 and WATM-1, in the case 5, T-cell pleomorphic lymphoma large cell type with HTLV-1 infection.

a) Lt-4. 3dO. Lymphoma cells show infiltrative proliferation in the dermis. Lt-4 reacted strongly nuclei of the lymphoma cells and the epidermal squamous cells. But weak stain of Lt-4 is also seen in the cytoplasm of the lymphoma cells.

b) WATM-1. x300. WATM-1 showed spotty stain dominantly in the cytoplasm of the lymphoma cells.

Figure 4 showed various stain of the modified ImmunoMax of WATM-1 in the T-cell MLs. In the case 2, HTLV-1-not-related T-cell pleomorphic medium-sized and large cell type, WATM-1 stained no lymphoma cells and showed only weak background spotty stain (Fig. 4a). In the case 11, HTLV-1-related T-cell anaplastic large cell lymphoma, a few large to giant cells showed extremely too fine spotty stains in nuclei to be evaluated as positive (Fig. 4b). In the case 1, HTLV-1-related T-cell pleomorphic lymphoma small cell type, the modified ImmunoMax of WATM-1 showed weak stains in many lymphoma cells (Fig. 4c) in comparison with the strong stains in the case 5, HTLV-related T-cell pleomorphic lymphoma large cell type (Fig. 4d).

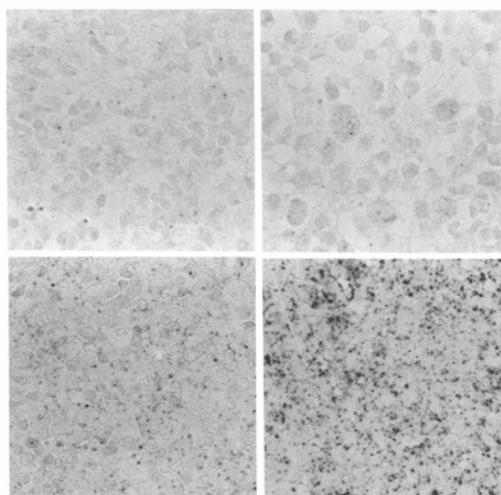


Figure 4. The modified ImmunoMax of anti-p40tax MoAb WATM-1

a) x300. No obvious stains except non-specific background spotty reaction in the case 2, T-cell pleomorphic lymphoma medium-sized and large cell type.

b) x300. Intranuclear weak and fine stains in lymphoma cells of the case 11, T-cell anaplastic large cell lymphoma.

c) x300. Weak stains in lymphoma cells of the case 1, T-cell pleomorphic lymphoma small cell type.

d) x300. Strong stains in lymphoma cells of the case 5, T-cell pleomorphic lymphoma large cell type.

Discussion

Immunohistochemistry (IHC) depends on the specific distribution of antigen labeled by MoAb in the target protein. The control stain of the IHC is necessary to see the specific distribution of the antigen in the target protein. But sometimes the amount of the target protein is quite too small to be detected by the routine IHC such as ABC, Elite-ABC and streptavidin biotin complex (sABC) method. Introducing to the routine IHC the antigen retrieval method^{8,14} and the ImmunoMax^{9,10} that is 1,000 times more sensitive than Elite-ABC¹¹, the IHC seems to get enough sensitivity to inform whether there is the target protein in the tissue section examined or not. But all the MoAbs can not be applied to the antigen-retrieval ImmunoMax method (the modified ImmunoMax). MoAb 6C2 against gp46env could not be applied to the antigen-retrieval method¹⁵ so that the other MoAbs against gp46env must be examined when the gp46env has to be detected by the modified ImmunoMax. MoAbs Lt-4 (table 1), WATM-1 and WATM-3 against p40tax could not label the all HTLV-1-related T-cell MLs so that these MoAbs could be applied to the modified ImmunoMax. In general, MoAb that yields a small amount of reaction products and a quite low level of background stain in the Elite ABC or in the sABC method, can be applied to the modified ImmunoMax.

The control stain is necessary in the modified ImmunoMax. This study concerned the instance when enough negative-control stain can not be performed. Introducing the capillary method with dryer-heating system (MicroProbe) to the IHC, it is easy to control reaction time and temperature in each process of the IHC¹⁴ and quantitative evaluation of the reaction products becomes possible. This study employed image analysis of color slide photos taken from the specimens treated by the quantitative modified ImmunoMax. Among the MoAbs against the same target protein, the smallest amount of antigen detected is expected to be specific for the target protein, when the background stain is quite weak or none. In this sense, this study predicted by the quantitative modified ImmunoMax that WATM-1 is specific for p40tax in the modified ImmunoMax. In order to get the HTLV-1-free cases of T-cell MLs in a HTLV-1-endemic area, the HTLV-1 infection was examined by means of PCR. Examining HBG in the extracted DNA by the PCR, comparison of the PCR for HTLV-1 proviral DNA and the PCR for HBG¹⁶ can inform whether T-cell ML cells are infected by HTLV-1 or not. Table 4 showed that there was only one HTLV-1-free case of T-cell ML (the case 2 in table 4) in the 15 cases examined. The modified ImmunoMax of WATM-1 in this case was the negative-control case. The positive stain of Lt-4 in lymphoma cells of the case 2 indicated that Lt-4 should not be applied to the modified ImmunoMax to detect p40tax, although the antigen-retrieval Elite ABC of Lt-4 could not label the all HTLV-1-related T-cell MLs (table 1). The negative stain of WATM-1 in lymphoma cells of the case 2 suggested that the modified ImmunoMax of WATM-1 has the specificity to detect p40tax even in the highly sensitive IHC.

The modified ImmunoMax of WATM-1 showed various positive stains in HTLV-1-related T-cell MLs, as shown in Fig. 4. Quite weak stain of WATM-1 that showed fine spotty intranuclear stain might detect the intranuclear p40tax² to transactivate host cell genes or to activate the long terminal repeat of integrated HTLV-1 proviral DNA to reproduce p40tax, as explained in Fig. 1, although the evaluation of the stain should be negative. The spotty or granular stains in the modified ImmunoMax of WATM-1 suggested a small amount of p40tax in HTLV-1-related T-cell MLs. In cytoplasm of many lymphoma cells of each HTLV-1-related T-cell ML, the stains varied in the intensity and the area would reflect the amount of p40tax. Because HTLV-1 p40tax can interact with cytoplasmic unit protein in host cells^{2,3}, the quantitative evaluation of the modified ImmunoMax of WATM-1 would predict the total function of p40tax in cytoplasm. Observation of nuclear p40tax fine spotty stain and quantitative evaluation of cytoplasmic p40tax stain would be applied to comparative molecular analysis of the appearance of HTLV-1 p40tax and the activation of host cell genes.

In the conclusion, the modified ImmunoMax of WATM-1 in T-cell MLs seems to be enough sensitive and specific to label HTLV-1-related cases and is expected to be applied to the histological analysis of pathogenesis in HTLV-1-related lesions¹¹.

Summary

Immunohistochemical detection of HTLV-1 pX protein p40tax is expected to show the activation of HTLV-1 proviral DNA and to give information about the pathogenesis of HTLV-1-related lesions. This study established the modified ImmunoMax of HTLV-1 p40tax, comparing the stain of three monoclonal

antibodies, Lt-4, WATM-1 and WATM-3. In two cases of HTLV-1-associated non-neoplastic lymphadenopathy (HANNLA) and one case of adult T-cell leukemia (ATLL), image analysis of the quantitative modified ImmunoMax of the three MoAbs showed that Lt-4 and WATM-1 are more specific for p40tax. In 15 cases of T-cell malignant lymphomas (T-cell MLs), in which HTLV-1 infection was examined by PCR, Lt-4 and WATM-1 were compared in the specificity against p40tax. In one HTLV-1-free T-cell ML, Lt-4 labeled lymphoma cells and nuclei of squamous epithelium and WATM-1 did not. Lt-4 reacted strongly nuclei of lymphoma cells in 7 of the 14 HTLV-1-related T-cell MLs. WATM-1 labeled the all 14 HTLV-1-related T-cell MLs except one case. WATM-1 showed various positive stains from intranuclear fine spotty stains in a few lymphoma cells to strong spotty stains in many lymphoma cells. Consequently, the modified ImmunoMax of WATM-1 against p40tax is expected to be applied to the surgical pathology of HTLV-1-related lesions and to the molecular analysis of HTLV-1 pathogenesis.

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Key words: Immunohistochemistry, ImmunoMax, DAKO CSA system, the modified ImmunoMax, HTLV-1, p40tax, Lt-4, WATM-1, WATM-3, T-cell malignant lymphoma

References

- 1, Takatsuki K, Yamaguchi A, Watanabe T et al: Adult T-cell leukemia and HTLV-1 related diseases. In: Advances in adult T-cell leukemia and HTLV-1 research. eds. Takatsuki K, Hinuma Y, Yoshida M. Japan Scientific Societies Press. Gann Monograph on Cancer Research No. 39. p.1-16, 1992
- 2, Yoshida M, Fujisawa J. Positive and negative regulation of HTLV-1 gene expression and their roles in leukemogenesis in ATL. In: Advances in adult T-cell leukemia and HTLV-1 research. eds. Takatsuki K, Hinuma Y, Yoshida M. Japan Scientific Societies Press. Gann Monograph on Cancer Research No. 39. p.217-236, 1992
- 3, Suzuki T, Kato S, Matsushima H, Yoshida M. HTLV-1 Tax protein interacts with cyclin-dependent kinase inhibitor p16INK4A and counteracts its inhibitory activity towards CDK4. EMBO J 15: 1607-1614, 1996
- 4, Kobayashi N. Virology of ATL. In: Adult T-cell leukemia/lymphoma. ed. Takatsuki K. Nishimura Shoten. Series of Hematology No. 6. p.122-155 (in Japanese)
- 5, Hasui K. Paraffin-immunohistochemistry of 226 non-Hodgkin's malignant lymphomas in the endemic area of human T-cell leukemia virus type 1. Acta Pathol Jpn 41: 350-362, 1991
- 6, Hasui A, Sato E. Human T-cell leukemia virus type 1 (HTLV-1) infection in malignant lymphomas in a HTLV-1-endemic area, Kagoshima, Japan: HTLV-1 in-situ-hybridization (ISH) analysis employing a highly biotinylated concatamer probe synthesized by polymerase chain reaction (PCR) for HTLV-1 proviral DNA pX Tax region. DENDRITIC CELLS 4: 125-137, 1994
- 7, Hasui K, Sato E, Sueyoshi K et al. Cold in-situ-hybridization of human T-cell leukemia virus type 1: Production of biotinylated probes by polymerase chain reaction and improvement of procedures. DENDRITIC CELLS 5: 27-35, 1995
- 8, Merz TL, Ricketts O, Schrimel S, Orscheschek K, Feller AC. Constant detection of surface and cytoplasmic immunoglobulin heavy and light chain expression in formalin-fixed and paraffin-embedded material. J Pathol. 170: 257-264, 1993

- 9, Merz H, Malisius R, Mannweiler S, Zhou R, Hartmann W, Orscheschek K, Moubayed P, Feller AC. Methods in Laboratory Investigation: ImmunoMax, a maximized immunohistochemical method for the retrieval and enhancement of hidden antigens. *hb Invest* 73: 149-156, 1995
- 10, Feller AC, Malisius R, Venske T, Merz H. New immunohistochemical methods for the visualization of formalin-sensitive antigens in routinely processed paraffin-embedded material. *Med. J. Kagoshima Univ.*, 47 (Suppl. 2): 33-38, 1995
- 11, Hasui K, Sato E, Tanaka Y, Yashiki S, Izumo S. Quantitative highly-sensitive immunohistochemistry of HTLV-1 p40tax and p27rex proteins in HTLV-1-associated non-neoplastic lymphadenopathy with estimation of HTLV-1 dose by polymerase chain reaction. *DENDRITIC CELLS* 7. in press.
- 12, Hasui K, Sueyoshi K, Kitajima S, Sato E. HTLV-1-associated non-neoplastic lymphadenopathy, Atypical follicular lesions of lymph nodes found in anti-human T-cell leukemia virus type 1 (HTLV-1) antibodies-positive subjects without neoplastic disorders. In: *Lymphoreticular cells, fundamentals and pathology. Proceedings of the second Japanese-Korean Lymphoreticular Workshop.* eds. Takahashi A Kim SH. Lymphoreticular Cell Foundation, Kumamoto, Japan. p. 239-251, 1992
- 13, Tanaka Y, Masuda M, Yoshida A et al. An antigenic structure of the trans-activator protein encoded by human T-cell leukemia virus type-1 (HTLV-1), as defined by a panel of monoclonal antibodies. *AIDS Res Hum Retroviruses* 8: 227-235, 1992
- 14, Hasui K, Jia HM, Jia XS, Nomoto M, Sato E. Antigen retrieval in paraffin-immunohistochemistry detecting monoclonality of immunoglobulin light chain in B-cell malignant lymphomas: Comparison with in-situ-hybridization. In: *Lymphoreticular cells and diseases. Proceedings of the fourth Japanese-Korean Lymphoreticular Workshop.* eds. Hara H, Lee JD. Lymphoreticular Study Group, Department of Pathology, Kochi Medical School, Nankoku, Japan. p.321-329, 1995
- 15, Hasui K. Personal data, unpublished.
- 16, Sueyoshi K. Detection of human T-lymphotropic virus type 1 (HTLV-1) and Epstein-Barr virus (EBV) in HTLV-1 seropositive malignant lymphomas. *Medical Journal of Kagoshima University* 48: 249-261, 1996