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TLS-CHOP represses miR-486 expression, inducing upregulation of a metastasis regulator PAI-1 in human myxoid liposarcoma

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ABSTRACT

Myxoid liposarcomas (MLSs) are characterized by t(12;16)(q13;p11) translocation and expression of TLS-CHOP chimeric oncoprotein. However, the molecular functions of TLS-CHOP have not been fully understood. On the other hand, microRNAs (miRNAs) comprise an abundant class of endogenous small non-coding RNAs that negatively regulate the expression of their target genes, and are involved in many biological processes. It is now evident that dysregulation of miRNAs is an important step in the development of many cancers. To our knowledge, however, there have been no reports of the miRNAs involved in MLS tumorigenesis and development. In this study, we have found that miR-486 expression was repressed in TLS-CHOP-expressed NIH3T3 fibroblasts and MLS tissues, and exogenous overexpression of miR-486 repressed growth of MLS cells. Thus, downregulation of miR-486 may be an important process for MLS. In addition, we have identified plasminogen activator inhibitor-1 (PAI-1) as a novel target gene of miR-486. PAI-1 is a unique type of serine protease inhibitor and is known to be one of the key regulators of tumor invasion and metastasis. Furthermore, knockdown of PAI-1 by a specific small interfering RNA (siRNA) inhibited growth of MLS cells, suggesting that increased expression of PAI-1 by miR-486 repression is critical for survival of MLS cells. Collectively, these results suggest a novel essential molecular mechanism that TLS-CHOP activates PAI-1 expression by repression of miR-486 expression in MLS tumorigenesis and development.

1. Introduction

MLS cases are associated with the chromosomal translocation t(12;16)(q13.3;p11.2), which creates a chimeric oncogene comprising part of the TLS (Translocated in Liposarcoma) gene, also known as FUS (Fused in Ewing's Sarcoma), found at 16p11.2 and part of the CHOP gene, also called DDIT3 (DNA-damage-inducible transcript 3) and GADD153 (growth arrest- and DNA damage-inducible gene 153), found at 12q13.3 [1–3]. Originally, TLS is an RNA-binding protein and is expressed constitutively. N-terminal part of TLS contains an autonomous transcriptional activation domain required for the oncogenic potential of the TLS-CHOP chimeric protein [4]. On the other hand, CHOP is a transcription factor belonging to the (c/EBP) family, and has a central role in endoplasmic reticulum (ER) stress and DNA damage response by inducing cell cycle arrest and apoptosis [5]. The resultant fusion gene TLS-CHOP encodes the N-terminal half of TLS fused to the complete sequence of CHOP. The TLS-CHOP chimeric protein is considered to function as an abnormal transcription factor, and several TLS-CHOP target genes that are seemed to be concerned with MLS development have been identified [6–9]. The definitive molecular mechanism of MLS tumorigenesis and progression, however, has not been fully elucidated up to now.

miRNAs are a class of 18–24 nucleotide RNA molecules that negatively regulate target mRNAs [10]. Dysregulation of miRNA expression can lead to cellular dedifferentiation, oncogenesis, tumor cell invasion and metastasis [11]. Recently, miR-486 was reported to function as a tumor suppressor by targeting the OLFM4 (Olfactomedin-4) antiapoptotic factor [12]. Downregulation of miR-486 has been observed in several cancers, breast, lung, gastric, hepatic, colon, ovarian, testicular and prostate cancers and lymphoma [12,13]. However, relationship between miR-486 and MLS has not been clarified.

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PAI-1, also known as serine/cysteine proteinase inhibitor clade E member 1 (SERPINE1), plays an important role in the plasminogen activator system, and is involved in various biological processes including thrombosis, wound healing, tumor angiogenesis and degradation of extracellular matrix components [14]. PAI-1 protein levels are usually increased in cancers [15], and high-level expression of PAI-1 is associated with a poor prognosis in a variety of cancers [16,17]. Furthermore, high physiological level of PAI-1 was shown to promote tumor invasion and angiogenesis [18], and siRNA specific for PAI-1 effectively reduced gastric peritoneal metastasis [19]. Thus, PAI-1 seems to have an important function in cancer progression and metastasis.

In this study, we have examined miRNA expression profiles in NIH3T3 cells with or without exogenous TLS-CHOP expression, and have found that repression of miR-486 expression by TLS-CHOP is necessary for MLS cell growth. In addition, we have identified a novel miR-486 target gene PAI-1 that may function in the molecular pathway in MLS. These results should contribute to understanding of the mechanisms for MLS tumorigenesis and development.

2. Materials and methods

2.1. Cell culture

The myxoid liposarcoma-derived cell line 2645/94 was kindly provided from Prof. David Ron (University of Cambridge, UK). NIH3T3 cells were obtained from the ATCC.

2.2. Vector construction and transfection

After polymerase chain reaction (PCR) amplification of a cDNA fragment containing the full-length coding region of TLS-CHOP type 2 derived from mRNA of the 2645/94 cells, we cloned the cDNA into pcDNA3.1(−) (Invitrogen, Carlsbad, CA, USA). 5 μg of the plasmid was transfected into NIH3T3 cells using FuGENE HD Transfection Reagent (Roche, Madison, Wisconsin, USA).

Retroviral expression vectors of CHOP and c/EBPα-CHOP were described previously [20]. NIH3T3 cells were infected with culture supernatants from virus-producing Plat-E cells [21] with polybrene.

2.3. Microarray analysis

MicroRNA microarray analysis was performed as previously described [22]. cDNA microarray analysis was performed as previously described [23]. The data presented in this manuscript have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE36966.

2.4. siRNA and miRNA transfection

2645/94 cells were transfected with siRNA or miRNA using HiPerFect Transfection Reagent (QIAGEN, Hilden, Germany). The nucleotide sequence of TLS-CHOP siRNA was previously described [9], and PAI-1 siRNA sequence is 5′-GGACAAAACUGGAGAUGCAdTdT-3′. Control siRNA and Synthetic miR-486 (BONAC Corporation, Kurume, Japan) were purchased, and we also used the control siRNA as negative control oligo in the miRNA transfection experiment.

2.5. Quantitative real-time PCR analysis

Quantitative real-time PCR analysis for miRNAs was performed as previously described [22]. For mRNA quantification, real-time PCR analysis was performed as described [24]. We used human PAI-1 specific primers, 5′-CCACAGCTGTCATAGTCTCA-3′ and 5′-GATGCATCTCCAGTTTTGTC-3′, and human GAPDH specific primers [25]. Statistical analysis of data were analyzed with Welch's t test. Values of $P < 0.05$ were considered statistically significant.

2.6. Western blotting

Western blot was performed as previously described [26]. Anti-CHOP mouse monoclonal antibody and anti-GAPDH (6C5) mouse monoclonal antibody were used [27].

2.7. Immunofluorescence staining

Immunofluorescence staining was performed as previously described [28]. The anti-CHOP antibody was applied at a concentration of 1:150. After washing with PBS, AlexaFluor 594-conjugated anti-mouse IgG (1:250, Life technologies) was applied.

2.8. In situ hybridization (ISH) and immunohistochemistry (IHC)

The Ethics Committee of Tokyo Medical University and Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital approved the research protocols for this study. ISH was performed as previously described [25]. The 5′ DIG-labeled ISH LNA probes used in this experiment is as follows: hsa-miR-486, 5′-UCCUGUACUGAGCUGCCCCGAG-3′; Scramble-ISH negative control sequence, 5′-GTGTAACACGTCTATACGCCCA-3′. Staining categories for ISH of miR-486, negative control staining (−) and positive staining (from + to +++) (Table 2), was determined as shown in Supplementary Fig. 1.

IHC was performed as previously described [27]. IHC analysis using anti-human PAI-1 (TJA6) monoclonal antibody (Sanbio B.V., Uden, The Netherlands).

2.9. Immunoprecipitation (IP)

To analyze Ago2 associated mRNA, co-IP experiments were performed using an anti-Argonaute 2 (Ago2) antibody (Wako Pure Chemical Industries, Tokyo, Japan) as previously described [25]. After that, RNA was subjected to quantitative real time PCR analysis using human PAI-1 specific primers and GAPDH specific primers.

3. Results

3.1. Identification of the microRNAs regulated by TLS-CHOP

To investigate alteration of microRNA expression profile by TLS-CHOP expression, we first constructed a TLS-CHOP expression vector. Although 11 types of TLS-CHOP variants are known up to now [3], the great majority (>50%) of MLSs have type 2 of TLS-CHOP [27]. Thus, we inserted the cDNA encoding full length of the type 2 variant into the expression vector pcDNA3.1(−). Then, after transient transfection of the resultant TLS-CHOP expression vector or parental pcDNA3.1(−) vector as a control into NIH3T3 cells, we performed microRNA microarray analysis of the cells (Fig. 1A and B). The results showed that only a small portion of miRNAs were increased or decreased more than 2-fold by exogenous TLS-CHOP expression (Table 1). Then, we focused on miR-486 which was the most significantly downregulated in the result of the microarray analysis (Table 1). Real-time PCR analysis confirmed the downregulation of miR-486 (Fig. 1C). Furthermore, we transfected 2645/94 cells with TLS-CHOP siRNA or a non-targeting control siRNA at a final concentration of 100 nM, and compared miR-486 expression levels in the cells with real-time PCR analysis. We found that TLS-CHOP knockdown by the specific siRNA led to 3-fold increase of miR-486 expression (Fig. 1D). These results indicate that TLS-CHOP represses miR-486 expression.

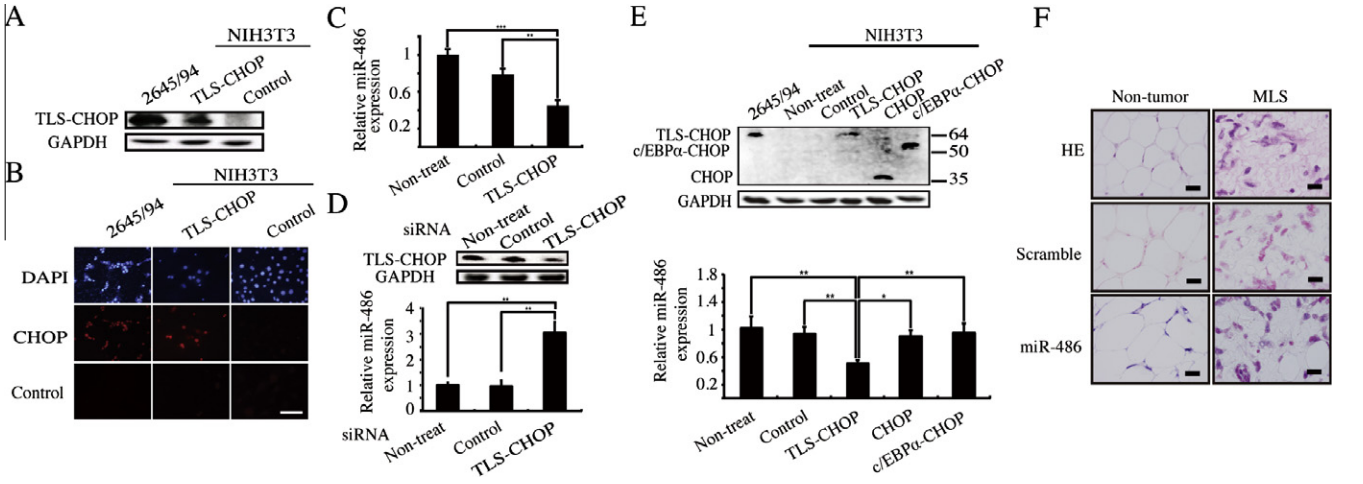

Fig. 1. TLS-CHOP represses miR-486 expression. NIH3T3 cells transfected with TLS-CHOP expression vector or negative control vector were harvested 48 h after transfection. The expression of transgene was confirmed by Western blotting using TLS-CHOP antibody (A) and Immunofluorescence staining using CHOP antibody (B). Nuclear staining with 4′, 6-diamidino-2-phenylindole (DAPI) (blue) is also shown. Scale bar, 50 μm. 2645/94 cells were also shown as a positive control. (C) Reduction of miR-486 expression in NIH3T3 cells by exogenous TLS-CHOP expression. Forty-eight hours after transfection with TLS-CHOP expression vector, RNA samples were extracted from the cells and subjected to quantitative real-time PCR analysis. Data were normalized to the miRNA level of non-treated cells that was arbitrarily set to 1 in the graphical presentation. Bars, SD. ** and *** indicate $P < 0.01$ and $P < 0.001$, respectively. (D) Induction of miR-486 expression in TLS-CHOP-knockdowned 2645/94 cells. 2645/94 cells transfected with TLS-CHOP siRNA or non-silencing control siRNA were harvested 72 h after transfection. Then, protein and RNA samples were prepared from the cells and subjected to Western blotting and quantitative real-time PCR analysis, respectively. Bars, SD. $^{**}P < 0.01$. Western blotting shows TLS-CHOP reduction by specific siRNA. GAPDH is showed as a loading control. (E) Relative expression level of miR-486 in NIH3T3 infected with retroviruses expressing CHOP or c/EBPα-CHOP determined by quantitative real-time PCR analysis. c/EBPα-CHOP is a fusion of CHOP to the activation domain of rat c/EBP protein. Bars, SD. * and ** indicate $P < 0.05$ and $P < 0.01$, respectively. (F) Expression of miR-486 in MLS and matched non-tumor tissues shown by in situ hybridization. Scale bar, 50 μm.

3.2. Repression of miR-486 expression is not caused by CHOP overexpression

Although TLS is expressed constitutively, CHOP is normally expressed at very low levels in normal cells [4,5]. However, TLS-CHOP that is expressed constitutively in MLS cells includes full length of CHOP protein. Thus, we next investigated whether the repression of miR-486 expression is caused by increased expression of CHOP or not. As shown in Fig. 1E, overexpression of intact CHOP did not repress miR-486 expression in NIH3T3 cells. In addition, c/EBPα-CHOP fusion protein, which acts similar to an intact functional complex of CHOP, also did not inhibit miR-486 expression (Fig. 1E). Thus, downregulation of miR-486 by TLS-CHOP seems to be a specific event by the fusion oncoprotein.

3.3. Low-level expression of miR-486 in human MLS tissues

Next, we examined miR-486 expression on a MLS tissue microarray which contains 4 cases of MLS tissues and 4 cases of matched non-tumor tissues by ISH. ISH showed that miR-486 expression levels in all of MLS tissue samples were lower than that in non-tumor tissues (Fig. 1F and Table 2). These results suggest that the repression of miR-486 expression by TLS-CHOP occurs not only in cultured cells but also within human MLS tissues.

3.4. Exogenous overexpression of miR-486 inhibits growth of MLS-derived cells

To investigate the functional significance of miR-486 repression in MLS, we transfected 2645/94 cells with synthetic miR-486 or negative control oligo at a final concentration of 100 nM. We observed that the exogenous overexpression of miR-486 inhibited growth of 2645/94 cells (Fig. 2). Thus, we consider that miR-486 has potential to function as a tumor suppressor, and is required to be repressed in MLS cells.

Table 1
microRNAs which are up-or down-regulated by TLS-CHOP.

Probe Name	TLS-CHOP	Control	TLS-CHOP/Control
mmu-miR-486	250.26	945.23	0.26
mmu-miR-543	201.25	540.56	0.37
mmu-miR-409	199.18	526.04	0.38
mmu-miR-201	273.54	699.60	0.39
mmu-miR-96	281.18	695.90	0.40
mmu-let-7d*	569.9	1394.47	0.41
mmu-miR-542-5p	471.78	1147.61	0.41
mmu-miR-540	568.47	1324.77	0.43
mmu-miR-499	244.17	530.75	0.46
mmu-miR-223	240.31	513.34	0.47
mmu-miR-362	191.33	401.78	0.48
mmu-miR-376c	184.81	382.30	0.48
mmu-miR-302c*	213.89	437.89	0.49
mmu-miR-302b*	208.15	416.60	0.50
mmu-miR-547	913.35	421.86	2.17
mmu-miR-140	1418.69	382.14	3.71
mmu-miR-374–5p	2903.4	662.45	4.38

Table 2
Clinical, Histological, Staining categoies for ISH of miR-486.

Case	Histology	ISH
		miR-486
1	MLS	++
2	MLS	++
3	MLS	++
4	MLS	+
5	Non-tumor	+++
6	Non-tumor	+++
7	Non-tumor	+++
8	Non-tumor	+++

Week (+), week-moderate (++), moderate-strong (+++).

3.5. PAI-1 is a novel target gene of miR-486 in human MLS

We next performed a whole human genome microarray analysis of 2645/94 cells transfected with synthetic miR-486 or control

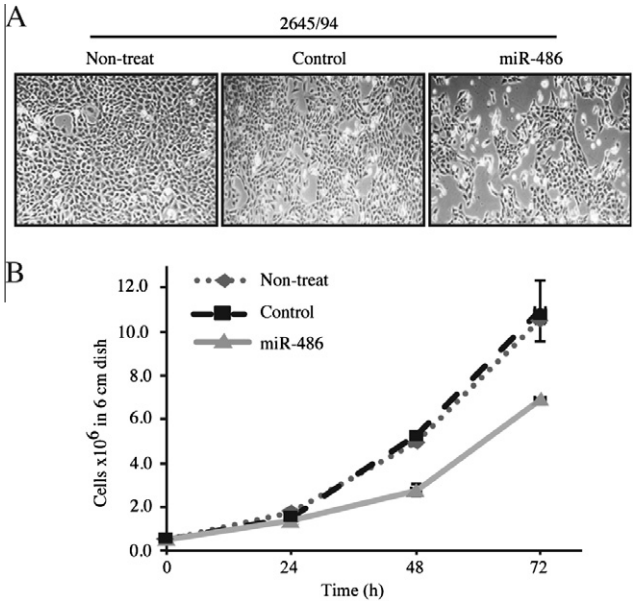

Fig. 2. Exogenous overexpression of miR-486 inhibited growth of MLS-derived 2645/94 cells. (A) Representative phase-contrast images of 2645/94 cells at 72 h after transfection of synthetic miR-486 or negative control oligo. (B) The cells transfected with miR-486 or control oligo were harvested at 0, 24, 48, and 72 h after transfection. Cell numbers in 6 cm dishes at each time point were counted using a hemocytometer. Bars, SD.

oligo to screen for genes whose expression levels were affected by miR-486 expression, and identified 1653 genes showing significant differences in probe intensity of at least 2-fold in the cells.

Then, we classified the 1653 genes according to their putative Gene Ontology (GO) – cellular component (CP) by using OBO-Edit software, AmiGO Database (http://amigo.geneontology.org/cgi-bin/amigo/go.cgi.). The cellular component GO analysis indicated that the genes associated with cellular membrane were significantly affected by exogenous miR-486 (Fig. 3A).

Next, we endeavored to predict the candidate miR-486 target genes by a computational approach. Microarray analysis showed that 1221 genes out of the 1653 genes were downregulated by exogenous miR-486 overexpression (Supplementary dataset 1). Because miRNAs inhibit translation or promote degradation of their target mRNAs [10], the 1221 downregulated genes are candidates of direct miR-486 target genes. Among the 1221 genes, only five genes, PAI-1, SORCS1, LTBP2, CDH7 and TWF1, were listed by more than two prediction programs (Target scan, miRanda and DIANA microT) (Fig. 3B and C). Then, to examine whether miR-486 actually downregulates the five genes or not, we performed real-time PCR analysis of RNA samples from 2645/94 cells transfected with miR-486 or control oligo, and only PAI-1 mRNA level was confirmed to be decreased by exogenous miR-486 expression (Fig. 3D).

Furthermore, we performed immunoprecipitation (IP) assays using an anti-Ago2 antibody. Mature miRNAs function in stable complexes with proteins of the Argonaute (ago) family, the core of the RNA-induced silencing complex (RISC) [29]. Initially miRNA targets RISC to mRNAs, and the RISC–mRNA interaction results in translational repression that may also be accompanied by mRNA destruction [30]. And Ago2-co-IP approach retains mRNAs containing miRNA binding sites [31]. Then, we examined PAI-1 mRNA levels in Ago2 IP samples from cell lysates of 2645/94 cells transfected with miR-486 or control oligo, and found that PAI-1 mRNA was

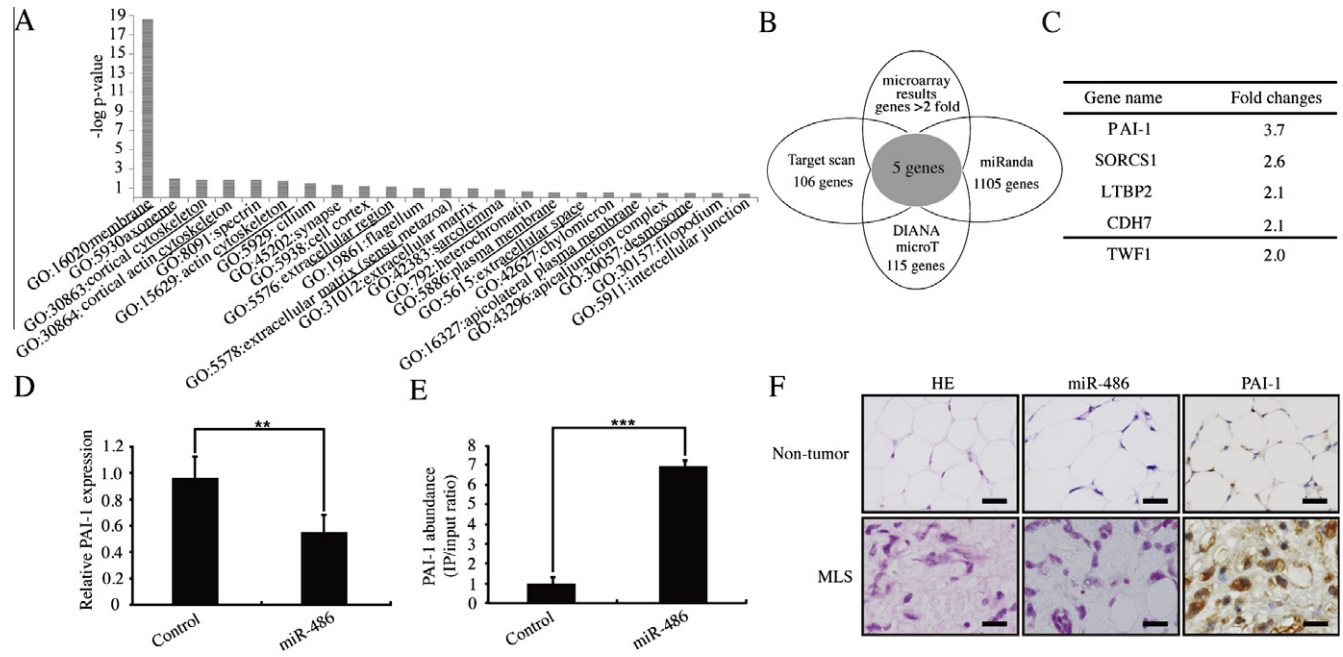

Fig. 3. Search for miR-486 target genes. (A) Classification of 1653 genes which exhibited more than two-fold changes in expression between 2645/94 cells transfected with synthetic miR-486 and control oligo by cellular component gene ontology. (B) The miR-486 target prediction by microarray analysis and three prediction algorithms, Target scan, miRanda, and DIANA microT. In the 1221 genes that were picked up by microarray analysis, 5 genes were also listed by more than two prediction algorithms. (C) List of 5 candidate target genes of miR-486. Fold changes of gene expression between 2645/94 cells transfected with synthetic miR-486 and control oligo are also shown. (D) Reduction of PAI-1 mRNA in miR-486-transfected 2645/94 cells. 2645/94 cells transfected with synthetic miR-486 or control oligo were harvested 48 h after transfection. Then, total RNA was extracted from the cells and subjected to quantitative real-time PCR analysis. Data were normalized to the mRNA level of the cells transfected with control oligo that was arbitrarily set to 1 in the graphical presentation. Bars, SD. $^{**}P < 0.01$. (E) Immunoprecipitation assay with an anti-Ago2 antibody. 2645/94 cells transfected with synthetic miR-486 or control oligo were harvested 17 h after transfection. After IP, RNA was extracted from the resultant samples, and was subjected to quantitative real-time PCR analysis. Bars, SD. $^{***}P < 0.001$. (F) Representative pictures of MLS tissue and matched non-tumor tissue of H&E-stained sections, in situ hybridization using LNA probe for miR-486, and immunohistochemistry with anti-PAI-1 antibody. Scale bar, 50 μm.

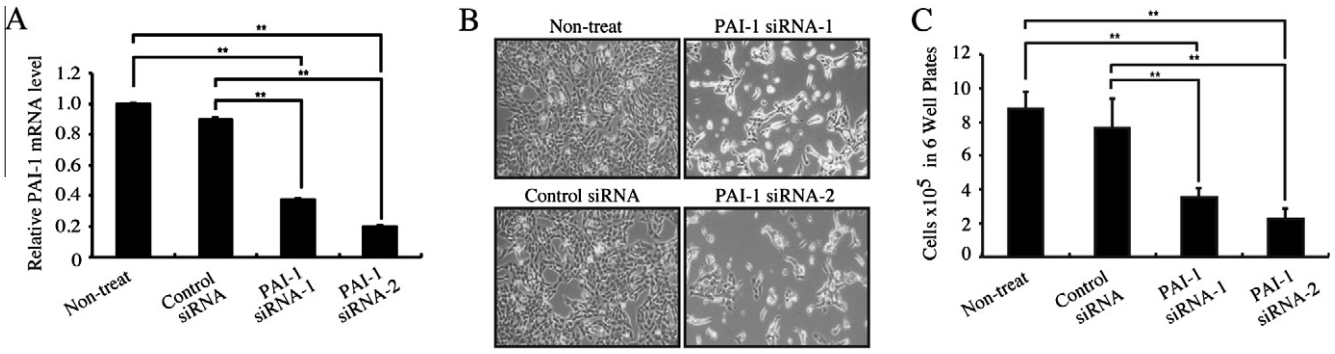

Fig. 4. Knockdown of PAI-1 expression in 2645/94 cells inhibited cell growth. (A) Reduction of PAI-1 transcript in 2645/94 cells by PAI-1 siRNA. 72 h after siRNA transfection, total RNA was extracted from 2645/94 cells transfected with PAI-1 siRNA or control siRNA, and subjected to quantitative real-time PCR analysis. Data were normalized to the mRNA level of non-treated cells that was arbitrarily set to 1 in the graphical presentation. Bars, SD. $^{**}P < 0.01$. (B) Representative phase-contrast images of 2645/94 cells at 72 h after siRNA transfection. (C) PAI-1 siRNA inhibited growth of 2645/94 cells. Cell numbers were determined at 72 h after siRNA transfection using a hemocytometer. Bars, SD. $^{**}P < 0.01$.

more abundant in the IP sample from miR-486-transfected cells than in control oligo-transfected cells (Fig. 3E). These results suggest that PAI-1 is a novel target of miR-486 in MLS cells. In addition, we confirmed elevated expression of PAI-1 protein in MLS tissue samples compared with non-tumor tissues by immunohistochemistry (Fig. 3F).

Finally, to obtain clues to the PAI-1 function in MLS cells, we transfected 2645/94 cells with PAI-1 siRNA at a final concentration of 50 nM. As shown in Fig. 4, knockdown of PAI-1 by the specific siRNA repressed growth of 2645/94 cells, suggesting that induction of PAI-1 expression by repression of miR-486 expression is a critical mechanism for proliferation of MLS cells.

4. Discussion

In this study, we have revealed that TLS-CHOP significantly represses miR-486 expression (Table 1, Fig. 1). In several human cancers including breast, lung, gastric, hepatic, colon, ovarian, testicular and prostate cancers and lymphoma, miR-486 has been already known to be downregulated [12,13]. In addition, Oh et al. reported that restoration of miR-486 expression by transfection of synthetic miRNA caused suppression of several pro-oncogenic traits and inhibition of miR-486 expression by transfection of miR-486 inhibitors (anti-miR-486) enhanced cellular proliferation in gastric cancer-derived cell lines [12]. Thus, miR-486 seems to have function as a tumor suppressor. We have also demonstrated that in MLS cells miR-486 expression was repressed (Fig. 1E, F, and Table 2), and exogenous overexpression of miR-486 inhibited MLS cell growth (Fig. 2). Therefore, we consider that repression of miR-486 expression may be a key process in MLS tumorigenesis and/or development. Although we focused only on miR-486 in this report, our microarray analysis demonstrated that expression of many other miRNAs might also be affected by TLS-CHOP (Table 1). Thus, it is also possible that at least some of them play a critical role in some processes in MLS. Further studies are required to elucidate all the details of the miRNA-involving processes. Nevertheless, to our knowledge this is the first report demonstrating that regulation of miRNA expression is involved in MLS tumorigenesis and/or development.

A previous study demonstrated that the tumor-suppressive effects of miR-486 are mediated through repression of OLFM4 in gastric cancer [12]. However, OLFM4 was not picked up as a candidate miR-486 target gene in our microarray analysis using 2645/94 cells (Fig. 3B and C). On the other hand, we have identified the PAI-1 gene as a novel target of miR-486 (Fig. 3C–F), and have demonstrated that PAI-1 knockdown by a specific siRNA lead to inhibit growth of cells (Fig. 4). Both OLFM4 and PAI-1 are known to promote tumor cell proliferation and invasion by inhibiting apoptosis [12,18,32,33]. Thus, OLFM4 and PAI-1 may play a similar role in gastric cancer and MLS, respectively.

Recently, we have revealed that TLS-CHOP represses melanoma differentiation-associated gene 7 (MDA-7)/interleukin-24 (IL-24) [9]. MDA-7/IL-24 is known as an anticancer cytokine, and exogenous expression of MDA-7/IL-24 induces growth arrest and apoptotic cell death in various human malignant cells. These molecular pathways may be function for maintenance of MLS cell proliferation. On the other hand, PAI-1 as a TLS-CHOP target identified in this report is suggested to function in cancer progression and metastasis [18]. This study may now lead to describe a vague outline of multistep mechanisms for MLS tumorigenesis, progression and metastasis. Further studies on the relationship between the mechanisms are required to make the outline more obvious.

In conclusion, we have revealed that TLS-CHOP induces the expression of PAI-1, a key regulator of tumor invasion and metastasis, through repression of miR-486 expression. We expect that understanding of this novel molecular mechanism by TLS-CHOP may contribute to the development of novel methods for MLS diagnosis and treatment.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.09.063.

References

[1] A. Crozat, P. Aman, N. Mandahl, D. Ron, Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma, Nature 363 (1993) 640–644.
[2] T.H. Rabbitts, A. Forster, R. Larson, P. Nathan, Fusion of the dominant negative transcription regulator CHOP with a novel gene FUS by translocation t(12;16) in malignant liposarcoma, Nat. Genet. 4 (1993) 175–180.
[3] M.P. Powers, W.L. Wang, V.S. Hernandez, K.S. Patel, D.C. Lev, A.J. Lazar, D.H. Lopez-Terrada, Detection of myxoid liposarcoma-associated FUS-DDIT3 rearrangement variants including a newly identified breakpoint using an optimized RT-PCR assay, Mod. Pathol. 23 (2010) 1307–1315.

[4] J. Perez-Losada, M. Sanchez-Martin, M.A. Rodriguez-Garcia, P.A. Perez-Mancera, B. Pintado, T. Flores, E. Battaner, I. Sanchez-Garcia, Liposarcoma initiated by FUS/TLS-CHOP: the FUS/TLS domain plays a critical role in the pathogenesis of liposarcoma, Oncogene 19 (2000) 6015–6022.
[5] C. Bento, M.K. Andersson, P. Aman, DDIT3/CHOP and the sarcoma fusion oncoprotein FUS-DDIT3/TLS-CHOP bind cyclin-dependent kinase 2, BMC Cell Biol. 10 (2009) 89.
[6] M. Kuroda, X. Wang, J. Sok, Y. Yin, P. Chung, J.W. Giannotti, K.A. Jacobs, L.J. Fitz, P. Murtha-Riel, K.J. Turner, D. Ron, Induction of a secreted protein by the myxoid liposarcoma oncogene, Proc. Natl. Acad. Sci. USA 96 (1999) 5025–5030.
[7] M.K. Andersson, M. Goransson, A. Olofsson, C. Andersson, P. Aman, Nuclear expression of FLT1 and its ligand PGF in FUS-DDIT3 carrying myxoid liposarcomas suggests the existence of an intracrine signaling loop, BMC Cancer 10 (2010) 249.
[8] P.A. Perez-Mancera, C. Bermejo-Rodriguez, M. Sanchez-Martin, F. Abollo-Jimenez, B. Pintado, I. Sanchez-Garcia, FUS-DDIT3 prevents the development of adipocytic precursors in liposarcoma by repressing PPARgamma and C/EBPalpha and activating eIF4E, PLoS One 3 (2008) e2569.
[9] K. Oikawa, M. Tanaka, S. Itoh, M. Takanashi, T. Ozaki, Y. Muragaki, M. Kuroda, A novel oncogenic pathway by TLS-CHOP involving repression of MDA-7/IL-24 expression, Br. J. Cancer (2012).
[10] H.W. Hwang, J.T. Mendell, MicroRNAs in cell proliferation, cell death and tumorigenesis, Br. J. Cancer 94 (2006) 776–780.
[11] A. Esquela-Kerscher, F.J. Slack, Oncomirs – microRNAs with a role in cancer, Nat. Rev. Cancer 6 (2006) 259–269.
[12] H.K. Oh, A.L. Tan, K. Das, C.H. Ooi, N.T. Deng, I.B. Tan, E. Beillard, J. Lee, K. Ramnarayanan, S.Y. Rha, N. Palanisamy, P.M. Voorhoeve, P. Tan, Genomic loss of miR-486 regulates tumor progression and the OLFM4 antiapoptotic factor in gastric cancer, Clin. Cancer Res. 17 (2011) 2657–2667.
[13] R. Navon, H. Wang, I. Steinfeld, A. Tsalenko, A. Ben-Dor, Z. Yakhini, Novel rank-based statistical methods reveal microRNAs with differential expression in multiple cancer types, PLoS One 4 (2009) e8003.
[14] H.R. Lijnen, Pleiotropic functions of plasminogen activator inhibitor-1, J. Thromb. Haemost. 3 (2005) 35–45.
[15] H.C. Kwaan, B. McMahon, The role of plasminogen-plasmin system in cancer, Cancer Treat. Res. 148 (2009) 43–66.
[16] P.A. Andreasen, R. Egelund, H.H. Petersen, The plasminogen activation system in tumor growth, invasion, and metastasis, Cell. Mol. Life Sci. 57 (2000) 25–40.
[17] S.B. Villadsen, J.B. Bramsen, M.S. Ostenfeld, E.D. Wiklund, N. Fristrup, S. Gao, T.B. Hansen, T.I. Jensen, M. Borre, T.F. Orntoft, L. Dyrskjot, J. Kjems, The miR-143/-145 cluster regulates plasminogen activator inhibitor-1 in bladder cancer, Br. J. Cancer 106 (2012) 366–374.
[18] K. Bajou, C. Maillard, M. Jost, R.H. Lijnen, A. Gils, P. Declerck, P. Carmeliet, J.M. Foidart, A. Noel, Host-derived plasminogen activator inhibitor-1 (PAI-1) concentration is critical for in vivo tumoral angiogenesis and growth, Oncogene 23 (2004) 6986–6990.
[19] N. Nishioka, T. Matsuoka, M. Yashiro, K. Hirakawa, K. Olden, J.D. Roberts, Plasminogen activator inhibitor 1 RNAi suppresses gastric cancer metastasis in vivo, Cancer Sci. 103 (2012) 228–232.
[20] H. Zinszner, R. Albalat, D. Ron, A novel effector domain from the RNA-binding protein TLS or EWS is required for oncogenic transformation by CHOP, Genes Dev. 8 (1994) 2513–2526.
[21] S. Morita, T. Kojima, T. Kitamura, Plat-E: an efficient and stable system for transient packaging of retroviruses, Gene Ther. 7 (2000) 1063–1066.
[22] M. Tanaka, K. Oikawa, M. Takanashi, M. Kudo, J. Ohyashiki, K. Ohyashiki, M. Kuroda, Down-regulation of miR-92 in human plasma is a novel marker for acute leukemia patients, PLoS One 4 (2009) e5532.
[23] R. Suzuki, M. Tanaka, M. Takanashi, A. Hussain, B. Yuan, H. Toyoda, M. Kuroda, Anthocyanidins-enriched bilberry extracts inhibit 3T3-L1 adipocyte differentiation via the insulin pathway, Nutr. Metab. (Lond.) 8 (2011) 14.
[24] K. Oikawa, K. Yoshida, M. Takanashi, H. Tanabe, T. Kiyuna, M. Ogura, A. Saito, A. Umezawa, M. Kuroda, Dioxin interferes in chromosomal positioning through the aryl hydrocarbon receptor, Biochem. Biophys. Res. Commun. 374 (2008) 361–364.
[25] A. Tsuchida, S. Ohno, W. Wu, N. Borjigin, K. Fujita, T. Aoki, S. Ueda, M. Takanashi, M. Kuroda, miR-92 is a key oncogenic component of the miR-17-92 cluster in colon cancer, Cancer Sci. 102 (2011) 2264–2271.
[26] K. Oikawa, T. Ohbayashi, J. Mimura, Y. Fujii-Kuriyama, S. Teshima, K. Rokutan, K. Mukai, M. Kuroda, Dioxin stimulates synthesis and secretion of IgE-dependent histamine-releasing factor, Biochem. Biophys. Res. Commun. 290 (2002) 984–987.
[27] K. Oikawa, T. Ishida, T. Imamura, K. Yoshida, M. Takanashi, H. Hattori, A. Ishikawa, K. Fujita, K. Yamamoto, J. Matsubayashi, M. Kuroda, K. Mukai, Generation of the novel monoclonal antibody against TLS/EWS-CHOP chimeric oncoproteins that is applicable to one of the most sensitive assays for myxoid and round cell liposarcomas, Am. J. Surg. Pathol. 30 (2006) 351–356.
[28] M. Kuroda, T. Ishida, M. Takanashi, M. Satoh, R. Machinami, T. Watanabe, Oncogenic transformation and inhibition of adipocytic conversion of preadipocytes by TLS/FUS-CHOP type II chimeric protein, Am. J. Pathol. 151 (1997) 735–744.
[29] S.M. Hammond, E. Bernstein, D. Beach, G.J. Hannon, An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells, Nature 404 (2000) 293–296.
[30] R.S. Pillai, S.N. Bhattacharyya, C.G. Artus, T. Zoller, N. Cougot, E. Basyuk, E. Bertrand, W. Filipowicz, Inhibition of translational initiation by Let-7 MicroRNA in human cells, Science 309 (2005) 1573–1576.
[31] F.V. Karginov, C. Conaco, Z. Xuan, B.H. Schmidt, J.S. Parker, G. Mandel, G.J. Hannon, A biochemical approach to identifying microRNA targets, Proc. Natl. Acad. Sci. USA 104 (2007) 19291–19296.
[32] X. Zhang, Q. Huang, Z. Yang, Y. Li, C.Y. Li, GW112 a novel antiapoptotic protein that promotes tumor growth, Cancer Res. 64 (2004) 2474–2481.
[33] Y. Chen, R.J. Kelm Jr., R.C. Budd, B.E. Sobel, D.J. Schneider, Inhibition of apoptosis and caspase-3 in vascular smooth muscle cells by plasminogen activator inhibitor type-1, J. Cell. Biochem. 92 (2004) 178–188.