Kageyama H, Soda M, Takeuchi K, Itami M, Iizasa T, Yoshino I, Mano H, Kimura H. EML4-ALK fusion gene assessment using metastatic lymph node samples obtained by endobronchial ultrasound-guided transbronchial needle aspiration. ***Clin Cancer Res***. 2010;16:4938-4945.

10) Takeuchi K, Yokoyama M, Ishizawa S, Terui Y, Nomura K, Marutsuka K, Nunomura M, Fukushima N, Yagyuu T, Nakamine H, Akiyama F, Hoshi K, Matsue K, Hatake K, Oshimi K. Lymphomatoid gastropathy: a distinct clinicopathologic entity of self-limited pseudomalignant NK-cell proliferation. ***Blood***. 2010;116:5631-5637.
11) Kodaira M, Takahashi S, Takeuchi K, Yuasa T, Saotome T, Yonese J, Fukui I, Hatake K. Sorafenib-induced erythema multiforme for metastatic renal cell carcinoma. ***Ann Oncol***. 2010;21:1563-1565.
12) Asai H, Yokoyama M, Terui Y, Ennishi D, Takeuchi K, Hatake K. Is statin use really associated with efficacy of rituximab? ***J Clin Oncol***. 2010;28:e424-425; author reply e427-428.
13) Hoshi R, Furuta N, Horai T, Takeuchi K, Ishikawa Y, Satoh Y. Implications for differential diagnosis of lung cancer-associated lymphadenopathy in lymphoepithelioid cell lymphoma (Lennert's lymphoma) arising simultaneously with lung cancer: a case report. ***Acta Cytol***. 2010;54:197-201.
14) Ichinohasama R, Oji Y, Yokoyama H, Takeuchi K, Fujiwara T, Ishizawa K, Taniguchi O, Tsuboi A, Oka Y, Sugiyama H. Sensitive immunohistochemical detection of WT1 protein in tumors with anti-WT1 antibody against WT1 235 peptide. ***Cancer Sci***. 2010;101:1089-1092.
15) Tsuji H, Tamura M, Yokoyama M, Takeuchi K, Mimura T. Ocular involvement by epstein-barr virus-positive diffuse large B-cell lymphoma of the elderly: a new disease entity in the world health organization classification. ***Arch Ophthalmol***. 2010;128:258-259.
16) Hiramatsu M, Ninomiya H, Inamura K, Nomura K, Takeuchi K, Satoh Y, Okumura S, Nakagawa K, Yamori T, Matsuura M, Morikawa T, Ishikawa Y. Activation status of receptor tyrosine kinase downstream pathways in primary lung adenocarcinoma with reference of KRAS and EGFR mutations. ***Lung Cancer***. 2010;70:94-102.
17) Mano H, Takeuchi K. EML4-ALK fusion in lung. ***Am J Pathol***. 2010;176:1552-1553; author reply 1553-1554.
18) Watanabe R, Tomita N, Takeuchi K, Sakata S, Tateishi U, Tanaka M, Fujita H, Inayama Y, Ishigatsubo Y. SUVmax in FDG-PET at the biopsy site correlates with the proliferation potential of tumor cells in non-Hodgkin lymphoma. ***Leuk Lymphoma***. 2010;51:279-283.
19) Kodaira M, Takahashi S, Yamada S, Ueda K, Mishima Y, Takeuchi K, Yamamoto N, Ishikawa Y, Yokoyama M, Saotome T, Terui Y, Hatake K. Bone metastasis and poor performance status are prognostic factors for survival of carcinoma of unknown primary site in patients treated with systematic chemotherapy. ***Ann Oncol***. 2010;21:1163-1167.
20) Ennishi D, Asai H, Maeda Y, Shinagawa K, Ikeda K, Yokoyama M, Terui Y, Takeuchi K, Yoshino T, Matsuo K, Hatake K, Tanimoto M. Statin-independent prognosis of patients with diffuse large B-cell lymphoma receiving rituximab plus CHOP therapy. ***Ann Oncol***. 2010;21:1217-1221.
21) Hyo R, Tomita N, Takeuchi K, Aoshima T, Fujita A, Kuwabara H, Hashimoto C, Takemura S, Taguchi J, Sakai R, Fujita H, Fujisawa S, Ogawa K, Motomura S, Suzuki R, Ishigatsubo Y. The therapeutic effect of rituximab on CD5-positive and CD5-negative diffuse large B-cell lymphoma. ***Hematol Oncol***. 2010;28:27-32.

H. 知的財産権の出願・登録状況

該当せず。

厚生労働科学研究費補助金（創薬基盤推進研究事業）
分担研究報告書
「環状 DNA を介するがん細胞のテロメア維持機構」に関する研究

分担研究者：　鍋谷　彰　　京都大学大学院生命科学研究科　助教

研究要旨：テロメア維持機構は、がん細胞の無限増殖能を保障する細胞機能の一つである。がん細胞のテロメア維持機構は、良く知られたテロメレース以外に ALT (alternative lengthening of telomeres) 経路が存在する。ALT 経路の分子的詳細は明らかになっていないが、これまで相同組換えに依存することが示唆されてきた。これまでの研究成果から、ALT 経路では環状テロメア DNA が鋳型となったローリングサークル型複製によるという仮説を立てている。これを再現および検出するための実験系として、外来の環状テロメア DNA モデルを用いがん細胞の染色体へ挿入を試み、得られたクローンのゲノム構造を解析した。その結果、少なくとも二種類の細胞において、導入した DNA が正向きのくり返し配列となって染色体末端に存在することが示された。本研究から、外来 DNA モデルを使った実験系が、ALT 経路の研究において有用であることが明らかとなった。

A　研究目的

がん細胞は、それ自身がもつ変異やエピジェネティックな変化により無限増殖能を獲得している。いくつかの細胞機能が無限増殖能に関係するが、その一つに染色体末端構造であるテロメアの維持機構が知られている。細胞の増殖に伴って起こるテロメア DNA の短小化（いわゆる末端複製問題）に拮抗するため、多くのがん細胞はテロメレースを発現してテロメア DNA を伸長している。一方肺がんを含むがん細胞の中には、テロメレースに依存しないテロメア維持機構を獲得しているものが存在する。これは ALT (alternative lengthening of telomeres) 経路と呼ばれてが、その分子的詳細について明らかになっていない。がん治療のために細胞のテロメア維持機構を標的とする場合には、テロメレースだけでなく ALT 経路も解明し、その抑制を目指す必要がある。このような背景から、ALT 経路の分子機構を解明するための基盤技術の確立が期待されている。

これまで ALT 経路は繰り返し配列であるテロメア DNA どうしの組換えによると考えられてきた。我々は最近、ALT 細胞には特徴的なテロメア DNA の一本鎖構造が存在することを明らかにした。この発見から、ALT 経路には染色体末端と環状 DNA の間で起こるローリングサークル型複製が関与する、という仮説を得た。本研究ではこの仮説を実証するためのモデル実験系を構築することを目的とした。

B　研究方法

環状テロメア DNA モデルを使ったローリングサークル型複製の検出実験系：

ローリングサークル型複製によるテロメア DNA の伸長合成は、次の様な各段階を順に進むことで起こっていると考えられる。初めのステップは、染色体末端にあるテロメア DNA の一本鎖末端が相同配列をもつ染色体外の環状テロメア DNA と対合する。第二のステップは、対合した部分から環状テロメア DNA を鋳型にして片側の鎖が連続的に合成される。最後は、新規に合成された一本鎖に相補的な鎖が合成され二本鎖となる。ローリングサークル型複製の最も特徴的な点は「環状 DNA を鋳型にした連続合成」であるため、これを検出できる実験系を作製した。

まず ALT 経路でテロメアを維持している細胞（ALT 細胞、本研究では代表として U2-OS 細胞を使用）に、テロメア配列（約 1.0 kb）とタグ配列（約 4.6 kb）の両方をもつ環状 DNA（環状テロメア DNA モデル、約 5.6 kb）を導入した。次にこのタグ配列に含まれる薬剤選択マーカー（Puromycin）を使い、薬剤耐性を示すクローンを得て、環状テロメア DNA モデルがゲノム中でどのような構造をとっているかを解析した。

C　研究結果

環状テロメア DNA モデルを導入した ALT 細胞から、Puromycin に耐性を示すク

ローンを6種類得ることができた。これらのクローンを増殖させ、細胞のゲノムDNAを調製し、挿入されたDNAモデルをサザン法により解析した。導入した環状モデルが鋳型となってテロメア伸長が起こった場合には、タグ配列が染色体末端に付加され、また環状モデルの全配列5.6 kbが正方向のくり返し構造をとることが期待される。これらについて解析を行い以下の結果を得た。

DNAモデルのくり返し構造：環状DNAモデルが挿入箇所で正方向のくり返し配列をとっている場合、このDNAに一カ所だけ認識部位が存在する制限酵素で切断すると、DNA一つの長さすなわち5.6 kbに相当する断片が生じるはずである。この断片が検出されるかをサザン法で検討したところ、3クローンが該当することがわかった。次にこれらについて、導入した環状DNAがそのまま染色体外配列として保持されているかを、二次元電気泳動法によって解析した。その結果、3クローンともに環状DNAは存在せず、導入したDNA由来の断片は染色体内に挿入されていることが明らかとなった。また検出された制限酵素断片のパターンから、この中の2クローンは複数のゲノム領域にモデルDNAが挿入されていることが明らかとなった。

染色体末端の形成：導入したDNAが染色体末端に存在するか否かは、BAL-31エキソヌクレアーゼに対する感受性によって検討した。DNAを末端からprocessiveに切断する酵素であるBAL-31によりゲノムDNAを処理に対し、染色体末端（すなわちテロメア領域）は他の染色体領域に比べて分解されやすい性質をもっている。得られた3クローンの中で、2クローンは明らかに導入したDNA部分がBAL-31に感受性を示した。1クローンについては、複数の挿入部位があるため判断が不可能であった。

以上の結果から、ALT細胞に環状テロメアDNAモデルを導入すると、そのくり返し配列が染色体の末端領域を形成することが可能であることが明らかとなった。

D&E. 考察及び結論

ALT経路に関わるDNA代謝の過程を再現して検出する実験系は、これまで全く確立されていなかった。本研究により、外来の環状テロメアDNAモデルが、ALT細胞の染色体末端に付加される過程を再現することができた。この結果は、外来DNAモデルを使った実験系が有用であることを示している。今回得られたクローンについて、挿入箇所の構造をさらに詳細に解析する必要がある。例えばローリングサークル型複製によりこの構造が生じた場合には、染色体末端に存在するDNAモデルと宿主細胞のゲノムDNAにはテロメア配列が存在するはずである。このような解析により、どのような反応で挿入が起こったかを知見を得ることができる。

本研究で使った実験系の問題点として、低頻度で定量的解析が非現実的なことが挙げられる。この問題を解決するために、例えば導入したDNAが染色体外の環状DNAとして安定に保持されるようにすると、染色体のテロメア伸長に関与する機会が増加することが期待できる。このような実験系はALT経路の解明だけでなく、個々のがん細胞においてそのテロメア維持機構を抑制する時の標的を知るための基本的な技術を提供する。

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

Nabetani, A. and Ishikawa, F. "Alternative lengthening of telomeres pathway: Recombination-mediated telomere maintenance mechanism in human cells." ***J. Biochem.*** 149(1): 5-14, 2011

H. 知的財産権の出願・登録状況

なし

自治医科大学　　間野博行　業績リスト

発表者名	論文タイトル名	発表誌名	巻号	ページ	出版年
Sasaki D, Imaizumi Y, Hasegawa H, Osaka A, Tsukasaki K, Choi YL, Mano H, Marquez V, Hayashi T, Yanagihara K, Moriwaki Y, Miyazaki Y, Kamihira S & Yamada Y.	Overexpression of enhancer of zeste homolog 2 with trimethylation of lysine 27 on histone H3 in adult T-cell leukemia/lymphoma as a target for epigenetic therapy	Haematologica	in press		2011
Iida A, Shinoe T, Baba Y, Mano H & Watanabe S.	Dicer plays essential roles for retinal development by regulation of survival and differentiation	Invest Ophthalmol Vis Sci	in press		2011
Takeuchi K, Soda M, Togashi Y, Ota Y, Sekiguchi Y, Hatano S, Asaka R, Noguchi M & Mano H.	Identification of a novel fusion, SQSTM1-ALK, in ALK-positive large B-cell lymphoma	Haematologica	in press		2010
Choi YL, Soda M, Yamashita Y, Ueno T, Takashima J, Nakajima T, Yatabe Y, Takeuchi K, Hamada T, Haruta H, Ishikawa Y, Kimura H, Mitsudomi T, Tanio Y & Mano H.	EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors	N Engl J Med	363	1734-1739	2010
Yamashita Y, Yuan J, Suetake I, Suzuki H, Ishikawa Y, Choi YL, Ueno T, Soda M, Hamada T, Haruta H, Takada S, Miyazaki Y, Kiyoi H, Ito E, Naoe T, Tomonaga M, Toyota M, Tajima S, Iwama A & Mano H.	Array-based genomic resequencing of human leukemia	Oncogene	29	3723-3731	2010
Zhang MJ, Franklin S, Li Y, Wang S, Ru X, Mitchell-Jordan SA, Mano H, Stefani E, Ping P & Vondriska TM.	Stress signaling by Tec tyrosine kinase in the ischemic myocardium	Am J Physiol Heart Circ Physiol	299	713-722	2010
Susaki K, Kitanaka A, Dobashi H, Kubota Y, Kittaka K, Kameda T, Yamaoka G, Mano H, Mihara K & Ishida T.	Tec protein tyrosine kinase inhibits CD25 expression in human T-lymphocyte	Immunol Lett	127	135-142	2010
Sakairi Y, Nakajima T, Yasufuku K, Ikebe D, Kageyama H, Soda M, Takeuchi K, Itami M, Iizasa T, Yoshino I, Mano H & Kimura H.	EML4-ALK Fusion Gene Assessment Using Metastatic Lymph Node Samples Obtained by Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration	Clin Cancer Res	16	4938-4945	2010
Osoegawa A, Nosaki K, Miyamoto H, Kometani T, Hirai F, Ondo K, Seto T, Sugio K, Choi YL, Soda M, Mano H & Ichinose Y.	Incidentally proven pulmonary "ALKoma"	Intern Med	49	603-606	2010
Nakajima T, Kimura H, Takeuchi K, Soda M, Mano H, Yasufuku K & Iizasa T.	Treatment of Lung Cancer with an ALK Inhibitor After EML4-ALK Fusion Gene Detection Using Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration	J Thorac Oncol	5	2041-2043	2010
Mano H & Takeuchi K.	EML4-ALK fusion in lung	Am J Pathol	176	1552-1553	2010
Hatanaka H, Tsukui M, Takada S, Kurashina K, Choi YL, Soda M, Yamashita Y, Haruta H, Hamada T, Ueno T, Tamada K, Hosoya Y, Sata N, Yasuda Y, Nagai H, Sugano K & Mano H.	Identification of transforming activity of free fatty acid receptor 2 by retroviral expression screening	Cancer Sci	101	54-59	2010

Hatanaka H, Takada S, Tsukui M, Choi YL, Kurashina K, Soda M, Yamashita Y, Haruta H, Hamada T, Tamada K, Hosoya Y, Sata N, Nagai H, Yasuda Y, Sugano K & Mano H.	Identification of the transforming activity of Indian hedgehog by retroviral expression screening	Cancer Sci.	101	60-64	2010

自治医科大学	杉山幸比古　業績リスト				
発表者名	論文タイトル名	発表誌名	巻号	ページ	出版年
Mizushina Y, Bando M, Hosono T, Mato N, Nakaya T, Ishii Y, Yamasawa H & Sugiyama Y	Clinical features of lymphangioleiomyomatosis complicated by renal angiomyolipomas	Intern Med	50	285-289	2011
Tanaka K, Ishihara T, Azuma A, Kudoh S, Ebina M, Nukiwa T, Sugiyama Y, Tasaka Y, Namba T, Ishihara T, Sato K, Mizushima Y & Mizushima T	Therapeutic effect of lecithinized superoxide dismutase on bleomycin-induced pulmonary fibrosis	Am J Physiol Lung Cell Mol Physiol	298	L348-L360	2010

自治医科大学　　遠藤俊輔　業績リスト

発表者名	論文タイトル名	発表誌名	巻号	ページ	出版年
三輪千尋、渡辺恭孝、白石守、工藤史明、遠藤俊輔、小山信一郎	経気管支肺生検で診断したPulmonary epithelioid hemangioendotheliomaの1例	気管支学	32	72-76	2010
中野知之、金井義彦、手塚憲志、坪地宏嘉、小山信一郎、遠藤俊輔	無症候性の後天性左上葉気管閉鎖症の1手術例	気管支学	32	314-317	2010
足立広幸、前原孝光、安藤耕平、益田宗孝、遠藤俊輔、岸本晃司	多発原発性肺癌手術例の検討	胸部外科	63	347-353	2010
遠藤俊輔、坂東政司、杉山幸比古	びまん性肺疾患と外科的肺生検	日本胸部臨床	69	S33-39	2010
Yamamoto S, Endo T, Tetsuka K, Endo S	A new technique for the examination of tracheal tumors the bronchoscopic turned around procedure	J Bronchol Intervent Pulmonol	17	273-275	2010
Nakano T, Endo S, Tsubochi H, Nokubi M, Watanabe Y, Koyama S	Thymic clear cell carcinoma	General Thoracic and Cardiovascular Surgey	58	98-100	2010
Yamamoto S, Tetsuka K, Sato Y, Endo S	Unsuspected tracheal web inhibits endotracheal intubation: report of a case	J Anesth	24	132-133	2010

東京大学　　鯉沼代造　業績リスト

発表者名	論文タイトル名	発表誌名	巻号	ページ	出版年
K Miyazono and D Koinuma	Arkadia--beyond the TGF-β pathway	J Biochem	149	1-3	2011
S Ehata, E Johansson, R Katayama, S Koike, A Watanabe, Y Hoshino, Y Katsuno, A Komuro, D Koinuma, MR Kano, M Yashiro, K Hirakawa, H Aburatani, N Fujita, and K Miyazono	Transforming growth factor-β decreases the cancer-initiating cell population within diffuse-type gastric carcinoma cells	Oncogene	in press		
Y Nagano, D Koinuma, K Miyazawa, and K Miyazono	Context-dependent regulation of the expression of c-Ski protein by Arkadia in human cancer cells	J Biochem	147	545-554	2010

癌研究会　　竹内賢吾　業績リスト

発表者名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tachibana T, Tomita N, Furuya M, Yamanaka S, Takeuchi K, Nakamura N, Fujita H, Ishigatsubo Y.	Aberrant CD20 expression in angioimmunoblastic T-cell lymphoma.	Internal Medicine.			in press.
Watanabe N, Noh JY, Narimatsu H, Takeuchi K, Yamaguchi T, Kameyama K, Kobayashi K, Kami M, Kubo A, Kunii Y, Shimizu T, Mukasa K, Otsuka F, Miyara A, Minagawa A, Ito K, Ito K.	Clinicopathological features of 171 cases of primary thyroid lymphoma: a long-term study involving 24,553 patients with Hashimoto's disease.	Br J Haematol.			in press.
Okuda C, Kim YH, Takeuchi K, Togashi Y, Masago K, Sakamori Y, Mio T, Mishima M.	Successful treatment with pemetrexed in a patient with mucinous bronchioloalveolar carcinoma: long-term response duration with mild toxicity.	J Thorac Oncol.	6	641-642	2011
Takeuchi K, Soda M, Togashi Y, Ota Y, Sekiguchi Y, Hatano S, Asaka R, Noguchi M, Mano H.	Identification of a novel fusion, SQSTM1-ALK, in ALK-positive large B-cell lymphoma.	Haematologica.			on line
Nakajima T, Kimura H, Takeuchi K, Soda M, Mano H, Yasufuku K, Iizasa T.	Treatment of Lung Cancer with an ALK Inhibitor After EML4-ALK Fusion Gene Detection Using Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration	J Thorac Oncol.	5	2041-2043	2010
Choi YL, Soda M, Yamashita Y, Ueno T, Takashima J, Nakajima T, Yatabe Y, Takeuchi K, Hamada T, Haruta H, Ishikawa Y, Kimura H, Mitsudomi T, Tanio Y, Mano H.	EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors.	N Engl J Med.	363	1734-1739	2010
Nishimori H, Takahashi S, Kiura K, Ennishi D, Kobayashi T, Sano K, Shinozaki E, Yokoyama M, Mishima Y, Terui Y, Chin K, Mizunuma N, Ito Y, Nishimura S, Takeuchi K, Ishikawa Y, Oguchi M, Tanimoto M, Hatake K.	Cancer of unknown primary site:a review of 28 cases and the efficacy of cisplatin/docetaxel therapy at a single institute in Japan.	Acta Med Okayama.	64	285-291	2010
Jokoji R, Yamasaki T, Minami S, Komuta K, Sakamaki Y, Takeuchi K, Tsujimoto M.	Combination of morphological feature analysis and immunohistochemistry is useful for screening of EML4-ALK-positive lung adenocarcinoma.	J Clin Pathol.	63	1066-1070	2010
Sakairi Y, Nakajima T, Yasufuku K, Ikebe D, Kageyama H, Soda M, Takeuchi K, Itami M, Iizasa T, Yoshino I, Mano H, Kimura H.	EML4-ALK fusion gene assessment using metastatic lymph node samples obtained by endobronchial ultrasound-guided transbronchial needle aspiration.	Clin Cancer Res	16	4938-4945	2010
Takeuchi K, Yokoyama M, Ishizawa S, Terui Y, Nomura K, Marutsuka K, Nunomura M, Fukushima N, Yagyuu T, Nakamine H, Akiyama F, Hoshi K, Matsue K, Hatake K, Oshimi K.	Lymphomatoid gastropathy: a distinct clinicopathologic entity of self-limited pseudomalignant NK-cell proliferation.	Blood	116	5631-5637	2010
Kodaira M, Takahashi S, Takeuchi K, Yuasa T, Saotome T, Yonese J, Fukui I, Hatake K.	Sorafenib-induced erythema multiforme for metastatic renal cell carcinoma.	Ann Oncol.	21	1563-1565	2010
Asai H, Yokoyama M, Terui Y, Ennishi D, Takeuchi K, Hatake K.	Is statin use really associated with efficacy of rituximab?	J Clin Oncol.	28	e424-425; author reply e427-428	2010
Hoshi R, Furuta N, Horai T, Takeuchi K, Ishikawa Y, Satoh Y.	Implications for differential diagnosis of lung cancer-associated lymphadenopathy in lymphoepithelioid cell lymphoma (Lennert's lymphoma) arising simultaneously with lung cancer: a case report	Acta Cytol.	54	197-201	2010
Ichinohasama R, Oji Y, Yokoyama H, Takeuchi K, Fujiwara T, Ishizawa K, Taniguchi O, Tsuboi A, Oka Y, Sugiyama H.	Sensitive immunohistochemical detection of WT1 protein in tumors with anti-WT1 antibody against WT1 235 peptide.	Cancer Sci	101	1089-1092	2010

Tsuji H, Tamura M, Yokoyama M, Takeuchi K, Mimura T.	Ocular involvement by epstein-barr virus-positive diffuse large B-cell lymphoma of the elderly: a new disease entity in the world health organization classification	Arch Ophthalmol.	128	258-259	2010
Hiramatsu M, Ninomiya H, Inamura K, Nomura K, Takeuchi K, Satoh Y, Okumura S, Nakagawa K, Yamori T, Matsuura M, Morikawa T, Ishikawa Y.	Activation status of receptor tyrosine kinase downstream pathways in primary lung adenocarcinoma with reference of KRAS and EGFR mutations.	Lung Cancer.	70	94-102	2010
Mano H, Takeuchi K.	EML4-ALK fusion in lung.	Am J Pathol.	176	1552-1553; author reply 1553-1554	2010
Watanabe R, Tomita N, Takeuchi K, Sakata S, Tateishi U, Tanaka M, Fujita H, Inayama Y, Ishigatsubo Y.	SUVmax in FDG-PET at the biopsy site correlates with the proliferation potential of tumor cells in non-Hodgkin lymphoma.	Leuk Lymphoma.	51	279-283	2010
Kodaira M, Takahashi S, Yamada S, Ueda K, Mishima Y, Takeuchi K, Yamamoto N, Ishikawa Y, Yokoyama M, Saotome T, Terui Y, Hatake K.	Bone metastasis and poor performance status are prognostic factors for survival of carcinoma of unknown primary site in patients treated with systematic chemotherapy.	Ann Oncol.	21	1163-1167	2010
Ennishi D, Asai H, Maeda Y, Shinagawa K, Ikeda K, Yokoyama M, Terui Y, Takeuchi K, Yoshino T, Matsuo K, Hatake K, Tanimoto M.	Statin-independent prognosis of patients with diffuse large B-cell lymphoma receiving rituximab plus CHOP therapy.	Ann Oncol.	21	1217-1221	2010
Hyo R, Tomita N, Takeuchi K, Aoshima T, Fujita A, Kuwabara H, Hashimoto C, Takemura S, Taguchi J, Sakai R, Fujita H, Fujisawa S, Ogawa K, Motomura S, Suzuki R, Ishigatsubo Y.	The therapeutic effect of rituximab on CD5-positive and CD5-negative diffuse large B-cell lymphoma.	Hematol Oncol.	28	27-32	2010

京都大学　　鍋谷彰　業績リスト

発表者名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nabetani, A. and Ishikawa, F.	Alternative lengthening of telomeres pathway: Recombination-mediated telomere maintenance mechanism in human cells	J. Biochem	149(1)	5-14	2011

Identification of a novel fusion, SQSTM1-ALK, in ALK-positive large B-cell lymphoma

Kengo Takeuchi,[1] Manabu Soda,[2] Yuki Togashi,[1] Yasunori Ota,[3] Yasunobu Sekiguchi,[4] Satoko Hatano,[1] Reimi Asaka,[1] Masaaki Noguchi,[4] Hiroyuki Mano[2,5]

[1]Pathology Project for Molecular Targets, Cancer Institute, Japanese Foundation for Cancer Research, Tokyo; [2]Division of Functional Genomics, Jichi Medical University, Tochigi; [3]Department of Pathology, Toranomon Hospital, Tokyo; [4]Department of Hematology, Urayasu Hospital, Juntendo University, Chiba; [5]CREST, Japan Science and Technology Agency, Saitama 332-0012, Japan

ABSTRACT

ALK-positive large B-cell lymphoma is a rare subtype of lymphoma, and most cases follow an aggressive clinical course with a poor prognosis. We examined an ALK-positive large B-cell lymphoma case showing an anti-ALK immunohistochemistry pattern distinct from those of 2 known ALK fusions, CLTC-ALK and NPM-ALK, for the presence of a novel ALK fusion; this led to the identification of SQSTM1-ALK. SQSTM1 is an ubiquitin binding protein that is associated with oxidative stress, cell signaling, and autophagy. We showed transforming activities of SQSTM1-ALK with a focus formation assay and an *in vivo* tumorigenicity assay using 3T3 fibroblasts infected with a recombinant retrovirus encoding SQSTM1-ALK. ALK-inhibitor therapies are promising for treating ALK-positive large B-cell lymphoma, especially for refractory cases. SQSTM1-ALK may be a rare fusion, but our data provide novel biological insights and serve as a key for the accurate diagnosis of this rare lymphoma.

Key words: ALK-positive, large B-cell lymphoma, fusion.

Citation: Takeuchi K, Soda M, Togashi Y, Ota Y, Sekiguchi Y, Hatano S, Asaka R, Noguchi M, Mano H. Identification of a novel fusion, SQSTM1-ALK, in ALK-positive large B-cell lymphoma. Haematologica 2011;96(03):000-000. doi:10.3324/haematol.2010.033514

Introduction

Anaplastic lymphoma kinase-positive large B-cell lymphoma (ALK+LBCL) is a rare subtype of lymphoma that was first described in 1997.[1] Approximately 50 cases have been reported to date,[2] with most cases (60%) following an aggressive clinical course.[3] In well-characterized cases, 3 genes have been reported as a fusion partner of *ALK*: *clathrin* (*CLTC-ALK*),[4-6] *nucleophosmin* (*NPM-ALK*),[7-8] and *SEC31A* (*SEC31A-ALK*).[9] In this paper, we report a case of ALK+LBCL that harbored a novel ALK fusion partner, sequestosome1 (SQSTM1).

Design and Methods

Materials

Biopsied specimens were fixed in 20% neutralized formalin and embedded in paraffin for conventional histopathological examination. We extracted DNA and total RNA from the snap-frozen specimens and subsequently purified the samples. Written informed consent was obtained from the patient. The study was approved by the Institutional Review Board of the Japanese Foundation for Cancer Research.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue was used. For antigen retrieval, we heated the slides for 40 min at 97°C in Target Retrieval Solution (pH 9.0; Dako), and subsequently detected the immune complexes with a dextran polymer reagent (EnVision+DAB system, Dako) and an AutoStainer instrument (Dako).

Isolation of ALK fusion cDNA

To obtain cDNA fragments corresponding to novel ALK fusion genes, we used an inverse reverse transcription-polymerase chain reaction (RT-PCR) method slightly modified from one previously reported.[10] Double-stranded cDNA was synthesized from 2 μg of total RNA with 1 pM of the primer ALKREVex22-23 (5′-TGGTTGAATTTGCTGATGATC-3′) and a cDNA Synthesis System (Roche), and was self-ligated by incubation overnight with T4 DNA ligase (TaKaRa Bio). We subjected the resulting circular cDNA to PCR (35 cycles of 94°C for 15 sec, 62°C for 30 sec, and 72°C for 1 min) with primers ALKREV3T (5′-CTGATGGAGGAGGTCTTGCC-3′) and ALKFWDex20-21 (5′-ATTCGGGGTCTGGGCCAT-3′) in a final volume of 20 μL. We subjected 1 μL of the 1:100 diluted reaction products to a second PCR step (the same settings as above), with primers ALKREV4T (5′-GGTTGTAGTCGGTCATGATGGTC-3′) and ALKFWDex21-22 (5′-AGTGGCTGTGAAGACGCTGC-3′) in a final volume of 20 μL. The resulting products were purified by gel extraction and directly sequenced in both directions with primers ALKFWDex20-21 and ALKREV4T.

The fusion point of *SQSTM1-ALK* cDNA was amplified by RT-PCR with primers SQSTM1 565F (5′-AAACACGGA-

Acknowledgments: we thank Drs. Masaru Hosone, Yuichi Sugisaki, Koji Izutsu, Shuji Momose, and Jun-ichi Tamaru for their advice. The nucleotide sequences of the cDNAs for SQSTM1-ALK have been deposited in the DDBJ/EMBL/GenBank databases under the accession number, AB583922. Manuscript received on xxxxxxxx. Revised version arrived on xxxxxxxxxxxxxxxx. Manuscript accepted on xxxxxxxxx.
Correspondence: Kengo Takeuchi, M.D., Ph.D. Pathology Project for Molecular Targets, Cancer Institute, Japanese Foundation for Cancer Research. 3-8-31 Ariake, Koto, Tokyo 135-8550, Japan. E-mail: kentakeuchi-tky@umin.net; Tel.: international +81-3-3520-0111; Fax: international +81-3-3570-0558.

CACTTCGGGT-3′) and ALK3078RR (5′-ATCCAGTTCGTCCTGTTCAGAGC-3′).

Full-length *SQSTM1-ALK* cDNA was obtained from the specimen by RT-PCR with primers SQSTM1v1-F90 (5′-CTCGCTATGGCGTCGCTCACCGTGAA-3′) and KA-W-cDNA-out-AS (5′-CCACGGTCTTAGGGATCCCAAGG-3′).

Fluorescence in situ *hybridization (FISH)*

We performed FISH analysis of the gene fusion for unstained slides (4 μm thick) with bacterial artificial chromosome (BAC) clone-derived DNA probes for *ALK* (RP11-984I21, RP11-62B19) and *SQSTM1* (RP11-55M16).

Transformation assay for ALK *fusion protein*

We analyzed the transforming activity of SQSTM1-ALK as described previously.[11-13] Briefly, cDNA for SQSTM1-ALK was inserted into the retroviral expression plasmid pMXS.[14] The resulting plasmid and similar pMXS-based expression plasmids for EML4-ALK variant 1 or NPM-ALK were used to generate recombinant ecotropic retroviruses, which were then used to infect mouse 3T3 fibroblasts. We evaluated formation of transformed foci after culturing the cells for 14 days. We subcutaneously injected the same set of 3T3 cells into nu/nu mice and examined tumor formation after 20 days.

PCR for IGH *gene rearrangement*

Genomic PCR was used for amplification of the rearranged *IGH* gene using the primers FR2A 5′-TGG(A/G)TCCG(A/C)CAG (C/G)C(C/T)(C/T)CNGG-3′ and LJH 5′-ACCTGAGGAGACGGTGACC-3′. Several clones were sequenced after subcloning the PCR product into pGEM-T-Easy Vector (Promega).

Results and Discussion

Case presentation

A 67-year old man was admitted with a tumor in the left side of his neck. A systemic workup revealed swelling of cervical, mediastinal, and hilar lymph nodes. Blood counts were within normal ranges. Lactose dehydrogenase was slightly elevated (223 IU/L) in peripheral blood with high IgG (2,425 mg/dL), normal IgA (157 mg/dL) and low IgM (32 mg/dL) levels.

Histopathological examination of the biopsied specimen from the cervical lymph node showed a diffuse infiltrate of tumor cells with a round, vesicular nucleus containing a centrally located large nucleolus. The cytoplasm was abundant (Figure 1A). These features may be consistent with immunoblasts or plasmablasts, but the size of tumor cells was large compared with typical immunoblasts and plasmablasts. Immunophenotypically, the tumor cells were negative for CD3, CD4, CD5, CD10, CD20, CD57, CD79a, and most cytokeratins (CK5/6, CK8, CK19, CK20); focally positive for CD30 and cytokeratins (AE1/AE3, CAM5.2, CK7, CK18) (Figure 1B); weakly positive for PAX5; and positive for CD138 (Figure

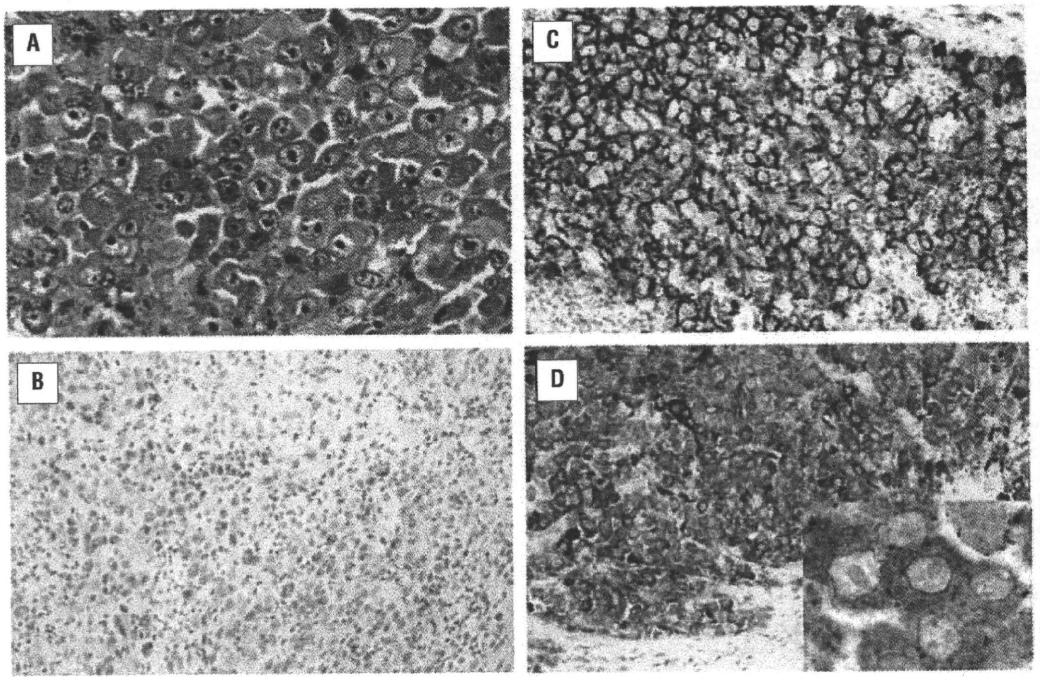

Figure 1. Histopathology of SQSTM1-ALK-positive large B-cell lymphoma. (A) The pattern of tumor infiltration was diffuse. The lymphoma cells were large with abundant cytoplasm and had round, vesicular nuclei, each containing a centrally located large nucleolus. These features may be consistent with immunoblasts or plasmablasts, but the size of tumor cells was extremely large compared with these typical cell types (40× objective). (B) Some lymphoma cells expressed cytokeratin (AE1/AE3) (20× objective). (C) Syndecan1/CD138 was strongly expressed (20× objective). (D) In anti-ALK immunohistochemistry, a diffuse cytoplasmic staining pattern with ill-demarcated spots was clearly shown (20× objective).

1C), EMA, and ALK (Figure 1D). The positivity of focal cytokeratin, which has been reported in a small proportion of ALK+LBCL cases,[15] and the cytomorphology of this case may have led to a misdiagnosis of undifferentiated metastatic carcinoma. The presence of *ALK* translocation was demonstrated by an ALK split FISH assay, which was performed at a commercial laboratory (*data not shown*). The tumor cells were positive for PAX5, which is suggestive of ALK+LBCL. However, we carefully excluded a possibility of metastasis of ALK-positive lung cancer[10] because the tumor cells were positive for some cytokeratins and immunohistochemistry for immunoglobulins was not evaluable due to background staining. Immunohistochemistry for TTF1 was negative; this is usually positive in ALK-positive lung cancers.[16] In addition, PCR and sequencing analyses revealed that *IGH* was monoclonally rearranged and somatically hypermutated (*data not shown*).

The patient was diagnosed as having ALK+LBCL and achieved complete remission after 6 cycles of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) treatment. Four months later, however, he relapsed.

Identification of SQSTM1-ALK

The 2 major ALK fusions in ALK+LBCL are CLTC-ALK and NPM-ALK, and they show a coarse granular cytoplasmic pattern and a nuclear and cytoplasmic pattern in anti-ALK immunohistochemistry, respectively. In the present case, anti-ALK immunohistochemistry showed a diffuse cytoplasmic staining pattern with ill-demarcated spots (Figure 1D), which was different from either of the former 2 patterns. Therefore, we carried out inverse RT-PCR to examine the presence of a novel fusion of *ALK*. We indeed isolated a cDNA containing the exon 5 of *SQSTM1* in-frame fused to the exon 20 of *ALK* (Figure 2A). A separate RT-PCR assay amplified the fusion point of *SQSTM1-ALK* cDNA (*data not shown*). To confirm the chromosome rearrangement, we performed *SQSTM1-ALK* fusion FISH. This result was consistent with the presence of a t(2;5)(p23.1;q35.3) leading to the generation of *SQSTM1-ALK* (Figure 2B). The complete sequences of SQSTM1-ALK are shown in the *Online Supplementary Figure S1*.

SQSTM1 is an ubiquitin binding protein that is associated with oxidative stress, cell signaling, and autophagy.[17-20] Autophagosomal membrane protein LC3/Atg8 binds SQSTM1 and makes SQSTM1-containing protein aggregate to the autophagosome.[21] Mutations within *SQSTM1* are identified in patients with Paget's disease of bone.[22]

SQSTM1 is located very near *NPM*, which is on 5q35.1. Therefore, the cytogenetic findings of the NPM-ALK-positive and the SOSTM1-ALK-positive lymphomas may be similar, and this may mean that SQSTM1-ALK occurrence in lymphoma may be underestimated. As mentioned, however, NPM-ALK and SQSTM1-ALK differ in terms of the anti-ALK immunostaining pattern. NPM has a nuclear transport signal, while SQSTM1 does not. Therefore, NPM-ALK shows a nuclear and cytoplasmic staining pattern while SQSTM1-ALK shows only a cytoplasmic staining pattern. ALK is a representative "promiscuous" molecule because of its various fusion partners. The subcellular localization of ALK fusions depends on the fusion partners. The anti-ALK immunohistochemical staining pattern is, therefore, a simple and useful means to identify the possible partner in a tested case and, in fact, has prompted the identification of many ALK fusion partners, including the present case.

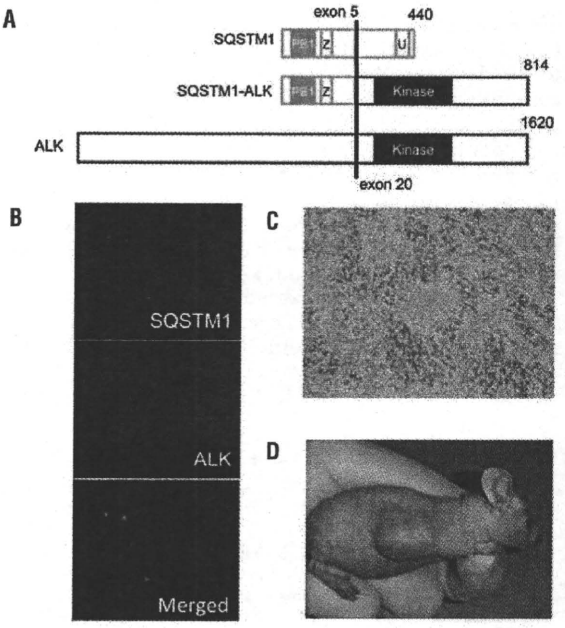

Figure 2. Discovery of *SQSTM-ALK* fusion gene. (A) A chromosome translocation, t(2;5)(p23.1;q35.3), generates a cDNA fusion in which exon 5 of *SQSTM* is joined to the *ALK* cDNA for the intracellular region of its encoded protein (containing the tyrosine kinase domain). Numbers indicate amino acid positions of each protein. PB1: Phox and Bem1p; Z: atypical zinc finger; U: ubiquitin-associated. (B) A section of the specimen for the present case was subjected to FISH with an *SQSTM1-ALK* fusion assay. Nuclei are stained blue with DAPI. (C) Murine 3T3 fibroblasts were infected with retroviruses expressing SQSTM1-ALK. The cells were photographed after culture for 14 days. (D) A nude mouse was injected subcutaneously with 3T3 cells infected as in (C), and tumor formation was examined after 20 days.

Transforming activities of SQSTM1-ALK

We generated a recombinant retrovirus encoding SQSTM1-ALK and used it to infect cultured 3T3 fibroblasts. Infection with the virus, but not with an empty virus, resulted in the formation of multiple transformed foci *in vitro* (Figure 2C). As control experiments for formation, EML4-ALK (variant 1) and NPM-ALK similarly produced transformed foci (*data not shown*). The same 3T3 cells were injected into nude mice for an *in vivo* tumorigenicity assay. As expected, 3T3 cells expressing SQSTM1-ALK developed subcutaneous tumors at all injection sites within an observation period of 20 days (Figure 2D), confirming the transforming potential of the novel fusion kinase, SQSTM1-ALK.

All ALK fusion partners identified so far except moesin (MSN) have a coiled-coil domain(s) in their sequences, and the domain is conserved in its fusion form. The coiled-coil domain allows the protein to homodimerize. The tyrosine kinase domain of the ALK fusions is constitutively phosphorylated and activated through homodimerization via

the coiled-coil domain. It has been speculated that the binding properties of MSN to cell membrane proteins lead to the dimerization of MSN-ALK proteins, enabling the constitutive phosphorylation of the chimeric MSN-ALK protein.[23] SQSTM1 does not harbor a coiled-coil domain and does not bind to membrane proteins. Instead, it has the Phox and Bem1p (PB1) domain in its N-terminus and forms heteromeric and homomeric complexes mediated by this domain.[24] Therefore, SQSTM1-ALK probably homodimerizes through the PB1 domain, leading to constitutive activation of the ALK kinase domain.

In conclusion, we reported a novel ALK fusion, SQSTM1-ALK, and its oncogenicity. ALK+LBCL is an aggressive lymphoma with poor prognosis;[3] ALK inhibitors are promising therapeutic agents for this condition. SQSTM1-ALK may be a rare fusion, but our data provide novel biological insights and may serve as a key to the accurate diagnosis of this rare lymphoma.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

References

1. Delsol G, Lamant L, Mariame B, Pulford K, Dastugue N, Brousset P, et al. A new subtype of large B-cell lymphoma expressing the ALK kinase and lacking the 2; 5 translocation. Blood. 1997;89(5):1483-90.
2. Beltran B, Castillo J, Salas R, Quinones P, Morales D, Hurtado F, et al. ALK-positive diffuse large B-cell lymphoma: report of four cases and review of the literature. J Hematol Oncol. 2009;2:11.
3. Laurent C, Do C, Gascoyne RD, Lamant L, Ysebaert L, Laurent G, et al. Anaplastic lymphoma kinase-positive diffuse large B-cell lymphoma: a rare clinicopathologic entity with poor prognosis. J Clin Oncol. 2009;27(25):4211-6.
4. Gascoyne RD, Lamant L, Martin-Subero JI, Lestou VS, Harris NL, Muller-Hermelink HK, et al. ALK-positive diffuse large B-cell lymphoma is associated with Clathrin-ALK rearrangements: report of 6 cases. Blood. 2003;102(7):2568-73.
5. De Paepe P, Baens M, van Krieken H, Verhasselt B, Stul M, Simons A, et al. ALK activation by the CLTC-ALK fusion is a recurrent event in large B-cell lymphoma. Blood. 2003;102(7):2638-41.
6. Chikatsu N, Kojima H, Suzukawa K, Shinagawa A, Nagasawa T, Ozawa H, et al. ALK+, CD30-, CD20- large B-cell lymphoma containing anaplastic lymphoma kinase (ALK) fused to clathrin heavy chain gene (CLTC). Mod Pathol. 2003;16(8):828-32.
7. Onciu M, Behm FG, Downing JR, Shurtleff SA, Raimondi SC, Ma Z, et al. ALK-positive plasmablastic B-cell lymphoma with expression of the NPM-ALK fusion transcript: report of 2 cases. Blood. 2003;102(7):2642-4.
8. Adam P, Katzenberger T, Seeberger H, Gattenlohner S, Wolf J, Steinlein C, et al. A case of a diffuse large B-cell lymphoma of plasmablastic type associated with the t(2;5)(p23;q35) chromosome translocation. Am J Surg Pathol. 2003;27(11):1473-6.
9. Van Roosbroeck K, Cools J, Dierickx D, Thomas J, Vandenberghe P, Stul M, et al. ALK-positive large B-cell lymphomas with cryptic SEC31A-ALK and NPM1-ALK fusions. Haematologica. 2010;95(3):509-13.
10. Takeuchi K, Choi YL, Togashi Y, Soda M, Hatano S, Inamura K, et al. KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. Clin Cancer Res. 2009;15(9):3143-9.
11. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature. 2007;448(7153):561-6.
12. Takeuchi K, Choi YL, Soda M, Inamura K, Togashi Y, Hatano S, et al. Multiplex reverse transcription-PCR screening for EML4-ALK fusion transcripts. Clin Cancer Res. 2008;14(20):6618-24.
13. Choi YL, Takeuchi K, Soda M, Inamura K, Togashi Y, Hatano S, et al. Identification of novel isoforms of the EML4-ALK transforming gene in non-small cell lung cancer. Cancer Res. 2008;68(13):4971-6.
14. Onishi M, Kinoshita S, Morikawa Y, Shibuya A, Phillips J, Lanier LL, et al. Applications of retrovirus-mediated expression cloning. Exp Hematol. 1996;24:324-9.
15. Reichard KK, McKenna RW, Kroft SH. ALK-positive diffuse large B-cell lymphoma: report of four cases and review of the literature. Mod Pathol. 2007;20(3):310-9.
16. Inamura K, Takeuchi K, Togashi Y, Hatano S, Ninomiya H, Motoi N, et al. EML4-ALK lung cancers are characterized by rare other mutations, a TTF-1 cell lineage, an acinar histology, and young onset. Mod Pathol. 2009;22(4):508-15.
17. Komatsu M, Kurokawa H, Waguri S, Taguchi K, Kobayashi A, Ichimura Y, et al. The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. Nat Cell Biol. 2010;12(3):213-23.
18. Kirkin V, McEwan DG, Novak I, Dikic I. A role for ubiquitin in selective autophagy. Mol Cell. 2009;34(3):259-69.
19. Seibenhener ML, Geetha T, Wooten MW. Sequestosome 1/p62--more than just a scaffold. FEBS Lett. 2007;581(2):175-9.
20. Bjorkoy G, Lamark T, Johansen T. p62/SQSTM1: a missing link between protein aggregates and the autophagy machinery. Autophagy. 2006;2(2):138-9.
21. Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, et al. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem. 2007;282(33):24131-45.
22. Laurin N, Brown JP, Morissette J, Raymond V. Recurrent mutation of the gene encoding sequestosome 1 (SQSTM1/p62) in Paget disease of bone. Am J Hum Genet. 2002;70(6):1582-8.
23. Tort F, Pinyol M, Pulford K, Roncador G, Hernandez L, Nayach I, et al. Molecular characterization of a new ALK translocation involving moesin (MSN-ALK) in anaplastic large cell lymphoma. Lab Invest. 2001;81(3):419-26.
24. Lamark T, Perander M, Outzen H, Kristiansen K, Overvatn A, Michaelsen E, et al. Interaction codes within the family of mammalian Phox and Bem1p domain-containing proteins. J Biol Chem. 2003;278(36):34568-81.

Published Ahead of Print on January 12, 2011, as doi:10.3324/haematol.2010.028605

haematologica
the hematology journal

Early Release Paper

Overexpression of enhancer of zeste homolog 2 with trimethylation of lysine 27 on histone H3 in adult T-cell leukemia/lymphoma as a target for epigenetic therapy

by Daisuke Sasaki, Yoshitaka Imaizumi, Hiroo Hasegawa, Akemi Osaka, Kunihiro Tsukasaki, Young Lim Choi, Hiroyuki Mano, Victor Marquez, Tomayoshi Hayashi, Katsunori Yanagihara, Yuji Moriwaki, Yasushi Miyazaki, Shimeru Kamihira, and Yasuaki Yamada

Haematologica 2010 [Epub ahead of print]

Citation: Sasaki D, Imaizumi Y, Hasegawa H, Osaka A, Tsukasaki K, Choi YL, Mano H, Marquez V, Hayashi T, Yanagihara K, Moriwaki Y, Miyazaki Y, Kamihira S, and Yamada Y. Overexpression of enhancer of zeste homolog 2 with trimethylation of lysine 27 on histone H3 in adult T-cell leukemia/lymphoma as a target for epigenetic therapy. Haematologica. 2010; 95:xxx doi:10.3324/haematol.2010.028605

Haematologica (pISSN: 0390-6078, eISSN: 1592-8721, NLM ID: 0417435, www.haematologica.org) publishes peer-reviewed papers across all areas of experimental and clinical hematology. The journal is owned by the Ferrata Storti Foundation, a non-profit organization, and serves the scientific community with strict adherence to the principles of open access publishing (www.doaj.org). In addition, the journal makes every paper published immediately available in PubMed Central (PMC), the US National Institutes of Health (NIH) free digital archive of biomedical and life sciences journal literature.

Official Organ of the European Hematology Association
Published by the Ferrata Storti Foundation, Pavia, Italy
www.haematologica.org

DOI: 10.3324/haematol.2010.028605

Overexpression of enhancer of zeste homolog 2 with trimethylation of lysine 27 on histone H3 in adult T-cell leukemia/lymphoma as a target for epigenetic therapy

Daisuke Sasaki,[1] Yoshitaka Imaizumi,[2] Hiroo Hasegawa,[1] Akemi Osaka,[1] Kunihiro Tsukasaki,[2] Young Lim Choi,[3] Hiroyuki Mano,[3] Victor E. Marquez,[4] Tomayoshi Hayashi,[5] Katsunori Yanagihara,[1] Yuji Moriwaki,[2] Yasushi Miyazaki,[2] Shimeru Kamihira,[1] and Yasuaki Yamada[1]

[1]Department of Laboratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [2]Department of Hematology and Molecular Medicine, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [3]Division of Functional Genomics, Jichi Medical University, Tochigi, Japan; [4]Chemical Biology Laboratory, National Cancer Institute, Frederick, MD, USA, and [5]Department of Pathology, Nagasaki University Hospital, Nagasaki, Japan

Funding

supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour, and Welfare of Japan (No. 04010119). For VEM, this research was supported in part by the Intramural Research Program of the NIH, Center for Cancer Research, NCI-Frederick.

DOI: 10.3324/haematol.2010.028605

Acknowledgments

The authors thank Sayaka Mori and Yuko Doi for excellent technical assistance.

Correspondence

Yasuaki Yamada, Department of Laboratory Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. Phone: international +81.2958197408.

Fax: international +81.959197422. E-mail: y-yamada@nagasaki-u.ac.jp

DOI: 10.3324/haematol.2010.028605

ABSTRACT

Background

Enhancer of zeste homolog 2 is a component of the Polycomb repressive complex 2 that mediates chromatin-based gene silencing through trimethylation of lysine 27 on histone H3. This complex plays vital roles in the regulation of development-specific gene expression.

Design and Methods

In this study, a comparative microarray analysis of gene expression in primary adult T-cell leukemia/lymphoma samples was performed, and the results were evaluated for their oncogenic and clinical significance.

Results

Significantly higher levels of *Enhancr of zeste homolog 2* and *RING1 and YY1 binding protein* transcripts with enhanced levels of trimethylation of lysine 27 on histone H3 were found in adult T-cell leukemia/lymphoma cells compared with those in normal CD4+ T-cells. Furthermore, there was an inverse correlation between the expression level of *Enhancer of zeste homolog 2* and that of miR-101 or miR-128a, suggesting that the altered expression of the latter miRNAs accounts for the overexpression of the former. Patients with high *Enhancer of zeste homolog 2* or *RING1 and YY1 binding protein* transcripts had a significantly worse prognosis than those without it, indicating a possible role

DOI: 10.3324/haematol.2010.028605

of these genes in the oncogenesis and progression of this disease. Indeed, adult T-cell leukemia/lymphoma cells were sensitive to a histone methylation inhibitor, 3-deazaneplanocin A. Furthermore, 3-deazaneplanocin A and histone deacetylase inhibitor panobinostat showed a synergistic effect in killing the cells.

Conclusions

These findings reveal that adult T-cell leukemia/lymphoma cells have deregulated Polycomb repressive complex 2 with overexpressed Enhancer of zeste homolog 2, and that there is the possibility of a new therapeutic strategy targeting histone methylation in this disease.

Introduction

The Polycomb group (PcG) proteins play critical roles in the regulation of development by repressing specific sets of developmental genes through chromatin modification.[1] They form two distinct multimeric complexes, Polycomb repressive complex 1 (PRC1) and PRC2, which bind to polycomb responsive elements (PRE), repress genes required for cell differentiation, and maintain pluripotency and self-renewal of embryonic stem cells and hematopoietic stem cells.[2,3] PRC2 consists of Enhancer of zeste homolog 2 (EZH2), which has histone methyltransferase activity, suppressor of zeste 12 (SUZ12), and embryonic ectoderm development (EED), which is required to maintain the integrity of PRC2.[1,4] Sequence-specific DNA binding protein YY1,