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Analysis of Notch1 protein expression in methotrexate associated lymphoproliferative disorders

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メトトレキサート (MTX) 関連リンパ増殖性疾患 (MTX-LPD) は、MTX 治療を受けた患者に発生するリンパ増殖性疾患である。発症機序はまだ解明されていないが、基礎にある自己免疫疾患、MTX の使用、エプスタイン・バーウイルス感染、加齢などの要因が複雑に絡み合っていると考えられている。

NOTCH 遺伝子は、胚発生過程における増殖や分化など、様々な基本的細胞プロセスを制御するシグナル伝達経路の受容体をコードしている。NOTCH1 の変異は、慢性リンパ性白血病/リンパ腫、マンツル細胞リンパ腫/リンパ腫を含む B 細胞腫瘍で報告されている。近年、NOTCH1 変異は、移植後のリンパ増殖性疾患や血管免疫芽球性 T 細胞リンパ腫の CD20 陽性細胞でも認められ、免疫不全におけるリンパ腫発生と関連している可能性があることが報告されている。本研究では MTX-LPD 症例 (組織学的に DLBCL 型 (n=24) および古典的ホジキンリンパ腫 (CHL) 型 (n=24)) および de novo リンパ腫症例 (組織学的に DLBCL 型 (n=24) および古典的ホジキンリンパ腫 (CHL) 型 (n=24)) において、核における Notch1 のタンパク質発現を免疫組織化学的に評価した。

その結果、MTX-LPD 症例では、CHL 症例よりも DLBCL 症例の方が Notch1 タンパク質の発現が有意に高かった ( $P < 0.001$ )。さらに DLBCL 形態の症例では、Notch1 の発現は de novo 群よりも MTX-LPD 群で高い傾向があったが、この差は有意ではなかった ( $P = 0.0605$ )。この結果から、NOTCH1 は MTX 使用下での B 細胞の増殖と腫瘍形成に関与している可能性が示された。今後遺伝子レベルでの検討を含め更なる検討が必要である。

**Table 1.** Demographics and clinical findings for MTX-LPD

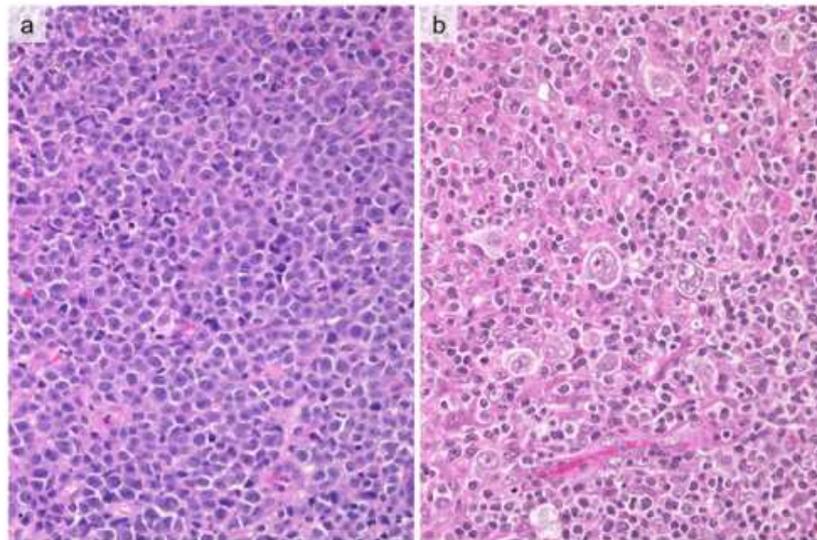
	MTX-LPD (n = 48)		<i>P</i>
	DLBCL type (n = 24)	CHL type (n = 24)	
Age, mean (range)	76.1 (62–87)	66.8 (41–82)	0.002*
Sex (male:female)	3:21	8:16	0.084
Underlying condition n (%)			
Rheumatoid arthritis	21 (87.5)	20 (83.3)	—
Hashimoto disease	1 (4.2)	0	—
SAPHO syndrome	0	1 (4.2)	—
Crohn disease	0	1 (4.2)	—
Unknown	3 (12.5)	2 (8.3)	—
Excised or biopsied site n (%)			
Lymph nodes	15 (62.5)	23 (95.8)	—
Axillary	6 (25.0)	3 (12.5)	—
Cervical	4 (16.7)	8 (33.3)	—
Subclavicular	0	3 (12.5)	—
Mediastinal	0	1 (4.2)	—
Inguinal	3 (12.5)	4 (16.7)	—
Peribronchial	1 (4.2)	0	—
Retroperitoneum	0	3 (12.5)	—
LN of unknown site	1 (4.2)	1 (4.2)	—
Soft tissue	2 (8.3)	0	—
Skin	2 (8.3)	0	—
Stomach	2 (8.3)	0	—
Tonsil	1 (4.2)	0	—
Gingiva	1 (4.2)	0	—
Orbit	1 (4.2)	0	—
Liver	0	1 (4.2)	—
Presence of EBV infection†			
Present	15 (62.5)	22 (91.7)	0.011*
Absent	8 (33.3)	1 (4.2)	

†The presence of EBV infection was evaluated by EBER in situ hybridization or LMP-1 immunostaining in proliferating atypical lymphocytes, and 23 cases each of DLBCL type and CHL type were evaluated. Significance was calculated using the Mann-Whitney U test. Fisher's exact analysis was used for the statistical analysis of nominal scales. \* $P < 0.05$ .

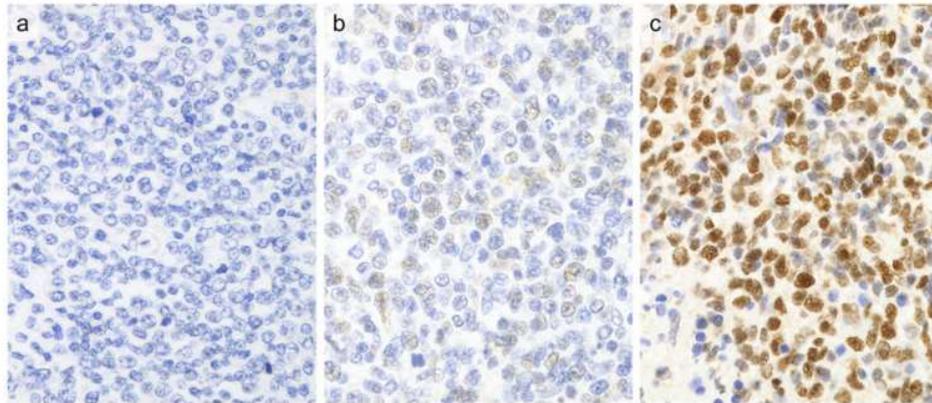
**Table 2.** Comparison of demographics and clinical findings between MTX-LPD and *de novo* lymphoma by morphology

	DLBCL morphology (n = 43)		P	CHL morphology (n = 39)		P
	MTX-LPD (n = 24)	<i>de novo</i> (n = 19)		MTX-LPD (n = 24)	<i>de novo</i> (n = 15)	
Age, mean (range)	76.1 (62–87)	69.8 (26–92)	0.239	66.8 (41–82)	49.1 (16–85)	0.032*
Sex (male:female)	3:21	15:4	<0.001**	8:16	11:4	0.017*
Excised or biopsied site n (%)						
Lymph nodes	15 (62.5)	19 (100)	—	23 (95.8)	14 (93.3)	—
Axillary	6 (25.0)	1 (5.3)	—	3 (12.5)	2 (13.3)	—
Cervical	4 (16.7)	13 (68.4)	—	8 (33.3)	8 (53.3)	—
Subclavicular	0	1 (5.3)	—	3 (12.5)	3 (20.0)	—
Mediastinal	0	0	—	1 (4.2)	0	—
Inguinal	3 (12.5)	3 (15.8)	—	4 (16.7)	1 (6.7)	—
Peribronchial	1 (4.2)	0	—	0	0	—
Retroperitoneum	0	0	—	3 (12.5)	0	—
Abdominal	0	1 (5.3)	—	0	0	—
Unknown LN	1 (4.2)	0	—	1 (4.2)	0	—
Soft tissue	2 (8.3)	0	—	0	1 (6.7)	—
Skin	2 (8.3)	0	—	0	0	—
Stomach	2 (8.3)	0	—	0	0	—
Tonsil	1 (4.2)	0	—	0	0	—
Gingiva	1 (4.2)	0	—	0	0	—
Orbit	1 (4.2)	0	—	0	0	—
Liver	0	0	—	1 (4.2)	0	—
Presence of EBV infection†						
Present	15 (62.5)	1 (5.3)	<0.001**	22 (91.7)	5 (33.3)	<0.001**
Absent	8 (33.3)	18 (94.7)		1 (4.2)	10 (66.7)	

†The presence of EBV infection was evaluated by EBER in situ hybridization or LMP-1 immunostaining in proliferating atypical lymphocytes, and 23 cases each of DLBCL type and CHL type in MTX-LPD were evaluated. Significance was calculated using the Mann–Whitney U test. Fisher's exact analysis was used for the statistical analysis of nominal scales. \* $P < 0.05$ , \*\* $P < 0.001$ .



**Fig. 1.** Typical histology of MTX-LPD cases included in this study. (a) DLBCL type: Diffuse proliferation of large atypical cells is observed (HE). (b) CHL type: Scattered large atypical cells with distinct nucleoli are observed in a background of small lymphocytes. Reed-Sternberg cell-like cells are also present (HE).



**Fig. 2.** Evaluation of Notch1 antibody expression in nuclei. (a) The nuclei of atypical cells showed no staining and were assessed as Score 0. (b) The nuclei showed weak positivity and were assessed as Score 1. (c) The nuclei showed strong positivity and were assessed as Score 2. For both Score 1 and Score 2, positivity was defined when more than 25% of tumor cells showed nuclear staining.

**Table 3.** Comparison of nuclear expression of Notch1 between DLBCL morphology and CHL morphology by pathogenesis

Notch1 positivity	MTX-LPD		<i>P</i>	<i>de novo</i>		<i>P</i>
	DLBCL type (n = 24)	CHL type (n = 24)		DLBCL (n = 19)	CHL (n = 15)	
2+	14	3		6	4	
1+	8	8		7	3	
0	2	13		6	8	
Positive rate (%) <sup>†</sup>	91.7	45.8	<0.001**	68.4	46.7	0.1766

<sup>†</sup> Positive rates were calculated for 1+ and 2+ cases.

Fisher's exact analysis and chi-square test was used for the statistical analysis of nominal scales. \*\**P* < 0.001.

**Table 4.** Comparison of nuclear expression of Notch1 between MTX-LPD and *de novo* lymphoma by morphology

Notch1 positivity	DLBCL morphology (n = 43)		<i>P</i>	CHL morphology (n = 39)		<i>P</i>
	MTX-LPD (n = 24)	<i>de novo</i> (n = 19)		MTX-LPD (n = 24)	<i>de novo</i> (n = 15)	
2+	14	6		3	4	
1+	8	7		8	3	
0	2	6		13	8	
Positive rate (%) <sup>†</sup>	91.7	68.4	0.0605	45.8	46.7	0.609

<sup>†</sup> Positive rates were calculated for 1+ and 2+ cases.

Fisher's exact analysis and chi-square test was used for the statistical analysis of nominal scales.

**Table 5.** Investigation of the correlation between the EBV infection status and Notch1 expression rate in the nucleus of tumor cells in each group

	MTX-LPD DLBCL type <sup>†</sup>		<i>P</i>	MTX-LPD CHL type <sup>‡</sup>		<i>P</i>	
	EBV infection (+) (n = 15)	EBV infection (-) (n = 8)		EBV infection (+) (n = 22)	EBV infection (-) (n = 1)		
Notch1 (+)	15	6	0.11	Notch1 (+)	11	0	1
Notch1 (-)	0	2		Notch1 (-)	11	1	
	<i>de novo</i> DLBCL		<i>P</i>	<i>de novo</i> CHL		<i>P</i>	
	EBV infection (+) (n = 1)	EBV infection (-) (n = 18)		EBV infection (+) (n = 5)	EBV infection (-) (n = 10)		
Notch1 (+)	0	13	0.32	Notch1 (+)	4	3	0.12
Notch1 (-)	1	5		Notch1 (-)	1	7	

<sup>†</sup> EBER-ISH was not performed in one case. <sup>‡</sup> EBER-ISH was not performed in one case.

Fisher's exact analysis was used for statistical analyses.