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Downregulation of Securin by the variant RNF213 R4810K (rs112735431, G>A) reduces angiogenic activity of induced pluripotent stem cell-derived vascular endothelial cells from moyamoya patients

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ABSTRACT

Moyamoya disease (MMD) is a cerebrovascular disease characterized by occlusive lesions in the circle of Willis. The RNF213 R4810K polymorphism increases susceptibility to MMD. Induced pluripotent stem cells (iPSCs) were established from unaffected fibroblast donors with wild-type RNF213 alleles, and from carriers/patients with one or two RNF213 R4810K alleles. Angiogenic activities of iPSC-derived vascular endothelial cells (iPSECs) from patients and carriers were lower (49.0 ± 19.4%) than from wild-type subjects ($p < 0.01$). Gene expression profiles in iPSECs showed that Securin was down-regulated ($p < 0.01$) in carriers and patients. Overexpression of RNF213 R4810K downregulated Securin, inhibited angiogenic activity (36.0 ± 16.9%) and proliferation of humanumbilical vein endothelial cells (HUVECs) while overexpression of RNF213 wild type did not. Securin expression was downregulated using RNA interference techniques, which reduced the level of tube formation in iPSECs and HUVECs without inhibition of proliferation. RNF213 R4810K reduced angiogenic activities of iPSECs from patients with MMD, suggesting that it is a promising *in vitro* model for MMD.

1. Introduction

Moyamoya disease (MMD) is an idiopathic cerebrovascular disease. It is characterized by occlusive lesions at the terminal portion of internal carotid arteries in the circle of Willis, with compensatory development of a fine vascular network that resembles "puffs of smoke" [1,2]. It is now recognized as one of the major causes of stroke in adults and children worldwide [3–6]. We recently identified *RNF213* as the susceptibility gene for MMD, and the p.R4810K (rs112735431, ss179362673: G>A; herein referred to as RNF213 R4810K) polymorphism as a founder variant commonly found in East Asian (Japanese, Korean and Chinese) patients [7]. RNF213 encodes a 591 kDa protein that exhibits ATPase and ubiquitin ligase activities. Although knockdown of RNF213 in zebrafish impaired angiogenesis, the physiological and biochemical functions of RNF213, and pathological consequences of MMD associated with RNF213 R4810K remain unknown [7].

The minor allele frequency of the founder RNF213 R4810K polymorphism in the general population is estimated to be 0.43–1.36% for East Asia, equivalent to a prevalence of 0.86–2.72% for carriers. RNF213 R4810K elevates the risk of MMD by more than 100-fold in carriers [7], with approximately 15 million individuals thought to be at extremely high risk [8]. The prevalence of patients with MMD (0.01%) is much lower than that for RNF213 R4810K carriers (3%) in Japan and Korea [3,4]. We have sought to determine the triggering factors that induce MMD in RNF213 R4810K carriers. Considering the social and economic dimensions of a large high-risk population in East Asia, determination of these MMD triggering

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factors is a high-priority issue. Such triggers are considered to act through RNF213 R4810K, but elucidation of these triggers has been hampered, mainly because of the lack of knowledge with respect to RNF213 R4810K pathology.

The primary aims of our study were to characterize RNF213 R4810K and the physiological functions of RNF213. To determine the pathological defects attributable to RNF213 R4810K, we tested whether vascular endothelial cells from patients with MMD have lowered angiogenic activities. Our hypothesis was based on reports of defective angiogenic activities for circulating endothelial progenitor cells in MMD patients [9]. We used induced pluripotent stem cell (iPSC) technology with the hope that it might yield useful *in vitro* disease models [10]. This approach is particularly useful for diseases in which the pathological processes have yet to be elucidated. Once an *in vitro* model has been established it is possible to reveal pathological clues about a disease; it can then be employed as a drug-screening tool, paving the way for translational research. We characterized *ex vivo* phenotypes of vascular endothelial cells differentiated from iPSCs (iPSECs) and conducted a series of *in vitro* experiments to understand the underlying mechanisms of MMD.

2. Methods

2.1. Participants

We studied three probands from three unrelated families with MMD. Diagnosis was made based on criteria from the Japanese Research Committee on MMD (Ministry of Health, Labour and Welfare, Japan) [11]. Participants consisted of six affected, or unaffected and unrelated subjects (Table 1 and Supplementary data). Genotyping revealed a AA genotype (homozygous for RNF213 R4810K) for two affected subjects, a GA genotype (heterozygous for RNF213 R4810K) in one affected and one unaffected subject, and a GG genotype (wild-type for RNF213 R4810K) for two unaffected subjects. We obtained written informed consent from all participants in this study. Our study was approved by the Institutional Ethical Review Board of Kyoto University.

2.2. Establishment of iPSECs

Dermal fibroblasts were isolated from arms and cultured. Induction of iPSCs was performed as described previously (Supplementary data) from primary fibroblasts for three MMD patients, an unaffected carrier and two controls (Table 1). We then induced the differentiation of iPSCs into vascular endothelial cells (Supplementary data). Angiogenic activity of iPSECs was assayed by tube formation. Gene expression profiles were determined using a GeneChip microarray (Human Gene 1.0 ST; Supplementary data).

Table 1
Summary of donor fibroblast information.

ID	Diagnosis	Gender	Age at onset	Age at biopsy	R4810K (G>A) of RNF213
Control 1	Healthy control	F	NA	81	GG
Control 2	Healthy control	F	NA	6	GG
Unaffected carrier	Healthy control	M	NA	36	GA
Patient 1	Familial MMD	F	10	43	GA
Patient 2	Familial MMD	F	55	63	AA
Patient 3	Familial MMD	F	50	64	AA

MMD, moyamoya disease.

2.3. Cell culture and transfection

Fibroblasts were maintained in Dulbecco's Minimal Essential Medium (DMEM; Invitrogen, Tokyo, Japan) containing 10% fetal bovine serum (FBS; Japan Bioserum, Hiroshima, Japan). The iPSCs were maintained in Primate ES medium (ReproCELL, Tokyo, Japan) and supplemented with 500 U/ml penicillin/streptomycin (Invitrogen) and 4 ng/ml recombinant human basic fibroblast growth factor (bFGF; WAKO, Tokyo, Japan) as previously reported [10,12]. Human umbilical vein endothelial cells (HUVECs; Lonza, Walkersville, MD, USA) were maintained in EGM-2 (Lonza). An mCherry-tagged wild-type RNF213 or an mCherry-tagged RNF213 R4810K was cloned into pcDNA3.1 (Invitrogen) (Supplementary data). The plasmids were introduced with an Amaxa Nucleofector Device (Lonza).

2.4. Assessment of angiogenic activity

Endothelial tube formation was assessed as described previously [13]. The iPSECs (5000 cells/well) or HUVECs (5000 cells/well or 20,000 cells/well) were seeded onto matrigel-coated (BD Biosciences, Bedford, MA, USA) 96-well plates. Cells were incubated for 12 h at 37 °C and digital images of tubes that formed were captured. For quantitation, tube area, total tube length and the number of tube branches were calculated using Image J software (National Institute of Health, USA). Parameters for assessing tube formation function were obtained from three or four independent tube formation assays.

2.5. RNA interference (RNAi)

Transfection of small interfering RNAs (siRNAs) was conducted using Dharmafect (#1 or #3; Dharmacon, Lafayette, CO, USA) following the manufacturer's recommendations. We also used Nucleofector instruments to transfect HUVECs and iPSECs according to the manufacturer's protocols. We purchased and used Securin siRNA (sc-37491; Santa Cruz Biotechnology, Santa Cruz, CA, USA), RNF213 siRNA 1 (sc-94184; Santa Cruz Biotechnology) and RNF213 siRNA 2 (s33568; Ambion, Austin, TX, USA), with control siRNA-A (sc-37007, Santa Cruz Biotechnology) and silencer select negative control #1 siRNA (Ambion) used as controls. To monitor knockdown of gene expression, real-time quantitative polymerase chain reaction (qPCR), immunostaining and/or western blotting assays were conducted.

2.6. Growth curves

Cell proliferation was assessed using colorimetric 3,4,5-dimethylthiazol-2-yl-2,5-diphenyl tetrazolium bromide (MTT) assays, which were carried out as described previously [14] unless otherwise specified. For growth curves for HUVECs in the overexpression experiment of RNF213 wild type and R4810K, at 2 days post-transfection, HUVECs were re-seeded at a density of 8×10^4 -cells/3.5-mm dish. Viable cells were assessed and counted each day using trypan blue (Nacalai Tesque) exclusion.

2.7. Western blotting

We used the CelLytic M (Sigma–Aldrich, St Louis, MO, USA) cell lysis buffer containing a protease inhibitor cocktail (Nacalai Tesque). In certain cases we also used a lysis buffered comprising 50 mM Tris–HCl (pH 8.0), 1% Nonidet P-40 and 150 mM NaCl. Samples were subjected to immunoblotting using the anti-RNF213

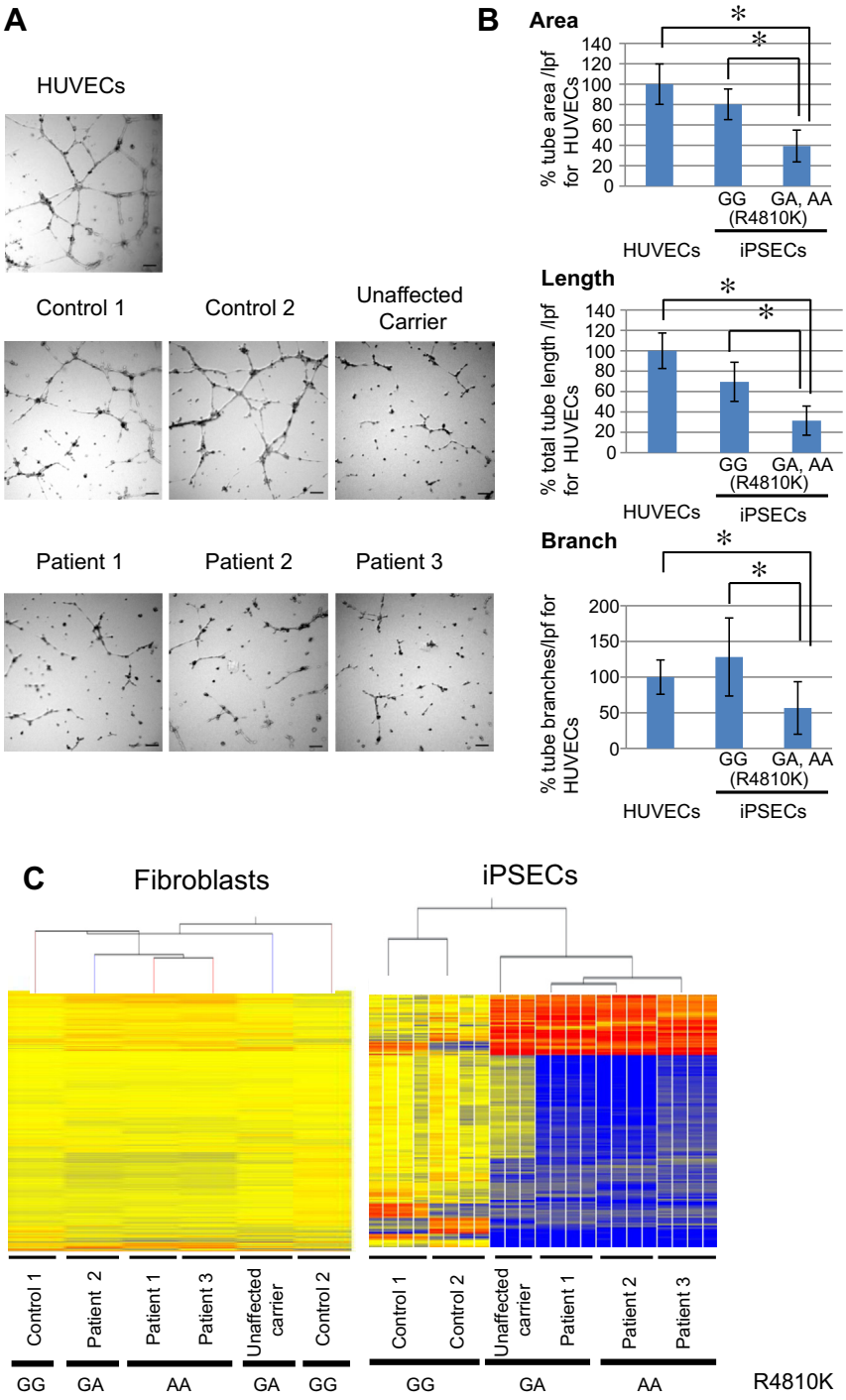

Fig. 1. Reduced angiogenic activities and differential gene expression in iPSECs. (A) Representative photomicrographs show the formation of tube-like structures in HUVECs and iPSECs from controls (GG genotype), the unaffected carrier, and patients (GA or AA genotypes). The scale bar indicates 100 μm. (B) Tube area, length and branch per low power field (lpf) were determined by matrigel assays and imaging analysis ($n = 3–4$, $^*p < 0.05$ using Student's t-test). Quantitative analysis of tube formation for iPSECs was performed between the GG genotype, and the GA and AA genotypes of p.R4810K on RNF213. HUVECs were used as a positive control. (C) Cluster analysis of fibroblasts from the control, unaffected carrier and MMD patients (single experiment) using microarrays (left panel). Cluster analysis of iPSECs from the unaffected carrier (three independent experiments), control and MMD patients (four independent experiments) using microarrays (right panel). Differentially regulated genes that were identified have been presented in Table S1.

antibody, which we generated (Supplementary data), or using anti-Securin (PTTG; Zymed, San Francisco, CA, USA), anti-β-tubulin (Sigma–Aldrich) or anti-β-actin (Abcam, Cambridge, UK) antibodies. Quantitation was conducted using Image J software.

2.8. Statistical analysis

Results are presented as the mean ± standard deviation (SD). Differences between groups were analyzed using analysis of variance (ANOVA), followed by Tukey's honestly significant difference

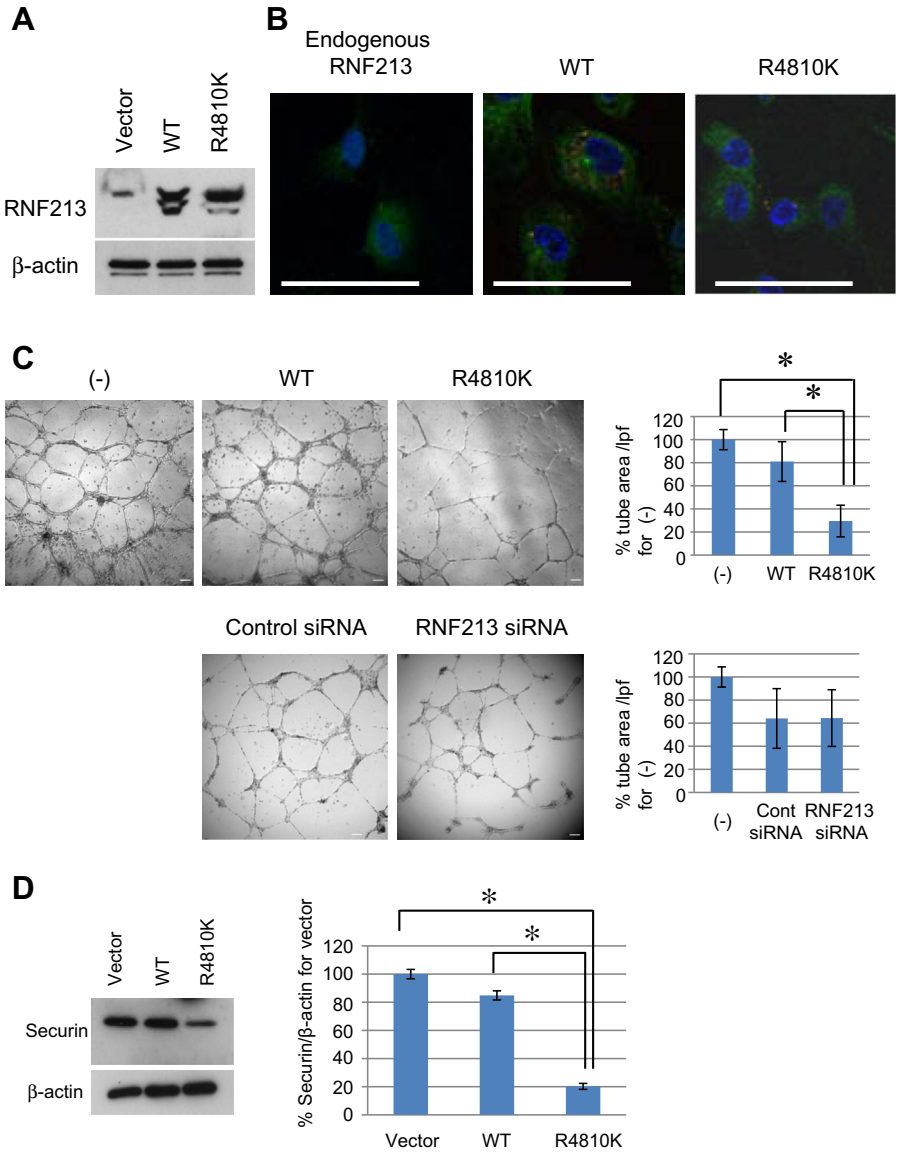

Fig. 2. Effects of RNF213 R4810K overexpression on the angiogenic activities of HUVECs. (A) Western blot analysis of endogenous and exogenous RNF213 in HUVECs. β-Actin was used as a loading control for all Western blots. (B) Subcellular localization of exogenous wild-type RNF213-mCherry (WT), RNF213 R4810K-mCherry (R4810K) and endogenous RNF213 in HUVECs. Left panel: HUVECs were stained for RNF213 (green) and DNA (blue). Middle and right panels: HUVECs transfected with RNF213-mCherry (red) were stained for tubulin (green) and DNA (blue). (C) Tube formation assays for HUVECs transfected with the RNF213 expression vector or RNF213 siRNA after 12 h of culture on matrigel. Non-transfected HUVECs (−) were used as a positive control. The scale bar indicates 100 μm. The tube areas for RNF213 overexpression ($n = 4$, $^*p < 0.05$ using Student's t-test) and siRNAs ($n = 3$) were quantified (right panel). Similar results were obtained for length and branch (data not shown). (D) Western blot analysis of Securin in HUVECs overexpressing wild-type RNF213 and RNF213 R4810K, with β-actin used as a loading control ($^*p < 0.01$ using Student's t-test). Data represents the mean of at least three independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

test for comparisons involving more than two means (SAS Institute Inc., Cary, NC, USA). A p-value less than 0.05 was considered statistically significant.

3. Results

3.1. iPS cell clones

We established iPSCs from three unaffected subjects and three patients with MMD (Table 1). We used qPCR to select iPSC clones where exogenously introduced genes were repressed (Fig. S1A). Genetic identity was confirmed by genotypes of short tandem repeat (STR) markers between donor fibroblasts and iPSCs derived from these cells (Fig. S1B). All six iPSC clones showed characteristics of human embryonic stem cell morphology. They also expressed pluripotency markers, including NANOG, OCT4, SOX2, SSEA4, TRA-1-60, TRA-1-81 and alkaline phosphatase (ALP) enzymatic activity (Fig. S1C). We confirmed that there was no expression of SSEA1. The pluripotent properties of the iPSCs were confirmed by examining methylation of the OCT4 and NANOG promoter regions (Fig. S1D), and by embryoid body (EB) formation (Fig. S1E).

3.2. Reduced angiogenic activities of iPSECs in subjects with RNF213 R4810K

The iPSECs derived from iPSCs showed typical morphological features. They exhibited a cobblestone-like appearance on culture dishes, and expressed eNOS, CD31, VE-Cadherin, vWF and CD34, while iPSCs did not (Fig. S2C and D). All these features correspond

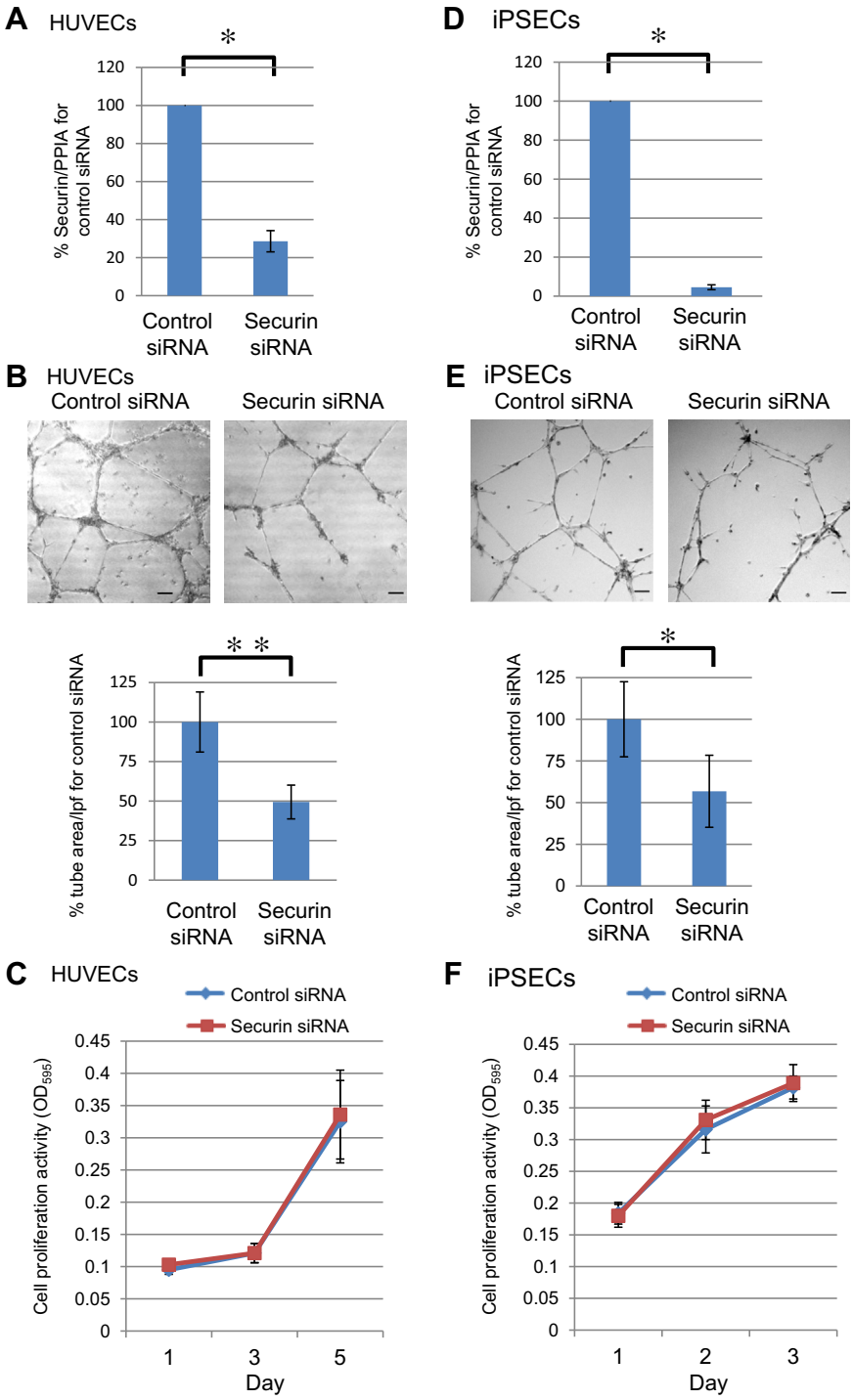

Fig. 3. The effect of depletion of Securin on angiogenic activities in HUVECs and iPSECs from a control subject. (A) RNA levels of Securin in HUVECs treated with control and Securin siRNA ($n = 3$, $^{*}p < 0.05$ using Student's t-test). (B) Representative photomicrographs (upper) and tube areas per low power field (lpf) (lower) of HUVECs treated with control and Securin siRNA ($n = 3$, $^{**}p < 0.01$ using Student's t-test). Similar results were obtained for length and branch (data not shown). The scale bar indicates 100 μm. (C) Time course of cell proliferation for HUVECs treated with control and Securin siRNA. (D) RNA levels of Securin in control iPSECs treated with control and Securin siRNA ($n = 3$, $^{*}p < 0.05$ using Student's t-test). (E) Representative photomicrographs (upper) and tube areas per lpf (lower) of control iPSECs treated with control and Securin siRNA ($n = 3$, $^{*}p < 0.05$ using Student's t-test). Similar results were obtained for length and branch (data not shown). The scale bar indicates 100 μm. (F) Time course of cell proliferation for control iPSCs treated with control and Securin siRNA.

to characteristics of vascular endothelial cells. We investigated iPSEC angiogenic activity by tube formation. The iPSECs from MMD patients and an unaffected carrier revealed significantly reduced tube areas compared with those from controls (wild-type RNF213; Fig. 1A and B and Fig. S3). Lower angiogenic activity was also confirmed by total tube length and numbers of branches (Fig. 1A and B, Fig. S3), but could not be attributed to the suppression of growth (Fig. S4).

3.3. Downregulation of mitotic phase-associated genes in iPSECs with RNF213 R4810K

To better understand whether RNF213 R4810K affects expression levels of specific genes related to reduced angiogenic activity, we used microarrays to identify differentially expressed genes in iPSECs and their parental fibroblasts. Cluster analysis of expression profiles demonstrated a clear difference for iPSECs with the RNF213 R4810K genotype (Fig. 1C).

In the iPSECs, the expression levels of 159 genes were differentially regulated by more than three-fold ($p < 0.01$). Our analysis revealed that 38 genes were up-regulated, and 121 were down-regulated (Table S1). Gene ontology analysis identified 161 significant terms ($p < 0.01$; Tables S2). Gene and ontology classifications revealed that many mitotic phase-associated genes were down-regulated (Tables S1 and S2) in iPSECs from donors with the RNF213 R4810K polymorphism. We confirmed the microarray results through qPCR assays for five genes (*Securin*, *BUB1*, *NDC80*, *PLK1* and *CDC20*) (Fig. S5).

3.4. Effects of RNF213 R4810K overexpression

Wild-type RNF213 and/or RNF213 R4810K proteins fused to the mCherry reporter (Fig. S6) were overexpressed in HUVECs (Fig. 2A and B). Plasmids were transfected into HUVECs by electroporation with gene transfer efficiencies around 72%. Overexpression of RNF213 R4810K reduced angiogenic activity in HUVECs (Fig. 2C) and significantly inhibited proliferation of HUVECs (Fig. S8). In contrast, neither overexpression nor depletion of wild-type RNF213 reduced angiogenic activity (Fig. 2C) or proliferation (Fig. S8). Localization of exogenous RNF213 R4810K was similar to that for the exogenous and endogenous wild-type RNF213, with these proteins observed in the cytoplasm around the nucleus (Fig. 2B). Overexpression of RNF213 R4810K significantly down-regulated the expression of Securin ($p < 0.01$; Fig. 2D), while overexpression of wild-type RNF213 had little effect on Securin.

3.5. Depletion of Securin affects angiogenic activities of HUVECs and iPSECs

Securin induces angiogenesis, and is an inhibitor of premature sister chromatid separation [15]. Depletion of Securin induces severe defects in cell migration by lowered microtubule nucleation [16], which results in lowered angiogenic activity. We then examined the effects of RNAi-mediated depletion of Securin on tube formation and proliferation using HUVECs and iPSECs carrying wild alleles. RNAi knockdown of Securin was shown to impair tube formation without inhibiting proliferation in both HUVECs (Fig. 3A–C) and iPSECs (Fig. 3D–F).

4. Discussion

In this study, we successfully differentiated iPSECs from the iPSCs of MMD patients and carriers harboring GA or AA genotypes of RNF213 R4810K. The iPSECs with GA or AA genotypes replicated lowered angiogenic activity for circulating endothelial progenitor cells in MMD patients [9]. To the best of our knowledge, we are the first to report the generation of MMD-specific iPSCs that are able to differentiate into iPSECs, which can then be used as an *in vitro* MMD model.

Reduced angiogenic activity was consistently observed in iPSECs from subjects that had RNF213 R4810K, and in HUVECs overexpressing RNF213 R4810K. Because gene expression of Securin, which is known to require for angiogenesis [15], was suppressed in iPSECs carrying RNF213 R4810K, we investigated the effects of RNAi-mediated knockdown of Securin on tube formation in HUVECs and iPSECs. Depletion of Securin inhibited tube formation consistently without inhibition of proliferation as we observed lowered tube formation of iPSECs from patients and the carrier. This indicated to us that reduced expression of Securin per se is responsible for lowered angiogenic activity. Because cell migration is known to be impaired when Securin is depleted [16], we postulate that the defect in migration might occur in iPSECs, thereby reducing angiogenic activity.

We did not investigate abnormalities of vascular smooth muscle cells (VSMCs), which could be considered a limitation of our work. A major pathological finding in MMD is the excessive proliferation of VSMCs. The interactions between ECs and VSMCs are known to play key roles in vascular structure and function of vessels. VSMC migration, proliferation, and differentiation are critical processes involved in intimal hyperplasia and are under regulation by endothelial cells [17]. We postulate that there is a defect in the crosstalk between ECs and VSMCs in patients.

Many mitosis-related genes were downregulated by RNF213 R4810K. We focused on Securin and determined that its downregulation may play a substantial role in lowered angiogenic activity of iPSECs from patients with MMD. However, in the present study, we did not investigate the effects of other downregulated genes. Furthermore, we did not investigate the mechanisms of Securin downregulation in iPSECs from MMD patients. Further studies are needed to substantiate the roles of RNF213 R4810K in downregulation of mitotic associated genes.

In conclusion, we observed that iPSECs from MMD patients had impaired angiogenic functions. RNF213 R4810K manifests as defects in angiogenesis by the downregulation of Securin expression. The resulting defects in angiogenesis are considered risk factors for MMD patients. Furthermore, our study demonstrated that iPSECs can serve as an *in vitro* MMD model, as they express a useful benchmark phenotype for high throughput screening, which can be applied to drug development and discovering MMD triggers.

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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.2013.07.004.

References

[1] J. Suzuki, A. Takaku, Cerebrovascular "moyamoya" disease. Disease showing abnormal net-like vessels in base of brain, Arch. Neurol. 20 (1969) 288–299.
[2] K. Takeuchi, K. Shimizu, Hypogenesis of bilateral internal carotid arteries, Brain Nerve 9 (1957) 37–43.
[3] S. Kuroda, K. Houkin, Moyamoya disease: current concepts and future perspectives, Lancet Neurol. 7 (2008) 1056–1066.
[4] W. Miao, P.L. Zhao, Y.S. Zhang, H.Y. Liu, Y. Chang, J. Ma, Q.J. Huang, Z.X. Lou, Epidemiological and clinical features of moyamoya disease in Nanjing, China, Clin. Neurol. Neurosurg. 112 (2010) 199–203.

[5] R.M. Scott, E.R. Smith, Moyamoya disease and moyamoya syndrome, N. Engl. J. Med. 360 (2009) 1226–1237.
[6] A. Veeravagu, R. Guzman, C.G. Patil, L.C. Hou, M. Lee, G.K. Steinberg, Moyamoya disease in pediatric patients: outcomes of neurosurgical interventions, Neurosurg. Focus 24 (2008) E16.
[7] W. Liu, D. Morito, S. Takashima, Y. Mineharu, H. Kobayashi, T. Hitomi, H. Hashikata, N. Matsuura, S. Yamazaki, A. Toyoda, K. Kikuta, Y. Takagi, K.H. Harada, A. Fujiyama, R. Herzig, B. Krischek, L. Zou, J.E. Kim, M. Kitakaze, S. Miyamoto, K. Nagata, N. Hashimoto, A. Koizumi, Identification of RNF213 as a susceptibility gene for moyamoya disease and its possible role in vascular development, PLoS ONE 6 (2011) e22542.
[8] W. Liu, T. Hitomi, H. Kobayashi, K.H. Harada, A. Koizumi, Distribution of moyamoya disease susceptibility polymorphism p.R4810K in RNF213 in East and Southeast Asian Populations, Neurol. Med. Chir. 52 (2012) 299–303.
[9] J.H. Kim, J.H. Jung, J.H. Phi, H.S. Kang, J.E. Kim, J.H. Chae, S.J. Kim, Y.H. Kim, Y.Y. Kim, B.K. Cho, K.C. Wang, S.K. Kim, Decreased level and defective function of circulating endothelial progenitor cells in children with moyamoya disease, J. Neurosci. Res. 88 (2010) 510–518.
[10] K. Takahashi, K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, S. Yamanaka, Induction of pluripotent stem cells from adult human fibroblasts by defined factors, Cell 131 (2007) 861–872.
[11] M. Fukui, Guidelines for the diagnosis and treatment of spontaneous occlusion of the circle of Willis ('moyamoya' disease). Research Committee on Spontaneous Occlusion of the Circle of Willis (Moyamoya Disease) of the Ministry of Health and Welfare, Japan, Clin. Neurol. Neurosurg. 99 (Suppl. 2) (1997) S238–S240.
[12] K. Amps, P.W. Andrews, G. Anyfantis, L. Armstrong, S. Avery, H. Baharvand, J. Baker, D. Baker, M.B. Munoz, S. Beil, N. Benvenisty, D. Ben-Yosef, J.C. Biancotti, A. Bosman, R.M. Brena, D. Brison, G. Caisander, M.V. Camarasa, J. Chen, E. Chiao, Y.M. Choi, A.B. Choo, D. Collins, A. Colman, J.M. Crook, G.Q. Daley, A. Dalton, P.A. De Sousa, C. Denning, J. Downie, P. Dvorak, K.D. Montgomery, A. Feki, A. Ford, V. Fox, A.M. Fraga, T. Frumkin, L. Ge, P.J. Gokhale, T. Golan-Lev, H. Gourabi, M. Gropp, G. Lu, A. Hampl, K. Harron, L. Healy, W. Herath, F. Holm, O. Hovatta, J. Hyllner, M.S. Inamdar, A.K. Irwanto, T. Ishii, M. Jaconi, Y. Jin, S. Kimber, S. Kiselev, B.B. Knowles, O. Kopper, V. Kukharenko, A. Kuliev, M.A. Lagarkova, P.W. Laird, M. Lako, A.L. Laslett, N. Lavon, D.R. Lee, J.E. Lee, C. Li, L.S. Lim, T.E. Ludwig, Y. Ma, E. Maltby, I. Mateizel, Y. Mayshar, M. Mileikovsky, S.L. Minger, T. Miyazaki, S.Y. Moon, H. Moore, C. Mummery, A. Nagy, N. Nakatsuji, K. Narwani, S.K. Oh, C. Olson, T. Otonkoski, F. Pan, I.H. Park, S. Pells, M.F. Pera, L.V. Pereira, O. Qi, G.S. Raj, B. Reubinoff, A. Robins, P. Robson, J. Rossant, G.H. Salekdeh, T.C. Schulz, et al., Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage, Nat. Biotechnol. 29 (2011) 1132–1144.
[13] M. Potente, L. Ghaeni, D. Baldessari, R. Mostoslavsky, L. Rossig, F. Dequiedt, J. Haendeler, M. Mione, E. Dejana, F.W. Alt, A.M. Zeiher, S. Dimmeler, SIRT1 controls endothelial angiogenic functions during vascular growth, Genes Dev. 21 (2007) 2644–2658.
[14] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.
[15] H. Ishikawa, A.P. Heaney, R. Yu, G.A. Horwitz, S. Melmed, Human pituitary tumor-transforming gene induces angiogenesis, J. Clin. Endocrinol. Metab. 86 (2001) 867–874.
[16] M.A. Moreno-Mateos, A.G. Espina, B. Torres, M.M. Gamez del Estal, A. Romero-Franco, R.M. Rios, J.A. Pintor-Toro, PTTG1/securin modulates microtubule nucleation and cell migration, Mol. Biol. Cell 22 (2011) 4302–4311.
[17] F. Milliat, A. Francois, M. Isoir, E. Deutsch, R. Tamarat, G. Tarlet, A. Atfi, P. Validire, J. Bourhis, J.C. Sabourin, M. Benderitter, Influence of endothelial cells on vascular smooth muscle cells phenotype after irradiation: implication in radiation-induced vascular damages, Am. J. Pathol. 169 (2006) 1484–1495.