Ishida T Hishizawa M Kato K Tanosaki R Fukuda T Takatsuka Y Eto T Miyazaki Y Hidaka M Uike N Miyamoto T Tsudo M Sakamaki H Morishima Y Suzuki R Utsunomiya A	Impact of GVHD on allogeneic HCT for adult T-cell leukemia-lymphoma focusing on preconditioning regimens: nationwide retrospective study.	Biol Blood Marrow Transplant	19(12)	1731-9	2013
Kinpara S Kijiyama M Takamori A Hasegawa A Sasada A Masuda T Tanaka Y Utsunomiya A Kannagi M	Interferon-α (IFN-α) suppresses human T-lymphotropic virus type-1 (HTLV-1) gene expression and cell cycling, while IFN-α combined with zidovudin induces p53 signaling and apoptosis in HTLV-1-infected cells.	Retrovirol	10	52	2013
Ohsugi T Wakamiya M Morikawa S Matsuura K Kumar JM, Kumasaka T Yamaguchi K	Invasion of histiocytic sarcoma into the spinal cord of HTLV-1 tax transgenic mice with HTLV-1-associated myelopathy/tropical spastic paraparesis-like disease.	Oncol Res	20	403-10	2013
Ohno N Kobayashi S Ishigaki T Yuji K Kobayashi M Sato K Watanabe N Tojo A Uchimaru K	Loss of CCR4 antigen expression after mogamulizumab therapy in a case of adult T-cell leukaemia-lymphoma.	Br J Haematol	163	683-5	2013

Odaka C Kato H Otsubo H Takamoto S Okada Y Taneichi M Okuma K Sagawa K Hoshi Y Tasaki T, Fujii Y, Yonemura Y Iwao N Tanaka A Okazaki H Momose S Kitazawa J Mori H Matsushita A Nomura H Yasoshima H Ohkusa Y Yamaguchi K Hamaguchi I	Online reporting system for transfusion-related adverse events to enhance recipient haemovigilance in Japan: a pilot study.	Transfus Apher Sci	48	95-102	2013
Ikebe E Kawaguchi A Tezuka K Taguchi S Hirose S Matsumoto T Mitsui T Senba K Nishizono A Hori M Hasegawa H Yamada Y Ueno T Tanaka Y Sawa H Hall W Minami Y Jeang KT Ogata M Morishita K Hasegawa H Fujisawa J Iha H	Oral administration of an HSP90 inhibitor, 17-DMAG, intervenes tumor-cell infiltration into multiple organs and improves survival period for ATL model mice.	Blood Cancer J	3	e132	2013

Ando H Sato T Tomaru U Yoshida M Utsunomiya A Yamauchi J Araya N Yagishita N Coler-Reilly A Shimizu Y Yudo K Nishioka K Nakajima T Jacobson S Yamano Y	Positive feedback loop via astrocytes causes chronic inflammation in virus-associated myelopathy.	Brain	136 (Pt 9)	2876-87	2013
Tamai Y Hasegawa A Takamori A Sasada A Tanosaki R Choi I Utsunomiya A Maeda Y Yamano Y Eto T Koh KR Nakamae H Suehiro Y Kato K Takemoto S Okamura J Uike N Kannagi M	Potential contribution of a novel Tax epitope-specific CD4+ T cells to graft-versus-Tax effect in adult T cell leukemia patients after allogeneic hematopoietic stem cell transplantation.	J Immunol	190	4382-92	2013
Ishihara M Araya N Sato T Tatsuguchi A Saichi N Utsunomiya A Nakamura Y Nakagawa H Yamano Y Ueda K	Preapoptotic protease calpain-2 is frequently suppressed in adult T-cell leukemia.	Blood	121 (21)	4340-7	2013

Nakano N Kusumoto S Tanaka Y Ishida T Takeuchi S Takatsuka Y Akinaga S Mizokami M Ueda R Utsunomiya A	Reactivation of hepatitis B virus in a patient with adult T-cell leukemia-lymphoma receiving the anti-CC chemokine receptor 4 antibody mogamulizumab.	Hepatol Res	Mar 26		2013
Kobayashi S Tian Y Ohno N Yuji K Ishigaki T Isobe M Ohfuchi-Tsuda M Oyaizu N Watanabe E Watanabe N Tani K Tojo A Uchimaru K	The CD3 versus CD7 plot in multicolor flow cytometry reflects progression of disease stage in patients infected with HTLV-I.	PLoS One	8	e53728	2013
Umekita K Hidaka T Miyauchi S Ueno S Kubo K Takajo I Hashiba Y Kai Y Nagatomo Y Okayama A	Treatment with anti-tumor necrosis factor biologics in human T-lymphotropic virus type 1 positive patients with rheumatoid arthritis.	Arthritis Care Res (Hoboken)	Oct 14		2013
Umekita K Umeki K Miyauchi S Ueno S Kubo K Kusumoto N Takajo I Nagatomo Y Okayama A	Use of anti-tumor necrosis factor biologics in the treatment of rheumatoid arthritis does not change human T-lymphotropic virus type 1 markers: a case series.	Mod Rheumatol	Nov 4		2013

Grassi MF Olavarria VN Kruschewsky Rde A Silva MT Yamano Y Jacobson S Taylor GP Martin F Galvão-Castro B	Utility of HTLV proviral load quantification in diagnosis of HTLV-1-associated myelopathy requires international standardization.	J Clin Virol	58(3)	584-6	2013
Nakano K Ando T Yamagishi M Yokoyama K Ishida T Ohsugi T Tanaka Y Brighty D-W Watanabe T	Viral interference with host mRNA surveillance, the nonsense-mediated mRNA decay (NMD) pathway, through a new function of HTLV-1 Rex: implications for retroviral replication.	Microbes Infect	15	491-505	2013
Iwanaga M Watanabe T Yamaguchi K	Adult T-cell leukemia: a review of epidemiological evidence.	Front Microbiol	3	322	2012
Ishida T Hishizawa M Kato K Tanosaki R Fukuda T Taniguchi S Eto T Takatsuka Y Miyazaki Y Moriuchi Y Hidaka M Akashi K Uike N Sakamaki H Morishima Y Kato K Suzuki R Nishiyama T Utsunomiya A	Allogeneic hematopoietic stem cell transplantation for adult T-cell leukemia-lymphoma with special emphasis on preconditioning regimen: a nationwide retrospective study.	Blood	120(8)	1734-41	2012
Uota S Dewan MZ Saitoh Y Muto S Itai A Utsunomiya A Watanabe T Yamamoto N Yamaoka S	An IκB kinase 2 inhibitor IMD-0354 suppresses the survival of adult T-cell leukemia cells.	Cancer Sci	103(1)	100-6	2012

Nishikawa H Maeda Y Ishida T Gnjatic S Sato E Mori F Sugiyama D Ito A Fukumori Y Utsunomiya A Inagaki H Old LJ Ueda R Sakaguchi S	Cancer/testis antigens are novel targets of immunotherapy for adult T cell leukemia/lymphoma.	Blood	119(13)	3097-104	2012
Nakahata S Saito Y Marutsuka K Hidaka T Maeda K Hatakeyama K Shiraga T Goto A Takamatsu N Asada Y Utsunomiya A Okayama A Kubuki Y Shimoda K Ukai Y Kurosawa G Morishita K	Clinical significance of CADM1/TSLC1/IgSF4 expression in adult T-cell leukemia/lymphoma.	Leukemia	26	1238-46	2012
Ishida T Joh T Uike N Yamamoto K Utsunomiya A Yoshida S Saburi Y Miyamoto T Takemoto S Suzushima H Tsukasaki K Nosaka K Fuzjiwara H Ishitsuka K Inagaki H Ogura M Akinaga S Tomonaga M Tobinai K Ueda R	Defucosylated anti-CCR4 monoclonal antibody (KW-0761) for relapsed adult T-cell leukemia-lymphoma: a multicenter Phase II study.	J Clin Oncol	30(8)	837-42	2012

Otani M Honda N Xia PC Eguchi K Ichikawa T Watanabe T Yamaguchi K Nakao K Yamamoto T	Distribution of Two Subgroups of Human T-Lymphotropic Virus Type 1 (HTLV-1) in Endemic Japan.	Trop Med Health	40	55-8	2012
Yamano Y Sato T	Clinical pathophysiology of human T-lymphotropic virus-type1-associated myelopathy/tropical spastic paraparesis.	Front Microbiol	3:389	1-10	2012
Kamihira S Iwanaga M Doi Y Sasaki D Mori S Tsurda K Nagai K Uno N Hasegawa H Yanagihara K Morinaga Y Tsukasaki K Taniguchi H	Heterogeneity in clonal nature in the smoldering subtype of adult T-cell leukemia: continuity from carrier status to smoldering ATL.	Int J Hematol	95	399-408	2012
Sugata K Satou Y Yasunaga J Hara H Suzutani T Ohshima K Utsunomiya A Mitsuyama M Matsuoka M	HTLV-1 bZIP factor impairs cell-mediated immunity by suppressing production of Th1 cytokines.	Blood	119(2)	434-44	2012
Kamoi K Mochizuki M	HTLV infection and the eye.	Curr Opin Ophthalmol	23	557-61	2012
Satou Y Utsunomiya A Tanabe J Nakagawa M Nosaka K Matsuoka M	HTLV-1 modulates the frequency and phenotype of FoxP3+CD4+ T cells in the HTLV-1 infected individuals.	Retrovirology	9(1)	46	2012

Fukuda RI Tsuchiya K Suzuki K Itoh K Fujita J Utsunomiya A Tsuji T	HTLV-I Tax regulates the cellular proliferation through the down-regulation of PIP3-phosphatase expressions via the NF-κB pathway.	Int J Biochem Mol Biol	3(1)	95-104	2012
Kamoi K Mochizuki M	HTLV-1 uveitis.	Front Microbiol	3	270	2012
Ichikawa T Taura N Miyaaki H Matsuzaki T Ohtani M Eguchi S Takatsuki M Soyama A Hidaka M Okudaira S Usui T Mori S Kamihira S Kanematsu T Nakao K	Human T-cell leukemia virus type 1 infection worsens prognosis of hepatitis C virus-related living donor liver transplantation.	Transpl Int	25	433-8	2012
Kanda J Hishizawa M Utsunomiya A Taniguchi S Eto T Moriuchi Y Tanosaki R Kawano F Miyazaki Y Masuda M Nagafuji K Hara M Takanashi M Kai S Atsuta Y Suzuki R Kawase T Matsuo K Nagamura-Inoue T Kato S Sakamaki H Morishima Y Okamura J Ichinohe T Uchiyama T	Impact of graft-versus-host disease on outcomes after allogeneic hematopoietic cell transplantation for adult T-cell leukemia: a retrospective cohort study.	Blood	119(9)	2141-8	2012

Ishihara K Sasaki D Tsuruda K Inokuchi N Nagai K Hasegawa H Yanagihara K Kamihira S	Impact of miR-155 and miR-126 as novel biomarkerson the assessment of disease progression and prognosis in adult T-cell leukemia.	Cancer Epidemiol	36	560-5	2012
Higashi Y Kawai K Yonekura K Takeda K Kanzaki T Utsunomiya A Kanekura K	Indication for random skin biopsy for the diagnosis of intravascular large B cell lymphoma.	Dermatology	224	46-50	2012
Yamagishi M Watanabe T	Molecular hallmarks of adult T cell leukemia.	Front Microbiol	17;3	334	2012
Yamagishi M Nakano K Miyake A Yamochi T Kagami Y Tsutsumi A Matsuda Y Sato-Otsubo A Muto S Utsunomiya A Yamaguchi K Uchimaru K Ogawa S Watanabe T	Polycomb-Mediated Loss of miR-31 Activates NIK-Dependent NF-kB Pathway in Adult T Cell Leukemia and Other Cancers.	Cancer Cell	21	121-35	2012

Katsuya H Yamanaka T Ishitsuka K Utsunomiya A Sasaki H Hanada S Eto T Moriuchi Y Saburi Y Miyahara M Sueoka E Uike N Yoshida S Yamashita K Tsukasaki K Suzushima H Ohno Y Matsuoka H Jo T Suzumiya J Tamura K	Prognostic index for acute- and lymphoma-type adult T-cell leukemia/lymphoma.	J Clin Oncol	30(14)	1635-40	2012
Ueno S Umeki K Takajo I Nagatomo Y Kusumoto N Umekita K Morishita K Okayama A	Proviral loads of human T-lymphotropic virus type 1 in asymptomatic carriers with different infection routes.	Int J Cancer	130	2318-26	2012
Haraguchi K Uto H Ohnou N Tokunaga M Tokunaga M Utsunomiya A Hanada S Tsubouchi H	Serum prohepcidin levels are potential prognostic markers in patients with multiple myeloma.	Exp Ther Med	4(4)	581-8	2012
Kannagi M Hasegawa A Takamori A Kinpara S Utsunomiya A	The roles of acquired and innate immunity in human T-cell leukemia virus type 1-mediated diseases.	Front Microbiol	3(323)	1-10	2012
Matsumoto C Igarashi M Furuta RA Uchida S Satake M Tadokoro K	Xenotropic murine leukemia virus-related virus proviral DNA not detected in blood samples donated in Japan.	Jpn J Infect Dis	65	334-6	2012

Kitazono T Okazaki T Araya N Yamano Y Yamada Y Nakamura T Tanaka Y Inoue M Ozaki S	Advantage of higher-avidity CTL specific for Tax against human T-lymphotropic virus-1 infected cells and tumors.	Cell Immunol	272(1)	11-7	2011
Umino A Nakagawa M Utsunomiya A Tsukasaki K Taira N Katayama N Seto M	Clonal evolution of adult T-cell leukemia/ lymphoma takes place in lymph node.	Blood	117(20)	5473-8	2011
Oka T Satou H Ouchida M Utsunomiya A Yoshino T	Cumulative epigenetic abnormalities in host genes with viral and microbial infection during initiation and progression of malignant lymphoma/leukemia.	Cancers (Basel)	3	568-81	2011
Watanabe T	Current status of HTLV-1 infection.	Int J Hematol	94	430-4	2011
Kannagi M Hasegawa A Kinpara S Shimizu Y Takamori A Utsunomiya A	Double control systems for human T-cell leukemia virus type 1 (HTLV-1) by innate and acquired immunity.	Cancer Sci	102(4)	670-6	2011

Koh H Nakamae H Hagihara K Nakane T Manabe M Hayashi Y Nishimoto M Umemoto Y Nakamae M Hirose A Inoue E Inoue A Yoshida M Bingo M Okamura H Aimoto R Aimoto M Terada Y Koh KR Yamane T Ohsawa M Hino M	Factors that contribute to long-term survival in patients with leukemia not in remission at allogeneic hematopoietic cell transplantation.	Exp Clin Cancer Res	30	36	2011
Araya N Takahashi K Sato T Nakamura T Sawa C Hasegawa D Ando H Aratani S Yagishita N Fujii R Oka H Nishioka K Nakajima T Mori N Yamano Y	Fucoidan therapy decreases the proviral load in patients with human T-lymphotropic virus type1-associated neurological disease.	Antivir Ther	16(1)	89-98	2011

Takamori A Hasegawa A Utsunomiya A Maeda Y Yamano Y Masuda M Shimizu Y Tamai Y Sasada A Zeng N Choi I Uike N Okamura J Watanabe T Masuda T Kannagi M	Functional impairment of Tax-specific but not cytomegalovirus-specific CD8+ cytotoxic T lymphocytes in a minor population of asymptomatic human T-cell leukemia virus type 1-carriers.	Retrovirology	7(8)	100(1-15)	2011
Ogata M Satou T Kawano R Yoshikawa T Ikewaki J Kohno K Ando T Miyazaki Y Ohtsuka E Saburi Y Kikuchi H Saikawa T Kadota J	High incidence of cytomegalovirus, human herpesvirus-6, and Epstein-Barr virus reactivation in patients receiving cytotoxic chemotherapy for adult T cell leukemia.	J Med Virol	83	702-9	2011
Araya N Sato T Yagishita N Ando H Utsunomiya A Jacobson S Yamano Y	Human T-lymphotropic virus type 1 (HTLV-1) and regulatory T cells in HTLV-1-associated neuroinflammatory disease.	Viruses	3(9)	1532-48	2011
Tian Y Kobayashi S Ohno N Isobe M Tsuda M Zaike Y Watanabe N Tani K Tojo A Uchimaru K	Leukemic T cells are specifically enriched in a unique CD3(dim) CD7(low) subpopulation of CD4(+) T cells in acute-type adult T-cell leukemia.	Cancer Sci	102	569-77	2011

Sasaki D Imaizumi Y Hasegawa H Osaka A Tsukasaki K Choi YL Mano H Marquez VE 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	96	712-9	2011
Kozako T Akimoto M Toji S White Y Suzuki S Arima T Suruga Y Matsushita K Shimeno H Soeda S Kubota R Izumo S Uozumi K Arima N	Target epitopes of HTLV-1 recognized by class I MHC-restricted cytotoxic T lymphocytes in patients with myelopathy and spastic paraparesis and infected patients with autoimmune disorders.	J Med Virol	8	501-9	2011
相良康子 後藤信代 井上由紀子 守田麻衣子 倉光球 大隈和 浜口功 入田和男 清川博之	抗HTLV-1抗体検査（ウエスタンブロット法）判定保留例の解析	日本輸血・細胞治療学会誌		印刷中	
齋藤　滋	HTLV-I抗体検査の理解	助産雑誌	68	17-21	2014
齋藤　滋 板橋家頭夫	シンポジウム2 「HTLV-I母子感染」 座長のまとめ	日本周産期・新生児医学会雑誌	48	4	2013
齋藤　滋	シンポジウム2 「HTLV-I母子感染」HTLV-I検査が全国で行なわれるようになった経緯	日本周産期・新生児医学会雑誌	48	5-7	2013

渡邉俊樹	特集：リンパ系腫瘍–最新の病態解析と治療–「成人T細胞白血病／リンパ腫の分子病態解析と治療の進歩」	最新医学	68(10)	40-7	2013
山野嘉久 佐藤知雄 宇都宮與	白血病　非定型白血病および特殊型　HTLV-1関連脊髄症（HAM）	別冊日本臨牀　新領域別症候群シリーズ　血液症候群（第2版）	23(Ⅲ)	195-9	2013
齋藤　滋	ヒト成人T細胞白血病ウイルス（HTLV-I） 母子感染予防対策	ペリネイタルケア	32	28-30	2013
吉田全宏 亀田和明 小川吉彦 金島広 中尾隆文 田邉順子 松岡雅雄 高　起良 山根孝久	末梢血でCD25陰性,リンパ節でCD25陽性を示した成人T細胞白血病/リンパ腫の1症例	日本検査血液学会雑誌	14巻2号	188-92	2013
山野嘉久 佐藤知雄	HTLV-1関連脊髄症（HAM）の病態・治療とバイオマーカー	日本臨牀	71(5)	870-5	2013
齋藤　滋	HTLV-Iと母子感染（解説）	日本産科婦人科学会誌	65	1658-63	2013
齋藤　滋	HTLV-I母子感染対策	産婦人科の実際	62	543-7	2013

山内淳司 八木下尚子 安藤仁 佐藤知雄 新谷奈津美 Ariella, Coler-Reilly 今井直彦 中澤龍斗 佐々木秀郎 柴垣有吾 安田隆 力石辰也 木村健二郎 山野嘉久	Human T-lymphotropic virus type 1感染者における腎移植の影響	日本臨床腎移植学会雑誌	1(1)	55-60	2013
渡邉俊樹	【成人T細胞白血病(ATL)】日本におけるHTLV-I/ATL研究,対策の歴史,現状	血液フロンティア	22	173-80	2012
山口一成	造血器腫瘍の疫学: 成人T細胞白血病・リンパ腫/HTLV-1感染の疫学. HTLV-1感染症の根絶に向けて.【造血器腫瘍学-基礎と臨床の最新研究動向】	日本臨床	70	32-6	2012
渡邉俊樹	総論 ATL研究の進展. 特集「ATLの基礎と臨床」	細胞	44	324-7	2012
岡山昭彦	特集/ストップ ザ 性感染症 性感染症—診断・治療 HTLV-1 感染	臨床と研究	89:7	907-10	2012
鮫島 梓 齋藤 滋	母児感染症の診断と管理	産婦人科の実際	61	1035-41	2012
岩永正子	ATLの疫学研究の現状と課題.特集「ATLの基礎と臨床」	細胞	44	8-11	2012
山野嘉久 佐藤知雄 安藤 仁 新谷奈津美 八木下尚子	HTLV-1 関連脊髄症 (HAM) の治療法を確立していくために—その現状と展望—	日本臨牀	70(4)	705-13	2012
齋藤 滋	HTLV-I母子感染対策のために助産師が知っておきたい知識	ペリネイタルケア	31	65-71	2012
齋藤 滋	座長のまとめ 教育講演10:「HTLV-I母子感染防止—長崎県における24年間の取り組み—」増崎英明	日本周産期・新生児医学会雑誌	47	772	2011

種部恭子 齋藤　滋 佐竹紳一郎 澤木　勝 十二町明 中山哲規 長谷川徹 布施秀樹	富山県における性感染症全数調査および定点の適正性に関する検討	日本性感染症学会誌	22	62-72	2011
齋藤　滋	妊婦健診における感染症スクリーニング検査	ロシュ・ダイアグノスティックス株式会社（リーフレット）			2011
山野嘉久 佐藤知雄 新谷奈津美 安藤 仁 八木下尚子	HAM専門外来の取り組み	神経内科	75（4）	387-92	2011
齋藤　滋	HTLV-I感染症	周産期医学	41	1099-103	2011
上平 憲	HTLV-1感染の特性と成人T細胞白血病-ウイルス母子感染対策	宝函	32	13-23	2011
安藤 仁 八木下尚子 新谷奈津美 佐藤知雄 山野嘉久	HTLV-1 関連脊髄症（HAM）の病態と治療	医療と検査機器・試薬	34(4)	472-7	2011
山野嘉久	HTLV-1キャリアー，HTLV-1-associated myelopathy(HAM)患者診療の現状と問題点	血液内科	63(1)	81-6	2011
渡邉俊樹	HTLV-1特命チームとHTLV-1/ATL研究	臨床血液	52	1439-47	2011
高崎由美 岩永正子 塚崎邦弘	話題：くすぶり型・慢性型成人T細胞白血病リンパ腫に対する無治療経過観察は適切な選択か?	血液内科	63	40-5	2011

IV. 研究成果の刊行物・印刷

Cancer Science

Adult T-cell leukemia cells are characterized by abnormalities of *Helios* expression that promote T cell growth

Satomi Asanuma,[1] Makoto Yamagishi,[1] Katsuaki Kawanami,[1] Kazumi Nakano,[1] Aiko Sato-Otsubo,[2] Satsuki Muto,[2] Masashi Sanada,[2] Tadanori Yamochi,[1] Seiichiro Kobayashi,[3] Atae Utsunomiya,[4] Masako Iwanaga,[5] Kazunari Yamaguchi,[6] Kaoru Uchimaru,[3] Seishi Ogawa[2] and Toshiki Watanabe[1,7]

[1]Graduate School of Frontier Sciences, The University of Tokyo; [2]Cancer Genomics Project, Graduate School of Medicine, The University of Tokyo; [3]Institute of Medical Science, The University of Tokyo, Tokyo; [4]Department of Hematology, Imamura Bun-in Hospital, Kagoshima; [5]Graduate School of Public Health, Teikyo University; [6]Department of Safety Research on Blood and Biological Products, National Institute of Infectious Diseases, Tokyo, Japan

(Received December 27, 2012/Revised April 11, 2013/Accepted April 15, 2013/Accepted manuscript online April 18, 2013/Article first published online May 19, 2013)

Molecular abnormalities involved in the multistep leukemogenesis of adult T-cell leukemia (ATL) remain to be clarified. Based on our integrated database, we focused on the expression patterns and levels of Ikaros family genes, *Ikaros*, *Helios*, and *Aiolos*, in ATL patients and HTLV-1 carriers. The results revealed profound deregulation of Helios expression, a pivotal regulator in the control of T-cell differentiation and activation. The majority of ATL samples (32/37 cases) showed abnormal splicing of Helios expression, and four cases did not express Helios. In addition, novel genomic loss in *Helios* locus was observed in 17/168 cases. We identified four ATL-specific short Helios isoforms and revealed their dominant-negative function. Ectopic expression of ATL-type Helios isoform as well as knockdown of normal Helios or Ikaros promoted T-cell growth. Global mRNA profiling and pathway analysis showed activation of several signaling pathways important for lymphocyte proliferation and survival. These data provide new insights into the molecular involvement of Helios function in the leukemogenesis and phenotype of ATL cells, indicating that Helios deregulation is one of the novel molecular hallmarks of ATL. (*Cancer Sci* 2013; 104: 1097–1106)

Adult T-cell leukemia (ATL) is a highly aggressive malignancy of mature CD4+ T cells and is caused by HTLV-1. After HTLV-1 infection, ATL is thought to develop following a multitude of events, including both genetic and epigenetic changes in the cells. Although many aspects of HTLV-1 biology have been elucidated, the detailed molecular mechanism of ATL leukemogenesis remains largely unknown.[1,2] Therefore, to precisely define the comprehensive abnormalities associated with ATL leukemogenesis, we previously carried out global mRNA and miRNA profiling of ATL cells derived from a large number of patients.[3,4] In this study, we focused on Ikaros family genes, especially Helios, on the basis of our integrated profiling of expression and gene copy number in ATL cells, which revealed the deregulated expression of this family of genes and genomic loss of *Helios* locus.

Ikaros family genes are specifically expressed in the hematopoietic system and play a vital role in regulation of lymphoid development and differentiation.[5–11] In addition, they are known to function as tumor suppressors during leukemogenesis according to several genetic studies carried out in mouse models.[12–15] Recently, many studies reported the deregulated splicing of Ikaros and the deletion of *Ikaros* locus in several human leukemias.[16–23] These abnormalities are associated with poor prognoses.[24–27] Helios is mainly expressed in the T-cell lineage.[10,11] Genomic changes and abnormal expression of Helios are also observed in some patients with T-cell malignancies.[18,28–31] However, in contrast to Ikaros, the substantial impact of aberrant Helios expression remains to be elucidated because of the absence of functional information, including the target genes of Helios.

In this study, we carried out a detailed expression analysis of Ikaros family genes in a large panel of clinical samples from ATL patients and HTLV-1 carriers and consequently identified a novel molecular characteristic, that is, abnormal splicing of Helios and loss of expression, which seems to be a significant key factor in leukemogenesis affecting the regulation of T-cell proliferation.

Materials and Methods

Cell lines and clinical samples. HeLa and 293T cells were cultivated in DMEM supplemented with 10% FCS. Human leukemic T cells, Jurkat, Molt-4, and CEM, ATL-derived, MT-1 and TL-Om1, and HTLV-1-infected MT-2 and Hut-102 cell lines were all maintained in RPMI-1640 with 10% FCS. The PBMCs from ATL patients of four clinical subtypes[32] and healthy volunteers were a part of those collected with informed consent as a collaborative project of the Joint Study on Prognostic Factors of ATL Development. The project was approved by the Institute of Medical Sciences, University of Tokyo Human Genome Research Ethics Committee (Tokyo, Japan). Clinical information of ATL individuals is provided in Table S1.

RNA isolation and RT-PCR analysis. The preparation of total RNA and synthesis of the first strand of cDNA were described previously.[3] The mRNAs of Ikaros family genes were examined by PCR with Platinum Taq DNA Polymerase High Fidelity (Invitrogen, Carlsbad, CA, USA). The PCR products were sequenced by automated DNA sequencer. Nested PCR amplification was carried out with diluted full-length PCR products by Accuprime Taq DNA polymerase High Fidelity (Invitrogen). Quantitative PCR was carried out as previously described.[3] The specific primer sets for each PCR are described in Table S2.

Immunoblot analysis. Cells were collected, washed with PBS, and lysed with RIPA buffer. For immunoprecipitation, cells were lysed with TNE buffer and incubated with specific antibody. Proteins samples were then analyzed by immunoblots with specific antibodies: anti-tubulin, anti-Ikaros, and anti-Helios antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse anti-FLAG antibody (M2) was from Sigma-Aldrich (St. Louis, MO, USA). Rabbit polyclonal anti-HA

[7]To whom correspondence should be addressed.
E-mail: tnabe@ims.u-tokyo.ac.jp

doi: 10.1111/cas.12181

antibody was from MBL (Nagoya, Japan). Anti-mouse, rabbit, and goat secondary antibodies were from Promega (Fitchburg, WI, USA).

Immunostaining. HeLa cells were cultured on coverslip slides and transfected with the indicated expression vectors by Lipofectamine LTX (Invitrogen). At 24 h post transfection, cells were washed three times with PBS, fixed in 4% paraformaldehyde, and permeabilized with 0.1% Triton X-100. Then, cells were stained with primary antibodies (diluted 1:500 to 1:2000). Alexa-488 or 546-conjugated secondary antibodies (Molecular Probes, Life Technologies, Carlsbad, CA, USA) were used for detection of specific targets, and DAPI was used for nuclear staining. Images were acquired by using a Nikon A1 confocal microscope (Nikon, Tokyo, Japan).

Electrophoretic mobility-shift assay. Experimental conditions and detail methods were previously reported.[3] For evaluation of DNA binding activity, 3–5 μg nuclear extracts from each transfectant were used per each lane of electrophoresis. The oligonucleotide sequences used as a probe are provided in Table S2.

Luciferase assay. The pGL4.10-firefly vector (Promega) containing *Hes1* promoter was used as a reporter vector and RSV-renilla vector was used as a control vector. HeLa cells were transiently transfected with these reporters and each Ikaros or/and Helios expression vector by Lipofectamine 2000 reagent (Invitrogen). The luciferase activities were quantified by the Dual-Luciferase Reporter Assay System (Promega) at 24 h post-transfection.

Retroviral construction and transduction. The FLAG-Hel-5 cDNA sequence was subcloned into retrovirus vector pRx-puro. Stable cell populations expressing Hel-5 were selected by puromycin. The shRNA-expressing retroviral vectors and virus production procedures have been established.[3] The shRNA sequences are listed in Table S2. Stable cell populations were obtained by puromycin or G418 selection.

Proliferation assays. Cells (0.5 or 1.0 × 10^4) were plated in 96-well plates with media supplemented with 10% or 0.2% FCS. The cell numbers were evaluated for 4 days by Cell Counting Kit-8 (Dojindo, Kumamoto, Japan). The averages of at least three independent experiments are shown.

Gene expression microarray analyses. Gene expression microarray used the 4 × 44K Whole Human Genome Oligo Microarray (Agilent Technologies, Santa Clara, CA, USA); detailed methods were previously reported.[3] Coordinates have been deposited in the Gene Expression Omnibus database with accession numbers GSE33615 (gene expression microarray), GSE33602 (copy number analyses), and GSE41796 (Jurkat models).

Results

Abnormal expression of short Helios transcripts in primary ATL cells. To characterize the gene expression signature in primary ATL cells, we previously carried out mRNA microarray analyses on a large number of samples. The comprehensive survey unveiled deregulated expression of Ikaros family genes; transcription levels of Ikaros and Aiolos were downregulated in ATL samples, whereas Helios was upregulated (Fig. S1). Thus, we examined the detailed expression patterns and levels of Ikaros family members in PBMCs derived from a panel of ATL patients and HTLV-1 carriers (Fig. 1a). Compared with control PBMCs from normal volunteers (Fig. 1b), the expression levels of Ikaros and Aiolos seemed to be downregulated in ATL samples, consistent with our microarray results. However, there were obvious abnormalities in the expression patterns of Helios. The main isoform of Helios was changed from full-length Hel-1 to Hel-2, which lacks exon 3 that contains the first N-terminal zinc finger in the DNA-binding domain. In addition, four ATL-specific Helios short transcripts were identified (Fig. 1c). Among them, Hel-5 and Hel-6 have been reported to be expressed in ATL.[29] We also identified two novel variants, Hel-v1 that lacks exons 3 and 4 and Hel-v2 that lacks exons 2, 3, and 6. These abnormal Helios variants were also expressed in the samples of high-risk HTLV-1 carriers, who subsequently developed ATL in the next few years. Furthermore, nested PCR revealed that Hel-5 or Hel-6 were expressed in a majority of ATL samples (17/22 acute cases, 10/10 chronic cases, and 5/5 smoldering cases; total, 32/37 cases) (Fig. 1d, upper panels), whereas Hel-v1 was expressed only in limited cases of ATL (Fig. 1d, lower panels). In four cases, Helios was not expressed. Collectively, our mRNA analysis showed that Helios expression was generally deregulated in ATL cells.

Genomic abnormalities at the *Helios* locus in primary ATL cells. To investigate the *Helios* locus in ATL, we retrieved data from our gene copy number analysis[3] and found that specific genomic deletion was accumulated at the *Helios* locus in ATL samples (17/168 cases, Fig. 2). All 17 cases were aggressive-type ATL (12/17 lymphoma types and 5/17 acute types). Furthermore, we found that two acute ATL cases in Figure 1(a) (#9 and #14), which showed severely deregulated or lost Helios expression, had a genomic deletion of the *Helios* locus.

Dimerization ability of ATL-type Helios isoforms with wild-type Helios or Ikaros. Consistent with a previously published report,[33] co-immunoprecipitation analyses confirmed that wild-type Hel-1 formed homodimers with themselves and heterodimers with wild-type Ikaros (Ik-1) protein (Fig. 3a, top panel, lane 1 and lane 4). In contrast, the dimerization activity of another artificial Helios mutant (Hel-ΔC), which lacks the dimerization domain at the C-terminal region, was dramatically declined (Fig. 3b, top panel, lane 1 and lane 4). We confirmed that all ATL-type Helios proteins could interact with Hel-1 and Ik-1, despite the fact that all of them lack various sets of the N-terminal exons (Fig. 3c–f).

Cytoplasmic localization of ATL-type Helios isoforms lacking exon 6. Ectopically expressed Hel-1 and Ik-1 were localized in the nucleus (Fig. 4a, top two panels). Regarding the ATL-type Helios isoforms, we found that Hel-5 and Hel-v1 were localized in the nucleus, whereas Hel-6 and Hel-v2, both of which lack exon 6, were substantially localized in the cytoplasm (Fig. 4a, middle four panels). We also confirmed the cytoplasmic localization of Hel-Δexon 6, which is an artificial Helios mutant lacking only exon 6 (Fig. 4a, bottom panel). Thus, exon 6 appears to be critical for nuclear localization of Helios proteins. Furthermore, defect of exon 6 led to disruption of the

Fig. 1. (On the next page) Abnormal expression of Helios mRNA in primary adult T-cell leukemia (ATL) cells. (a) Expression analysis of Ikaros family genes in PBMCs by full-length RT-PCR (Acute, $n = 22$; Chronic, $n = 10$; Smoldering, $n = 5$; HTLV-1 carriers, $n = 5$; High-risk carriers, $n = 4$). To detect and distinguish alternative splicing variants, PCR analyses were carried out with the sense and antisense primer sets designed in the first and final exons of each full-length transcript of Ikaros family genes. Obtained cDNAs were cloned and their sequences were analyzed. The samples acute #4, 4′, and 4″ were derived from the same patient, but were studied independently. (b) Expression of Ikaros family genes in PBMCs from normal volunteers ($n = 10$). (c) Schematic representation of Hel-1, Hel-2, and ATL-type Helios isoforms identified in this study. Hel-variant 1 (Hel-v1) and Hel-variant 2 (Hel-v2) are novel isoforms in ATL. Arrows indicate primer locations of full-length PCR for Helios. Ex, exon; F1–F6, functional zinc-finger domains. (d) Nested PCR with specific primer sets, which were designed at exon junction of exon 1–5 or exon 2–5 for detection of Hel-5 and Hel-6 (upper panel), or detection of Hel-v1 (lower panel), respectively. Arrows indicate primer locations.

doi: 10.1111/cas.12181