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Hypermethylation of *MST1* in IgG4-related autoimmune pancreatitis and rheumatoid arthritis

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ABSTRACT

The serine/threonine kinase Mst1 plays important roles in the control of immune cell trafficking, proliferation, and differentiation. Previously, we reported that Mst1 was required for thymocyte selection and regulatory T-cell functions, thereby the prevention of autoimmunity in mice. In humans, *MST1* null mutations cause T-cell immunodeficiency and hypergammaglobulinemia with autoantibody production. *RASSF5C(RAPL)* is an activator of *MST1* and it is frequently methylated in some tumors. Herein, we investigated methylation of the promoter regions of *MST1* and *RASSF5C(RAPL)* in leukocytes from patients with IgG4-related autoimmune pancreatitis (AIP) and rheumatoid arthritis (RA). Increased number of CpG methylation in the 5′ region of *MST1* was detected in AIP patients with extrapancreatic lesions, whereas AIP patients without extrapancreatic lesions were similar to controls. In RA patients, we detected a slight increased CpG methylation in *MST1*, although the overall number of methylation sites was lower than that of AIP patients with extrapancreatic lesions. There were no significant changes of the methylation levels of the CpG islands in the 5′ region of *RASSF5C(RAPL)* in leukocytes from AIP and RA patients. Consistently, we found a significantly down-regulated expression of *MST1* in regulatory T cells of AIP patients. Our results suggest that the decreased expression of *MST1* in regulatory T cells due to hypermethylation of the promoter contributes to the pathogenesis of IgG4-related AIP.

1. Introduction

Mst1 (also known as Stk4), a ste20-like kinase, regulates a wide variety of cellular functions including immune cell trafficking, cell proliferation and apoptosis [1,2]. We previously demonstrated that MST1 and RASSF5C (RAPL) are effecter proteins of the small GTPase Rap1 that regulates integrin activation and cellular morphology. Mst1 forms a complex with Rap1 and Rassf5c in response to chemokine and antigen receptor stimulation, leading to conformational changes and clustering of LFA-1 [3–6]. This complex also induces polarized lymphocyte morphology. In addition, Rassf5c negatively regulates lymphocyte proliferation through the G1 cyclin inhibitor p27Kip [7].

Gene-targeting of Mst1 or Rassf5c in mice resulted in hypoplastic lymphoid organs with defective lymphocyte homing and interstitial migration due to integrin dysfunction [8,9]. Interestingly, mice deficient in either Mst1 or Rassf5c also exhibited autoimmune phenotypes. The absence of Rassf5c in mice caused lupus-like glomerulonephritis often accompanied with B-cell lymphoma. On the other hand, Mst1-deficient mice developed distinct types of autoimmunity, namely an increase of effector/memory lymphocytes with a production of organ-specific autoantibodies. Furthermore, leukocyte infiltration was observed in multiple organs including the pancreas, liver, stomach, lung, and kidney.

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We found that the absence of Mst1 caused a breakdown of checkpoint controls of self-tolerance. Mst1-deficient mice exhibited impaired thymocyte selection and thymic Treg development due to defective thymocyte migration and self-antigen recognition in the medulla [10]. In addition, the antigen-specific suppression by Mst1-deficient Tregs was severely impaired both *in vitro* and *in vivo*. These defects in Treg suppression were due to impaired antigen-dependent interactions with dendritic cells and immunological synapse formation [11]. In agreement with the mouse model, human germline mutations of *STK4/MST1* caused T-cell immunodeficiency with recurrent infections as well as autoimmune manifestations including an increase of effector-memory lymphocytes, autoantibody production, and hypergammablubulinemia [12–14].

MST1 and *RASSF5C* have CpG islands in their promoter regions [15,16], suggesting epigenetic regulation of these genes. Therefore, we explored the possibility that epigenetic abnormalities of these genes could also lead to the phenotypes of autoimmune diseases. For this purpose, we examined CpG methylation of *MST1* and *RASSF5C* in IgG4-related autoimmune pancreatitis (AIP). IgG4-related AIP is characterized by a frequent elevation of serum IgG4 and infiltration of IgG4-positive plasma cells into the pancreas and other organs, therefore it is categorized as a pancreatic manifestation of a novel systemic IgG4-related disease (IgG4-RD) [17,18]. IgG4-RD includes a wide variety of diseases that were formerly diagnosed as Mikulicz's disease, hypophysitis, Riedel thyroiditis, interstitial pneumonitis, interstitial nephritis, prostatitis, lymphadenopathy retroperitoneal fibrosis, inflammatory aortic aneurysm, and inflammatory pseudotumor. The detailed pathogenesis of IgG4-RD remains unknown, but some immunological abnormalities have been reported including innate immunity [19,20], regulatory T cells (Tregs) [21–24] and aberrant Th1/2 balance [25–27] and regulatory B cells [28].

In this study, we sought to elucidate the epigenetic regulation of IgG4-related AIP by examining the methylation levels of CpG sites in the 5′ promoter region of *MST1* and *RASSF5C* in leukocytes, and also examine the expression levels of MST1 in Treg from patients with IgG4-related AIP.

2. Materials and methods

2.1. Patients and mice

A total of 20 patients (15 males and 5 females; aged 51–77 years, mean age 67.6) were diagnosed with IgG4-related AIP according to the international consensus diagnostic criteria for autoimmune pancreatitis guidelines of the International Association of Pancreatology. AIP patients were further categorized as those with or without extrapancreatic lesions (lacrimal gland, thyroid gland, and kidney). RA patients (6 males and 15 females, aged 21–85 years, mean age 58.5) and healthy controls (9 males and 1 female, aged 26–54 years, mean age 37.6) were also studied for comparison. This study was approved by the Kansai Medical University ethics committee and all patients provided informed consent. The demographics of the patients are shown in Table 1. Mst1-deficient mice was described before [10].

2.2. Bisulfite treatment

Blood samples were taken from the patients before drug treatments and centrifuged for 10 min at 1000 g to pellet the cells from the plasma. The buffy coat containing leukocytes was carefully removed and washed with PBS. The cells were suspended in lysis solution (10 mM Tris HCl pH 8.0, 0.4 M NaCl, 10 mM EDTA, 1% SDS and 200 μg/ml proteinase K) and incubated overnight at 50 °C. Genomic DNA was isolated using either phenol-CIA (CIA = 24:1 chloroform: isoamyl alcohol) extraction or column extraction (NucleoSpin Blood XL, Macherey-Nagal). Genomic DNA was digested with EcoRI, Hind III and Sph I, and then precipitated. Digested DNA (0.5–1.0 μg) in 19 μl of distilled water was treated with 1 μl of 6 N NaOH and incubated at 37 °C for 15 min. These samples were reacted with 120 μl of freshly prepared bisulfite solution (107 μl of 2.02 M sodium metabisulfite, 7 μl of 10 mM hydroquinone and 6 μl of 6 N NaOH) and overlaid with mineral oil. The bisulfite reaction was performed with 15 cycles of 30 s at 95 °C for denaturing and 15 min at 50 °C for reaction. The free bisulfite was removed using the Wizard DNA Clean-Up System (Promega). Then, DNA was eluted with 50 μl of TE buffer pH 8.0, followed by a 5 min incubation with 2.5 μl of 6 N NaOH. Samples were neutralized with 35 μl of 5 M NH_4OAc and precipitated with ethanol and glycogen. The efficiency of bisulfite reaction was greater than 97%.

2.3. PCR amplification and sequencing of CpG islands of MST1 and RASSF5C

After bisulfite treatment, the 5′ flanking regions of *MST1* and *RASSF5C* were amplified by PCR using Ex Taq DNA polymerase (Takara Bio). The primer sequences were as follows: 5′-TTTTAGTTTGTGAAATGGGATTTAGGATTT-3′ (forward) and 5′-CCA-TACCCRTCCTCTTAACCAATAA-3′ (reverse) (R represents A and G) for *MST1*, and 5′-ATTTTGTAGAGGAAGTGGTTTTAGAATTGT-3′ (forward) and 5′-ACRTAAACCCCTAACTCTAAACCC-3′ (reverse), for *RASSF5C*. PCR conditions were as follows: 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 59 °C (MST1) or 57 °C (RASSF5C) for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 10 min. PCR products were purified using Wizard SV Gel and PCR Clean-Up System (Promega), and then cloned into pGEMT easy plasmids (Promega) using a DNA ligation kit (Takara Bio). At least ten independent clones were sequenced for each sample using an ABI PRISM 3130xl Genetic Analyzer.

2.4. Intracytoplasmic staining of MST1

Leukocytes were separated from blood of twelve AIP patients (7 males and 5 females; aged 39–81 years, mean age 64.2) by density gradient centrifugation using Ficoll–Paque PLUS (GE Healthcare). Cells were permeabilized by CytoPerm/Fix (BD Pharmacies) according to the manufacture's protocol, and stained with APC conjugated anti-human CD4 antibody, anti human FoxP3-PE-Cy7 (eBioscience), rabbit anti-mouse/human MST1 polyclonal antibody (Upstate), followed by anti-rabbit IgG(H + L)-AlexaFlour 488 (Lifetechnology). A FACS Canto II (BD Biosciences) was used to collected data, which were analyzed with a FlowJo software. To confirm the specificity of the intracytoplasmic staining, T cells were isolated from wild-type and Mst1-deficient mice using panT isolation kit according to the manufacture's protocol, and subjected to immunostaining and FACS analysis as above.

2.5. Statistical analysis

PRISM (GraphPad Software) was used to conduct Mann–Whitney's U tests for the statistical analysis of the sequencing results. Corrected p values of <0.05 were considered statistically significant.

Table 1
IgG4-related diseases.

Patient ID	Age/sex	IgG <1700 (mg/dl)	IgG4 <135 (mg/dl)	Extrapancreatic lesion
1	62/F	417	338	Mediastinal LNS[a], thyroiditis
2	66/M	4384	2070	Nephropathy, Kuttner tumor
3	75/F	1814	241	Mediastinal LNS, sialoadenitis
4	69/M	1770	249	Mikulicz disease, thyroiditis
5	62/F	2754	1110	Sialoadenitis, mediastinal LNS
6	68/M	1802	264	Mikulicz disease
7	72/M	3274	1870	Mediastinal LNS
8	63/M	NT[b]	NT	Mikulicz disease
9	70/F	2195	588	Mikulicz disease
10	57/F	2200	472	Hypothyroidism
11	76/M	1942	254	Retroperitoneal fibrosis
12	66/M	2493	1450	(−)
13	69/M	4060	1170	(−)
14	74/M	NT	226	(−)
15	51/M	2065	479	(−)
16	77/M	NT	NT	(−)
17	65/M	2073	487	(−)
18	77/M	NT	NT	(−)
19	70/M	1622	407	(−)
20	62/M	1699	676	(−)

[a] Lymph node swelling.
[b] Not tested.

3. Results

3.1. CpG sites in MST1 and RASSF5C

MST1 and *RASSF5* are located on 20q11.2–q13.2 and 1q32, respectively. CpG plots identify the CpG islands in the 5′ regions of both *MST1* (exon 1) and *RASSF5* (exon 1C) (Fig. 1), thus suggesting a potential for epigenetic regulation of these genes by methylation. To examine the methylation levels of *MST1* and *RASSF5*, genomic DNA was isolated from the peripheral leukocytes of healthy controls, IgG4-related AIP and RA patients before drug treatments. The samples were subjected to bisulfite sequencing to determine the frequencies and locations of the methylated CpG sites (Fig. 1). The amplified genomic regions of *MST1* and *RASSF5* contained 43 and 40 CpG sites, respectively. We sequenced a minimum of 10 clones of each sample.

3.2. Methylation of CpG sites in MST1

We examined methylation of 43 CpG sites in the amplified genomic region of *MST1* in healthy controls and AIP patients (Figs. 1A and 2). In AIP patients, the frequency of methylated CpG sites tended to be increased compared to that of healthy controls. although they were not statistically significant (Fig. 2A). Importantly, the number of the methylation sites in AIP patients were not correlated with age ($R^2 = 0.0825$, Fig. 2B), indicating that the increase of CpG methylation in AIP patients was not due to aging.

To gain insight into the enhanced methylation in various phenotypes of AIP, AIP patients further divided into two groups based on the presence or absence of extrapancreatic lesions and compared to healthy controls. AIP patients without extrapancreatic lesions exhibited similar CpG methylation of *MST1* to healthy controls. In contrast, CpG methylation was increased in AIP patients with extrapancreatic lesions (Fig. 2A). The incidence of methylated CpG sites was significantly increased in AIP patients with extrapancreatic lesions relative to healthy controls ($p = 0.0133$) and to AIP patients without extrapancreatic lesions ($p = 0.0245$). Furthermore, a low but significant correlation was observed between the number of extrapancreatic lesions and that of methylated CpG sites ($R^2 = 0.2534$) (Fig. 2C). Thus, the increase of CpG methylation in AIP patients could reflects pathological conditions.

3.3. Methylation patterns of MST1 in RA patients

In RA patients, the frequency of methylated CpG sites was increased, which was not statistically significant, compared to healthy controls (Fig. 3A). The frequency of CpG methylation in RA patients was not correlated with age (Fig. 3B).

3.4. Methylation status of RASSF5C

Rassf5c associates with and activates Mst1 kinase [4], and *RASSF5C* is frequently methylated in some tumors. Therefore, we used bisulfite sequencing to examine the methylation status of 40 CpG sites in the 5′ region of *RASSF5C* in AIP and RA patients (Fig. 1B). We found the frequency (Fig. 3C) and location (data not shown) of CpG methylation sites in the 5′ region of *RASSF5C* were not significantly changed in AIP patients with or without extrapancreatic lesions relative to healthy controls as well as RA patients.

3.5. Decreased Mst1 expression in regulatory T cells of AIP patients

Since *MST1* CpG methylation was significantly elevated in AIP patients with extrapancreatic lesions, we examined Mst1 expression in T cells from AIP patients. For this purpose, MST1 expression levels in T cells from additional twelve patients and ten healthy controls were evaluated with intracytoplasmic staining followed by FACS analysis. We confirmed the specificity of the immunostaining using wild-type and Mst1-deficient T cells. While in wild-type T cells mean fluorescence intensities (MFI) clearly increased compared to control antibody, Mst1-deficient T cells did not exhibit significant levels of immunostaining (Fig. 4A). Having confirmed the specificity of the immunostaining for MST1, The MFI of MST1 was measured in $CD4^+FoxP3^+$ regulatory T cells (Treg) and $CD4^+FoxP3^-$ conventional T cells (Tconv) and expressed as ratios of MST1 levels of Treg relative to those of Tconv (Fig. 4B). We found

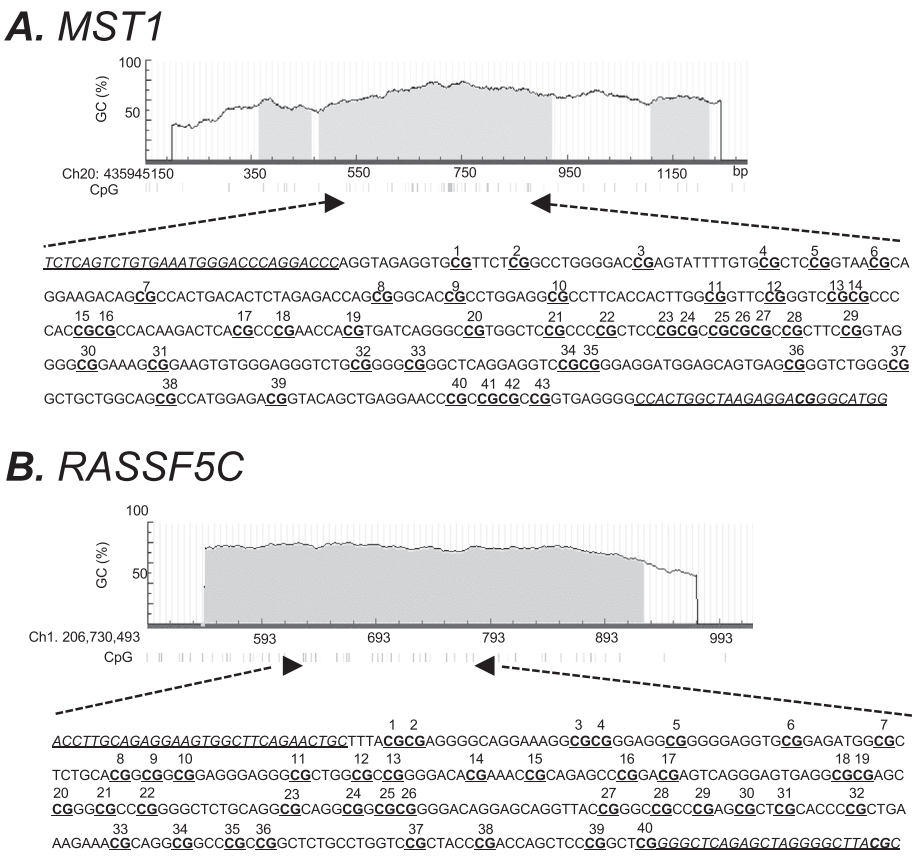

Fig. 1. CpG islands in the 5′ region of *MST1* and *RASSF5C*. CpG plots of the 5′ region of *MST1* (A) *and RASSF5C* (B). The percentage of GC contents in the 5′ region of *MST1* and *RASSF5C* were plotted using MethPrimer software. Nucleotide sequences of the CpG sites of *MST1* and *RASSF5C* (*RAPL*) are shown. The CpG sites (underlined) and PCR primers (bold lines) for bisulfite-sequencing are indicated.

that in healthy controls MST1 levels in Treg were higher than those in Tconv, whereas MST1 levels were comparable in two T cell subsets of AIP patients. Consequently, the MST1 ratios in Treg of the AIP patients were significantly lower than those of the control (Fig. 4C). We also analyzed MST1 expressions in the subgroups of AIP with or without extrapancreatic lesion. Although MST1 expressions in both groups were significantly lower than those of healthy controls, there were no further decrease of MST1 levels in AIP with extrapancreatic lesions. MST1 expressions did not correlate to age, as is the case shown in methylation assays (Fig. 4D).

4. Discussion

MST1 has CpG islands in its 5′ promoter region, thus suggesting that *MST1* expression is regulated by DNA methylation. Understanding that Mst1 deficiency leads to autoimmune-like phenotypes in mice and humans, we explored the possibility that epigenetic abnormalities of *MST1* are an underlying mechanism that contributes to the phenotypes observed in autoimmune diseases. Therefore, we examined CpG methylation levels in total peripheral blood leukocytes and MST1 expression in Treg from patients that were diagnosed with IgG4-related AIP.

IgG4-related AIP is characterized by steroid-sensitive leukocyte infiltration into the pancreas as well as an elevation of serum IgG4 levels. Most IgG4-related AIP patients have lesions in not only pancreas but also other organs. Interestingly, IgG4-related AIP patients with extrapancreatic lesions exhibited a significant increase in the frequency of CpG methylation sites in the 5′ region of *MST1* in their leukocytes. This was of interest because in a previous study, we found that in aged mice with Mst1 deficiency was linked to autoimmune phenotypes associated with leukocyte infiltration in multiple organs and autoantibody production. The increased levels of CpG methylation was modest, ranging between 5 and 10%, suggesting that only a subset of peripheral blood leukocytes harbored hypermethylated CpG sites in *MST1*.

In line with these findings, MST1 expression levels were significantly decreased in Foxp3$^+$ Treg from IgG4-RD patients. We previously demonstrated that a deficiency of Mst1 caused the dysfunction of regulatory T cells in the periphery [29]. Those defects likely contribute to autoimmune pathogenesis of Mst1-deficient mice, as previously shown by the association of Treg dysfunction to the induction of multi-organ autoimmune diseases following the disruption of Treg-related genes [30–33]. Several studies have indicated alterations in Treg population of IgG4-RD patients [21–24]. In IgG4-related AIP patients, the number of CD4$^+$CD25$^+$CD45RA$^+$ naïve Tregs was decreased, whereas the number of CD4$^+$CD25$^+$CD45RA$^-$ effector/memory Treg was increased [21]. Similar to AIP patients, Mst1-deficient mice had a decreased number of thymic Tregs whereas the frequency of CD62L$^-$FoxP3$^+$-induced Tregs were increased in the periphery [10]. In addition, the antigen-specific suppression by Mst1-deficient Tregs was severely impaired both *in vitro* and *in vivo*. These defects in Treg suppression were due to impaired antigen-dependent interactions with dendritic cells and immunological synapse formation [29]. The methylation levels of MST1 in PBL was not significantly augmented in IgG4-RD patients without extrapancreatic lesions, whereas MST1 protein levels in Treg from these patients were decreased. This might be due to the experimental

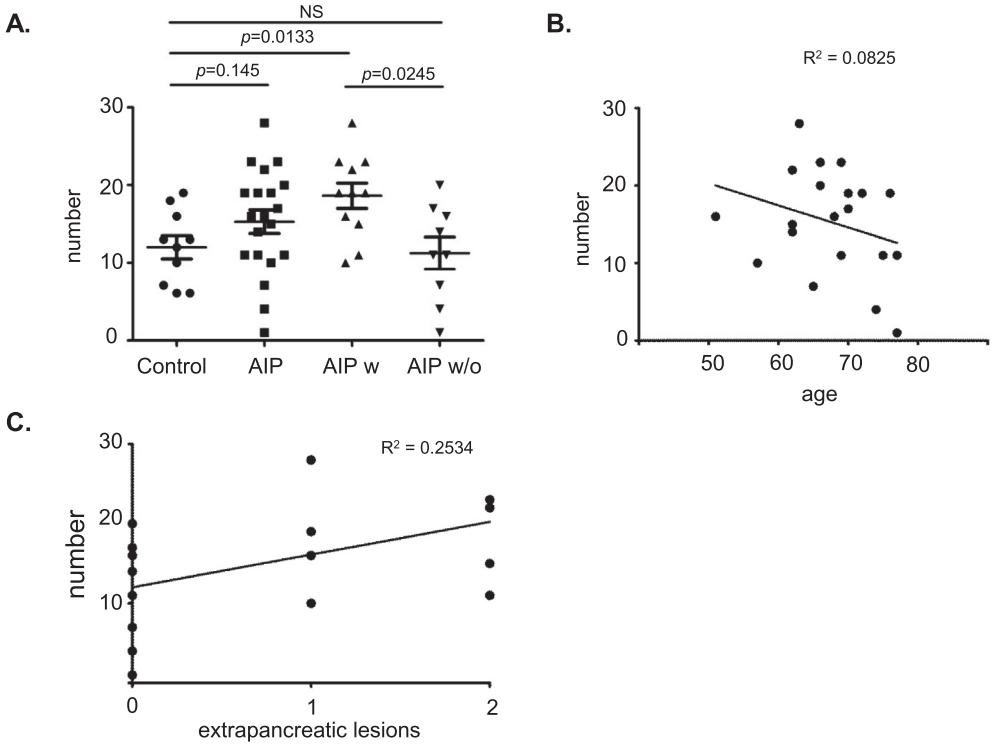

Fig. 2. Increased methylation of CpG sites in AIP patients with extrapancreatic lesions. A. Average methylation frequencies for all CpG sites in the 5′ region of *MST1* from healthy controls and IgG4-related AIP patients. AIP patients are divided into two groups with extrapancreatic lesions (AIP w) or without extrapancreatic lesions (AIP w/o) for further analysis. B. A correlation between age and the total number of methylation at all CpG sites in IgG4-related AIP patients. C. A correlation between the number of methylated CpG sites and extrapancreatic lesions.

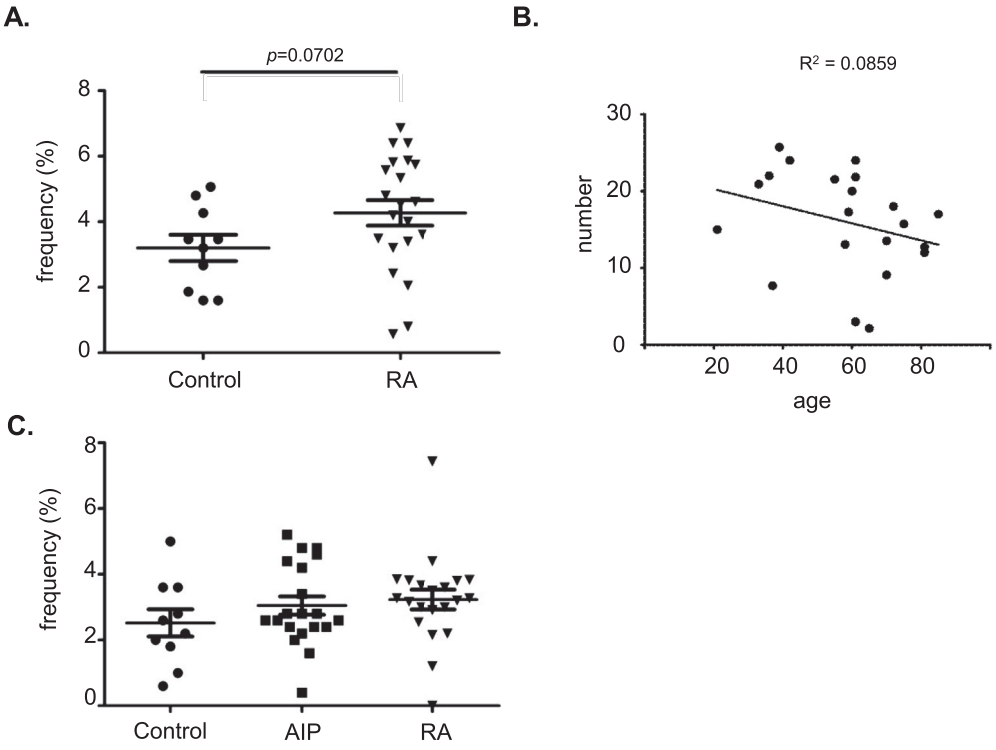

Fig. 3. Methylation of CpG sites of *MST1* in RA patients and Methylation of *RASSF5C*. A. Average frequencies of methylation at CpG sites in the 5′ region of *MST1* from healthy controls and RA patients. B. A correlation between age and the total number of all methylated CpG sites in RA patients. C. Average frequencies of methylation at CpG sites in the 5′ region of *RASSF5C* in healthy controls, IgG4-related AIP patients with or without extrapancreatic lesions, and RA patients.

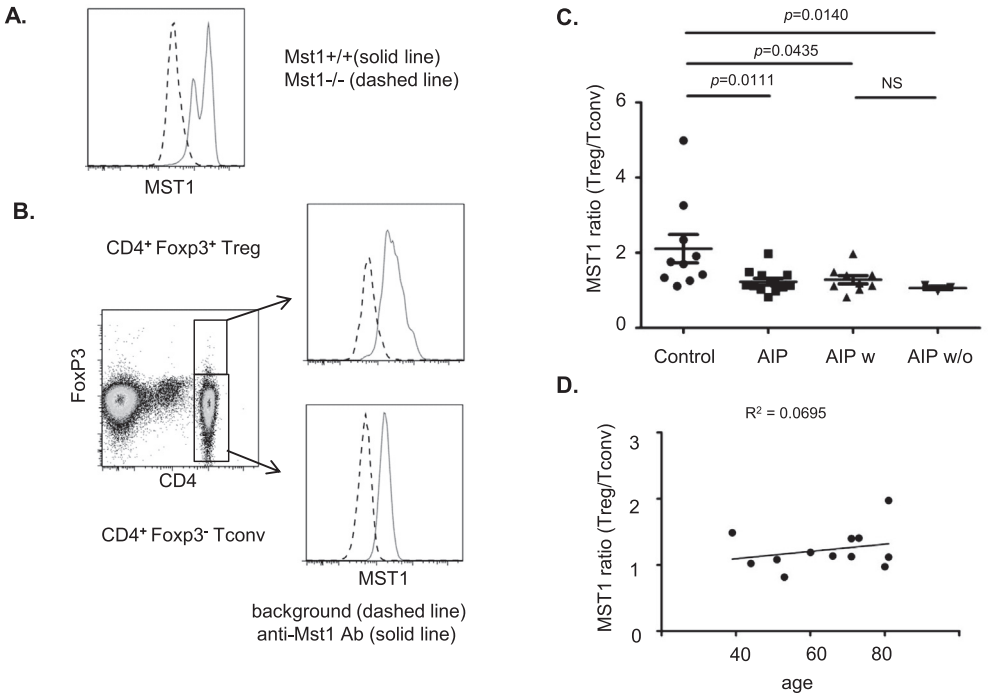

Fig. 4. Decreased expression of *MST1* in Tregs of AIP patients. A. The intensity of the anti-human and murine MST1 antibody in lymphocytes from wild type and MST1 knockout mice. B. Representative flow cytometry gates on T cell subsets. Mean fluorescent intensities of intracytoplasmic MST1 staining were measured in $CD4^{+}FoxP3^{+}$ Treg and $CD4^{+}FoxP3^{-}$ Tconv. C. The ratios of MFI of MST1 in Treg relative to Tconv are shown on healthy controls and AIP patients. AIP patients are divided into two groups with extrapancreatic lesions (AIP w) or without extrapancreatic lesions (AIP w/o). D. A correlation between age and the MST1 expression ratio in Treg of AIP patients.

conditions that genomic DNA for the methylation assays was derived from total leukocytes, as small numbers of Treg was not sufficient to perform methylation assays. Alternatively, MST1 down-regulation in Treg could occur without direct DNA methylation of *MST1,* which were subsequently stabilized epigenetically, leading to the inflammatory lesions in non-pancreatic tissues.

Germline null mutations of *MST1* in humans led to primary T-cell immunodeficiency with recurrent infections as well as autoimmune phenotypes [11–13]. To date, there have been no reported cases of IgG4-RD with immunodeficiency and genetic transmission. We speculate that modest suppression of *MST1* expressions with age is sufficient to maintain basal immune functions that are capable of limiting microbe invasion, but may lead to impaired self-tolerance. Indeed, full *MST1* expression is required to mediate stable adhesion during negative selection of thymocytes as well as Treg suppression functions [29]. On the other hand, Mst1 is down-regulated in effector T cells, indicating that Mst1 is not required for effector T-cell functions. Therefore, the compromised expression of *MST1* affects self-tolerance rather than immune defense.

RA is characterized with inflammatory lesions of major joints. The frequency of methylated CpG sites in 5′ region of *MST1* of leukocytes from RA patients was slightly elevated, but not significantly. The distinct methylation level of AIP and RA could reflect differences of pathological conditions of the immune disorders.

Rassf5c-deficient mice have lupus-like autoimmune diseases and frequent occurrence of B-cell lymphoma [7]. Epigenetic silencing of *RASSF5C* was reported in some human tumors [16,34]. However, methylation of CpG sites in *RASSF5C* was not increased in AIP and RA patients. Our findings suggested that RASSF5C was not involved in the pathogenesis of AIP nor RA. Furthermore, this supports the notion that our observed increase of CpG methylation in *MST1* is not secondary to global enhancement of methylation activity, but rather it is specifically related to *MST1* regulation in the autoimmune disease.

In summary, AIP patients exhibited decreased expression of *MST1* in Tregs. AIP with extrapancreatic lesions also showed a significantly increased frequency of CpG sites in *MST1*. This increased frequency of methylated CpG sites was correlated with the number of affected organs in AIP patients. These results reveal the putative role of MST1 as a pathological mechanism driving IgG4-RD.

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References

[1] J. Avruch, M. Praskova, S. Ortiz-Vega, et al., Nore1 and RASSF1 regulation of cell proliferation and of the MST1/2 kinases, Methods Enzymol. 407 (2006) 290–310.
[2] F. Qin, J. Tian, D. Zhou, et al., Mst1 and Mst2 kinases: regulations and diseases, Cell. Biosci. 3 (2013) 31.
[3] K. Katagiri, A. Maeda, M. Shimonaka, et al., RAPL, a Rap1-binding molecule that mediates Rap1-induced adhesion through spatial regulation of LFA-1, Nat. Immunol. 4 (2003) 741–748.

[4] K. Katagiri, M. Imamura, T. Kinashi, Spatiotemporal regulation of the kinase Mst1 by binding protein RAPL is critical for lymphocyte polarity and adhesion, Nat. Immunol. 7 (2006) 919–928.
[5] K. Harvey, N. Tapon, The Salvador-Warts-Hippo pathway – an emerging tumour-suppressor network, Nat. Rev. Cancer 7 (2007) 182–191.
[6] L.J. Saucedo, B.A. Edgar, Filling out the Hippo pathway, Nat. Rev. Mol. Cell. Biol. 8 (2007) 613–621.
[7] K. Katagiri, Y. Ueda, T. Tomiyama, et al., Deficiency of Rap1-binding protein RAPL causes lymphoproliferative disorders through mislocalization of p27kip1, Immunity 34 (2011) 24–38.
[8] K. Katagiri, N. Ohnishi, K. Kabashima, et al., Crucial functions of the Rap1 effector molecule RAPL in lymphocyte and dendritic cell trafficking, Nat. Immunol. 5 (2004) 1045–1051.
[9] Y. Ebisuno, K. Katagiri, T. Katakai, et al., Rap1 controls lymphocyte adhesion cascade and interstitial migration within lymph nodes in RAPL-dependent and -independent manners, Blood 115 (2010) 804–814.
[10] Y. Ueda, K. Katagiri, T. Tomiyama, et al., Mst1 regulates integrin-dependent thymocyte trafficking and antigen recognition in the thymus, Nat. Commun. 3 (2012) 1098.
[11] N.T. Nehme, J. Pachlopnik Schmid, F. Debeurme, et al., MST1 mutations in autosomal recessive primary immunodeficiency characterized by defective naive T-cell survival, Blood 119 (2012) 3458–3468.
[12] H. Abdollahpour, G. Appaswamy, D. Kotlarz, et al., The phenotype of human STK4 deficiency, Blood 119 (2012) 3450–3457.
[13] A. Crequer, C. Picard, E. Patin, et al., Inherited MST1 deficiency underlies susceptibility to EV-HPV infections, PLoS One 7 (2012) e44010.
[14] H. Hamano, S. Kawa, A. Horiuchi, et al., High serum IgG4 concentrations in patients with sclerosing pancreatitis, N. Engl. J. Med. 344 (2001) 732–738.
[15] C. Seidel, U. Schagdarsurengin, K. Blumke, et al., Frequent hypermethylation of MST1 and MST2 in soft tissue sarcoma, Mol. Carcinog. 46 (2007) 865–871.
[16] D. Macheiner, G. Heller, S. Kappel, et al., NORE1B, a candidate tumor suppressor, is epigenetically silenced in human hepatocellular carcinoma, J. Hepatol. 45 (2006) 81–89.
[17] H. Umehara, K. Okazaki, Y. Masaki, et al., A novel clinical entity, IgG4-related disease (IgG4RD): general concept and details, Mod. Rheumatol. 22 (2012) 1–14.
[18] H. Umehara, A. Nakajima, T. Nakamura, et al., IgG4-related disease (IgG4-RD) and its pathogenesis-crosstalk between innate and acquired immunity, Int. Immunol. 26 (11) (2014) 585–595.
[19] T. Watanabe, K. Yamashita, S. Fujikawa, et al., Involvement of activation of toll-like receptors and nucleotide-binding oligomerization domain-like receptors in enhanced IgG4 responses in autoimmune pancreatitis, Arthritis Rheum. 64 (2012) 914–924.
[20] T. Watanabe, K. Yamashita, T. Sakurai, et al., Toll-like receptor activation in basophils contributes to the development of IgG4-related disease, J. Gastroenterol. 48 (2013) 247–253.
[21] H. Miyoshi, K. Uchida, T. Taniguchi, et al., Circulating naïve and CD4+CD25high regulatory T cells in patients with autoimmune pancreatitis, Pancreas 36 (2008) 133–140.
[22] M. Koyabu, K. Uchida, H. Miyoshi, et al., Analysis of regulatory T cells and IgG4-positive plasma cells among patients of IgG4-related sclerosing cholangitis and autoimmune liver diseases, J. Gastroenterol. 45 (2010) 732–741.
[23] T. Kusuda, K. Uchida, H. Miyoshi, et al., Involvement of inducible costimulator- and interleukin 10-positive regulatory T cells in the development of IgG4-related autoimmune pancreatitis, Pancreas 40 (2011) 1120–1130.
[24] K. Uchida, T. Kusuda, M. Koyabu, et al., Regulatory T cells in type 1 autoimmune pancreatitis, Int. J. Rheumatol. 2012 (2012) 795026.
[25] Y. Zen, T. Fujii, K. Harada, et al., Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis, Hepatology 45 (2007) 1538–1546.
[26] R. Akitake, T. Watanabe, C. Zaima, et al., Possible involvement of T helper type 2 responses to Toll-like receptor ligands in IgG4-related sclerosing disease, Gut 59 (2010) 542–545.
[27] A. Tanaka, M. Moriyama, H. Nakashima, et al., Th2 and regulatory immune reactions contribute to IgG4 production and the initiation of Mikulicz disease, Arthritis Rheum. 64 (2012) 254–263.
[28] K. Sumimoto, K. Uchida, T. Kusuda, et al., The role of CD19+ CD24high CD38high and CD19+ CD24high CD27+ regulatory B cells in patients with type 1 autoimmune pancreatitis, Pancreatol.: Off. J. Int. Assoc. Pancreatol. 14 (2014) 193–200.
[29] T. Tomiyama, Y. Ueda, T. Katakai, et al., Antigen-specific suppression and immunological synapse formation by regulatory T cells require the Mst1 kinase, PLoS One 8 (2013) e73874.
[30] R. Bacchetta, L. Passerini, E. Gambineri, et al., Defective regulatory and effector T cell functions in patients with FOXP3 mutations, J. Clin. Invest. 116 (2006) 1713–1722.
[31] S. Le Bras, R.S. Geha, IPEX and the role of Foxp3 in the development and function of human Tregs, J. Clin. Invest. 116 (2006) 1473–1475.
[32] K. Lahl, C. Loddenkemper, C. Drouin, et al., Selective depletion of Foxp3+ regulatory T cells induces a scurfy-like disease, J. Exp. Med. 204 (2007) 57–63.
[33] W. Ouyang, O. Beckett, Q. Ma, et al., Foxo proteins cooperatively control the differentiation of Foxp3+ regulatory T cells, Nat. Immunol. 11 (2010) 618–627.
[34] A.M. Richter, G.P. Pfeifer, R.H. Dammann, The RASSF proteins in cancer; from epigenetic silencing to functional characterization, Biochim. Biophys. Acta 1796 (2009) 114–128.