BioFormosa (2007) 42(1): 25-33

Studies of *trans* RNA Interference of *SCA8* CTG Trinucleotide Repeats Expansion

Hsuan-Yuan Lin, Wei-Ling Hung, Shih-Huan Kao, Jui-Hung Chang, Ming-Liang Li*, Guey-Jen Lee-Chen*
Department of Life Science, National Taiwan Normal University
Taipei, Taiwan

(Received: 27 November 2006, accepted: 2 April 2007)

ABSTRACT

The mutation causing spinocerebellar ataxia type 8 (SCA8) has been identified as a CTG repeats expansion in the 3' region of the *SCA8* gene, which overlaps the 5' region of the *Kelch-like 1* (*KLHL1*) gene. To examine the *trans* RNA interference, the human *SCA8* cDNA with 0, 23, 88 and 157 CTA/CTG combined repeats as well as full-length *KLHL1* and 5' 254-bp CAG repeats-containing *TBP* cDNA were cloned and expressed in human embryonic kidney 293 cells for fluorescence activated cell sorting (FACS) and RNA fluorescent *in situ* hybridization (RNA FISH) analyses. FACS analysis of cells co-expressing *SCA8* and GFP-tagged *KLHL1* revealed that *SCA8* may function as a negative regulator of *KLHL1*. Co-expression of *SCA8* and 5' CAG repeats-containing *TBP* cDNA fragment also demonstrated repeat length-dependent down-regulation on the expression of CAG repeats-containing RNA. When compared to that of *SCA8* transcripts without combined repeats, the suppressive activity for both *KLHL1* and CAG repeats-containing cDNA fragment was significantly different for *SCA8* transcripts carrying 157 combined repeats. RNA FISH experiments further revealed ribonuclear foci formation in cells carrying expanded 88 and 157 combined repeats. The results indicate that the pathogenesis of SCA8 may mediate through the ribonuclear foci formation and mis-regulation of *KLHL1* and CAG repeats-containing RNA expression.

Key words: Spinocerebellar ataxia type 8, CTG repeats expansion, ribonuclear foci, *trans* RNA interference

Introduction

The spinocerebellar ataxias (SCAs) comprise a heterogeneous group of disorders involving progressive degeneration of the cerebellum, brainstem, and spinal tract (Wullner, 2003). Of all SCAs, SCA8 presents a molecular trait that distinguishes it from other dominant ataxias: its being attributed to the CTG triplet expansion on a non-translated gene on chromosome 13q21, with 110~130 CTA/CTG combined repeats in the affected individuals (Koob *et al.*, 1999). Since this original description, the sizing of SCA8 alleles has been clarified in various populations, with unrelated expanded alleles ranging from 68 to 800 repeats found in familial and sporadic ataxia patients (Day *et al.*, 2000; Ikeda *et al.*, 2000a, 2000b, 2004; Juvonen *et al.*, 2000; Silveira *et al.*, 2000; Worth *et al.*, 2000; Cellini *et al.*, 2001; Tazon *et al.*, 2002; Topisirovic *et al.*, 2002; Izumi *et al.*, 2003; Zeman *et al.*, 2004). The incomplete penetrance (Juvonen *et al.*, 2000; Worth *et al.*, 2000; Tazon *et al.*, 2002) as well as the size of the expanded repeats that were not correlated among affected and healthy individuals (Stevanin *et al.*, 2000; Sobrido *et al.*, 2001; Schols *et al.*, 2003; Sulek *et al.*, 2004) have argued strongly that expansion may not be a susceptibility factor for the disorder. Nevertheless, the ectopic expression of *SCA8* RNA in *Drosophila* induced late-onset, progressive degeneration in the photoreceptor and pigment cells of flies (Mutsuddi *et al.*, 2004).

The pathogenic role of SCA8 expansion remains uncertain. The *SCA8* transcripts are ubiquitously expressed in various brain tissues and no extended open reading frames are present (Koob *et al.*, 1999). The 5' end of the *SCA8* transcripts overlap the transcription and translation start sites, as well as the first splice donor sequence of the *Kelch-like 1* (*KLHL1*) gene, a predicted actin-

*Corresponding author: Guey-Jen Lee-Chen (E-mail: t43019@ntnu.edu.tw) and Ming-Liang Li (E-mail: t43010@ntnu.edu.tw); FAX: 886-2-29312904

binding protein (Nemes *et al.*, 2000). This antisense/sense transcriptional organization is evolutionarily conserved in both human and mouse, although no CTG repeats are found in the mouse *SCA8* gene (Benzow and Koob, 2002). Thus *SCA8* may operate as an endogenous anti-sense RNA to regulate *KLHL1* expression. The causative agent for myotonic dystrophy (DM1) is also known to be a CTG expansion in the 3'-UTR of the *DMPK* gene (Carango *et al.*, 1993). The expanded CUG repeat in the *DMPK* RNA formed extended hairpin loops to impair nuclear cytoplasmic transport, resulting in nuclear retention and ribonuclear foci formation to sequester CUG-binding proteins or affect the transport of other CAG repeats-containing RNA (Taneja *et al.*, 1995; Hamshere and Brook, 1996; Davis *et al.*, 1997; Koch and Leffert, 1998; Sasagawa *et al.*, 1999; Timchenko *et al.*, 2001; Fardaei *et al.*, 2002). Hence a toxic RNA gain-of function mechanism analogous to DM1 was proposed (Mutsuddi *et al.*, 2004; Ranum and Day, 2004). In addition, using a transgenic mouse model in which the human *SCA8* mutation is present on a BAC, a newly discovered gene, *ataxin 8* (*ATXN8*), which encodes a nearly pure polyglutamine expansion protein in the CAG direction, was reported (Moseley *et al.*, 2006). The studies of BAC *SCA8* transgenic mice revealed 1C2-positive intranuclear inclusions in Purkinje and brainstem neurons, indicating polyglutamine expansion protein in the CAG direction with the pathogenesis of the disease (Moseley *et al*., 2006).

In this study, we cloned the human *SCA8* cDNA with 0, 23, 88 and 157 combined triplet repeats as well as *KLHL1* and 5' CAG repeats-containing *TBP* cDNA to investigate the molecular mechanisms underlying SCA8 using cell culture studies. We showed that the expansion of *SCA8* expansion mutation resulted in ribonuclear foci formation and mis-regulation of *KLHL1* and CAG repeats-containing RNA expression.

Materials and Methods

Reverse transcription-polymerase chain reaction (RT-PCR) and cDNA constructs

Human cerebellum polyadenylated RNA (200 ng) (Clontech) was reverse transcribed using the SuperScript™ III reverse transcriptase (Invitrogen). Sense and antisense primers used for amplification of *SCA8* cDNA were 5'-ATCCTTCACCTGTTGCCT-3' and 5'-GCTTGTGAGGACTGAGAATG-3', respectively. The 1.3-kb full-length, $(CTA)_{11}(CTG)_{12}$ combined repeats (CR_{23}) containing cDNA (including exons D, C2, C1, B, and A) (Nemes *et al.*, 2000) was cloned into pGEM-T Easy vector (Promega) and sequenced. The cloned *SCA8* cDNA containing 88 CTA/CTG combined repeats was made by replacing a 178 bp *Nla*III-*Afl*II fragment with a 373 bp fragment from the PCR clone of a Parkinson's disease (PD) patient [$(CTA)_8$CCACTA CTGCTACTGCTA$(CTG)_{74}$] (CR_{88}) (Wu *et al.*, 2004). The repeat number was further expanded to 157 [$(CTA)_8$CCACTACTGCTA CTGCTA$(CTG)_{67}$ CTA$(CTG)_{65}$CTA$(CTG)_9$] (CR_{157}) by ligating *Fnu*4HI partially digested fragments. The interruption of the CTG repeat tract by CCA and CTA is similar to that reported seen in SCA8 patients (Moseley *et al.*, 2000). To construct the clone without combined repeats (CR_0), a *Dra*I site was added to the 5' end of CTA/CTG repeats by site-directed mutagenesis using primer 5'-CCCTG GGTCCTTCATGTTAGAAAACCTGGCTTTAAA $A(CTA)_8$C-3' and a 273-bp *Dra*I fragment containing 88 CTA/CTG combined repeats was removed. Then the *SCA8* cDNA with 0, 23, 88 or 157 combined repeats was placed in the *Not*I site of the pEF-IRES/hrGFP vector (EF, elongation factor 1α promoter, a kind gift from Dr Kwang-Soo Kim of the Harvard Medical School) (Chung *et al.*, 2002) in which the *Kpn*I fragment containing 3' region of the internal ribosome entry segment (IRES) sequence and the humanized *Renilla* green fluorescent protein (*hrGFP*) gene were removed to generate pEF-SCA8.

The 3.2 kb *KLHL1* cDNA was amplified using sense (5'-CATGTCAGGCTCTGGGCGAAAAG-3') and antisense (5'-TGGGCGATGAGAATATGAAG TCTG-3') primers. After cloning and sequencing, the 2.2 kb *KLHL1* coding sequences and the EGFP gene from pEGFP-N1 (Clontech) were fused in-frame and inserted into the *Not*I site of the modified pEF-IRES/hrGFP vector to generate pEF-KLHL1-EGFP. The construct was verified by DNA sequencing.

The 1.1 kb *TATA binding protein* (*TBP*) cDNA containing 36 CAA/CAG repeats [$(CAG)_3(CAA)_3(CAG)_9$CAACAGCAA$(CAG)_{16}$CAACAG] was amplified using sense (5'-CTGGTTTGCCAAGAA GAAAGTG-3') and antisense (5'-AGGCAAGGG TACATGAGAGCCA-3') primers. After cloning and sequencing, the 5' 254-bp cDNA fragment was

placed between the *Eco*RI and *Pst*I sites of the pIRES2-EGFP vector (Clontech). The resulting pCMV-$(CAG)_{36}$-IRES-EGFP contains the 5' region of *TBP* cDNA upstream to the IRES and EGFP gene. The construct was verified by DNA sequencing.

Expression studies

Human embryonic kidney (HEK)-293 cells cultivated in DMEM containing 10% FCS were transfected with the various cDNA constructs using a lipofection procedure. pEGFP-N1 was used as a negative control to show the specificity of SCA8 *trans* RNA interference. Forty-eight hours later, cells were harvested for fluorescence activated cell sorting (FACS) analysis. The amounts of GFP expressed were analyzed in a FACStar flow cytometer (Becton-Dickinson), equipped with an argon laser operating at 530 nm. A forward scatter gate was established to exclude dead cells and cell debris from the analysis. 10^4 cells were analyzed in each sample.

Fluorescent in situ hybridization (FISH)

To examine the ribonuclear foci, cells grown on coverslips were transfected, washed, and fixed for 15 min at room temperature in 4% formaldehyde and 10% acetic acid. After 0.1% Triton X-100 treatment for 10 min, a Cy3-$(CAG)_{10}$ (Operon) or Cy5-CTGCGACTCCGCTGGAAACT CTTCAGCCA (unique to *SCA8*) oligonucleotide probe was added at 37 ^{o}C for 2 hr for FISH experiments (http://www.singerlab.org/protocols). Nuclei were detected using DAPI (4'-6-diamidino-2-phenylindole). Fluorescent signals are visualized using a Leica TCS confocal laser scanning microscope optimized for simultaneous dual fluorescent imaging.

Results

Regulation of KLHL1 and CAG repeat-containing RNA expression by SCA8

The genomic organizations of the *SCA8* and *KLHL1* coding regions are overlapped and orientated in reversed directions (Benzow and Koob, 2002). As the expressions of both genes are brain-specific, it has been suggested that *SCA8* may function as an anti-sense regulator of *KLHL1* (Nemes *et al.*, 2000). To examine this, we constructed the human *SCA8* cDNA with 0, 23, 88, or 157 combined repeats (pEF-SCA8) as well as *KLHL1* cDNA tagged with EGFP (pEF-KLHL1-EGFP) (Fig. 1A). After co-transfection into HEK-293 cells for two days, FACS was performed to evaluate the expression of the *KLHL1* fusion gene. KLHL1-EGFP fusion protein production from cells co-transfected with *SCA8* carrying 0 ~ 157 combined repeats was significantly reduced (29%, 35%, 38% and 55% of the levels of *KLHL1* fusion gene, $P < 0.05$) (Fig. 1B, gray box). The difference between co-transfecting *SCA8* carrying 0 and 157 repeats constructs were significant (29% vs. 55%, $P = 0.03$). The results suggested that *SCA8* may function as a negative regulator of *KLHL1*, and the length of CUG repeats affects this down-regulation.

The expanded CUG repeats within *DMPK* transcripts were able to interact with CAG repeats located within the *TBP* or androgen receptor mRNA (Hamshere and Brook, 1996; Sasagawa *et al.*, 1999). To examine if *SCA8* RNA could pair with CAG repeats from the *TBP* gene transcript, we placed the 5' *TBP* $(CAG)_{36}$-containing cDNA fragment upstream to the IRES-mediated translation of EGFP gene [pCMV-$(CAG)_{36}$-IRES-EGFP] (Fig. 1A). After co-transfecting equal amount of pCMV-$(CAG)_{36}$-IRES-EGFP and pEF-SCA8-CR_0, -CR_{23}, -CR_{88}, or CR_{157} constructs into HEK-293 cells for two days, EGFP protein production was 97%, 84%, 80% ($P > 0.05$), and 71% ($P = 0.01$) of the levels in cells transfecting pCMV-$(CAG)_{36}$-IRES-EGFP and pEF vector (Fig. 1B, black box). The difference between co-transfecting *SCA8* carrying 0 and 157 repeats constructs were also significant (97% vs. 71%, $P = 0.04$). These results suggested that *SCA8* RNA may down regulate the protein expression of CAG repeat-containing RNA gene, and the length of CUG repeats affects this down-regulation.

To demonstrate the specificity of SCA8 *trans* RNA interference, pEGFP-N1 was used to co-transfect with pEF-SCA8 constructs. As shown in Fig. 1B, 93%, 98%, 98% and 106% of the levels of the EGFP protein expression were detected as compared to that of co-transfecting pEGFP-N1 and pEF vector (white box, $P > 0.05$). The difference between co-transfecting *SCA8* carrying 0 and 157 repeats constructs were not significant (93% vs. 106%, $P = 0.09$).

Ribonuclear foci formation on SCA8 CUG expansion

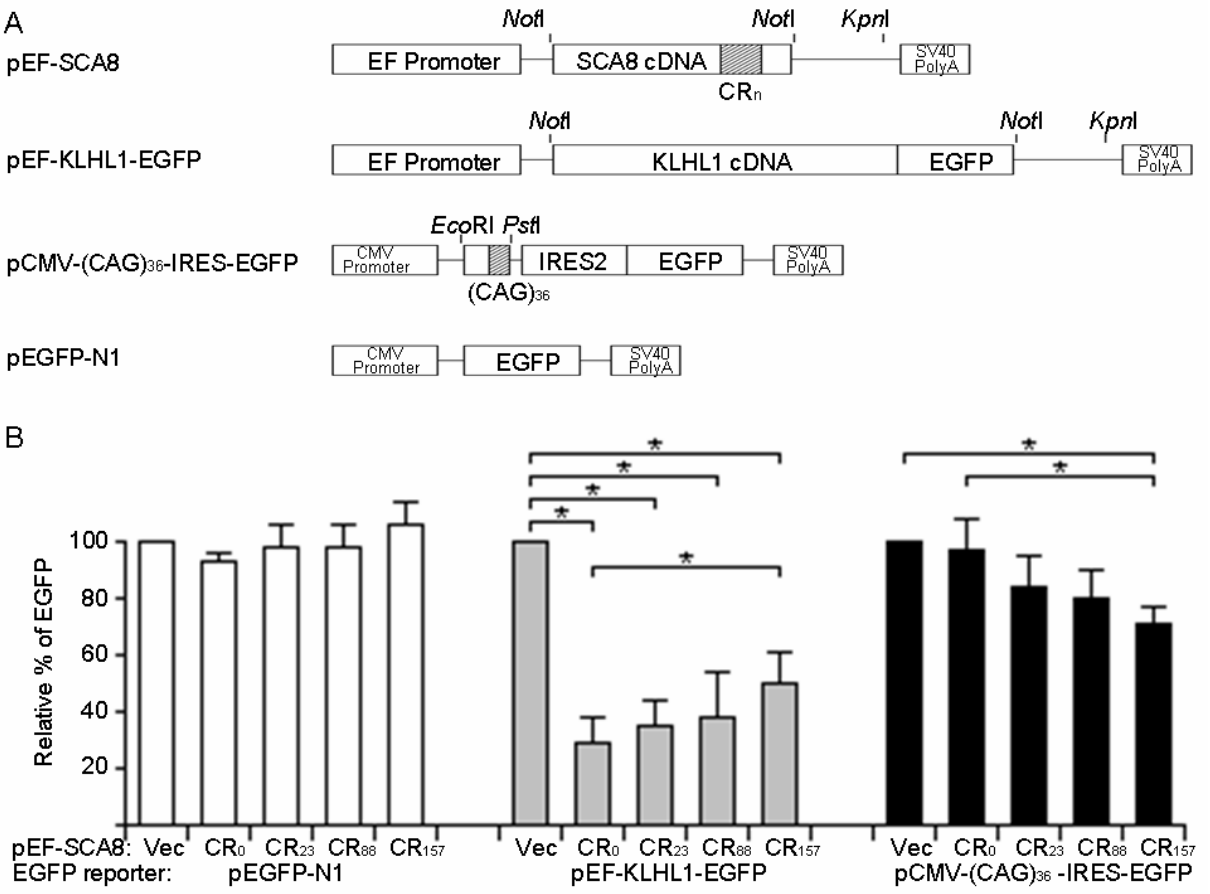

Figure 1. Regulation of the *KLHL1* and CAG repeat-containing transcript expression by *SCA8*. A. The pEF-SCA8, pEF-KLHL1-EGFP, pCMV-$(CAG)_{36}$-IRES-EGFP and pEGFP-N1 constructs. pEF-SCA8: *SCA8* alleles of 0, 23, 88 and 157 CTA/CTG combined repeats (CR_n) were placed in the *Not*I site of the pEF-IRES/hrGFP vector in which the *Kpn*I fragment containing IRES/hrGFP gene was removed. pEF-KLHL1-EGFP: the in-framed KLHL1-EGFP fusion gene was inserted into the *Not*I site of the modified pEF vector. pCMV-$(CAG)_{36}$-IRES-EGFP: the 5' region of *TBP* cDNA was placed between the *Eco*RI and *Pst*I sites of the pIRES2-EGFP vector. pEGFP-N1 from Clontech was used as a negative control to show the specificity of SCA8 *trans* RNA interference. B. Co-transfection of pEGFP-N1 (white box), pKLHL1-EGFP (gray box), pCMV-$(CAG)_{36}$-IRES-EGFP (black box) and pEF-SCA8 constructs in HEK-293 cells. The amounts of EGFP expressed were analyzed by FACS analysis 48 hr after transfection. Levels of EGFP were expressed as percentages of EGFP reporter, which was set at 100%. Each value is the mean ± SD of three independent experiments each performed in duplicate. An asterisk (*) depicts significant difference ($P < 0.05$) between comparisons.

Since the mutant *DMPK* transcripts accumulated in the nuclei of DM patient cells and aggregated to form distinct foci (Taneja *et al.*, 1995; Davis *et al.*, 1997; Koch and Leffert, 1998), we investigated whether the expanded CUG repeats form ribonuclear foci as seen in DM1. The pEF-SCA8 carrying 23, 88, or 157 triplet repeats was transiently transfected into HEK-293 cells and FISH experiments using a Cy3-labeled $(CAG)_{10}$ oligonucleotide probe was performed two days later. As shown in Fig. 2, no ribonuclear foci were seen in cells expressing *SCA8* CR_{23}. However, distinct ribonuclear foci, mostly perinuclear, were observed in cells expressing expanded *SCA8* CR_{88} and CR_{157}. Similar results were obtained using an oligonucleotide probe specific to *SCA8*. Since this probe can only bind to the *SCA8* RNA in single copy, the inability to detect ribonuclear foci with CR_{23} is not an artifact of the copy number of the repeats in the *SCA8* CR_{88} or CR_{157}.

Discussion

Clinical and genetic studies have shown that SCA8 is very slowly progressive and highly incomplete penetrance, in addition to its rare

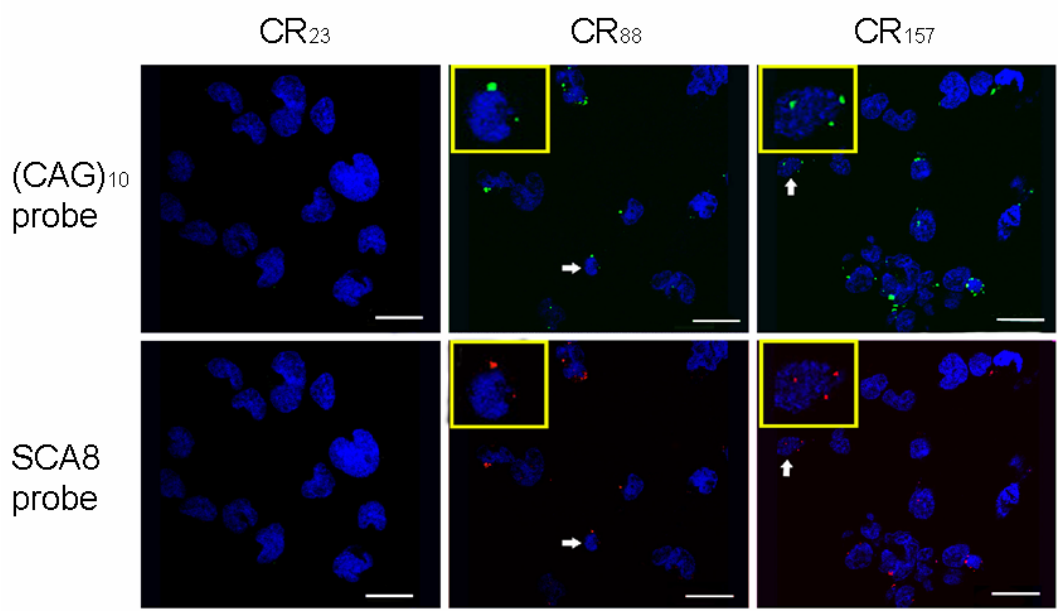

Figure 2. Ribonuclear foci formation with *SCA8* CUG expansion. HEK-293 cells were transfected with *SCA8* constructs carrying 23, 88 or 157 combined repeats (CR_{23}, CR_{88}, and CR_{157}) and analyzed 48 hr later by RNA-FISH using a Cy3 labeled $(CAG)_{10}$ (green, upper panel) or Cy5 labeled *SCA8* unique sequence (red, lower panel) oligonucleotide probe. Nuclei were counterstained with DAPI (blue). (The scale bar = 20 μm).

occurrence in individuals with a much larger repeat expansion (Koob *et al.*, 1999; Juvonen *et al.*, 2000; Worth *et al.*, 2000; Tazon *et al.*, 2002; Mosemiller *et al.*, 2003). Thus the pathogenic mechanisms underlying SCA8 are expected to be complicated. In this study we investigate the *trans* RNA interference of *SCA8* mutation using a cell model. We found that: 1) *SCA8* may down-regulate the expression of actin-binding *KLHL1*; 2) expanded CUG repeats may down-regulate the expression of CAG repeats-containing RNA; and 3) expanded CUG repeats form ribonuclear foci. The implications of each finding in the pathogenesis of SCA8 are discussed as the following.

Down-regulation of KLHL1

The 5' ends of the *SCA8* and *KLHL1* transcripts are complementary (Nemes *et al.*, 2000), suggesting that *KLHL1* and *SCA8* can interact with each other. In addition, the genomic organizations and dynamic expression profiles of *SCA8*/*KLHL1* gene pair are well conserved in both humans and mice (Benzow and Koob, 2002), which strongly indicate the preservation of a significant biological function. We co-transfected cells with *KLHL1* and *SCA8* carrying various numbers of triplet repeats to investigate whether or not *SCA8* interacts with *KLHL1*, and if CTG expansion alters the interactions. The expression of *KLHL1* was reduced as expected when *SCA8* was expressed in the same cells (29 ~ 55% of the levels of *KLHL1*-EGFP gene, Fig. 1B). As the levels of both *KLHL1* and *SCA8* transcripts were not significantly reduced by RT-PCR and gel semi-quantitation (data not shown), the repression of *KLHL1* by *SCA8* was likely mediated through formation of double-stranded RNA, which blocks the translation initiation. Although the repression of *KLHL1* is CTG repeats-independent, *SCA8* with 157 copies of CTA/CTG combined repeats showed significantly reduced repression of *KLHL1* expression compared to *SCA8* with 0 copy of combined repeats (29% vs. 55%, $P = 0.03$). Thus the pathogenesis of SCA8 may mediate through the mis-regulation of *KLHL1*.

Down-regulation of CAG repeat-containing gene

Previously *trans* RNA interference was shown *in vitro* between CUG repeats over 140 and CAG repeats over 35 (Sasagawa *et al.*, 1999). In our cell model, we showed that the CUG repeats as short as 23 in the *SCA8* down regulated the expression of 36 CAG triplet repeats from the *TBP* gene (84%, Fig. 1B), although the levels of *TBP* transcripts were not significantly reduced ($P = 0.14$). The anti-sense

RNA interference effect is positively correlated with the length of CTG repeats. The RNA interference underlying pathogenesis of CTG expansion has also been supported by *in vitro* RNA–RNA binding experiments and an *in vivo* study (Watanabe *et al.*, 2004). The *in vitro* study demonstrated that the incubation of expanded CUG repeats with CUGBP1 RNA generated a higher molecular weight band. The *in vivo* study showed that the amount of CUGBP1 mRNA containing several CAG repeat sequences was reduced in muscle biopsy samples from 10 DM1 patients. The expanded CUG repeats in the *SCA8* mRNA may also anneal to other mRNAs with sufficiently long stretches of CAG repeats to result in abnormal RNA processing.

Ribonuclear foci formation

Formation of discrete ribonuclear foci of the expanded repeat RNA is a distinctive and consistent feature of affected muscle in DM1 and DM2 patients. A CTG expansion in the 3'-UTR sequesters CUG binding proteins from their normal cellular functions, leading to abnormal RNA splicing of several genes has been suggested for the RNA foci associated pathogenesis (Ranum and Day, 2002). Using a cell system, we have shown that the *SCA8* cDNA carrying 88 or 157 combined repeats form ribonuclear foci (Fig. 2). Most of the RNA foci formed are located near nuclear membrane, which may be compatible with the observation by Koch and colleague that the hairpin structure formed by long CUG repeats (> 44) cannot pass through nucleic pores (Koch and Leffert, 1998). The ribonuclear foci observed in the nucleus may also result in transcriptional dysfunction as indicated by the *DMPK* mutant RNA leaches transcription factors from chromatin in *MyoD*-generated myocytes from DM1 subjects (Ebralidze *et al.*, 2004).

In conclusion, our data provide several lines of evidence that may explain the expanded CTG leading to neuronal dysfuntion in SCA8. Although the *in vitro* cell culture study may not truly reflect the situation *in vivo*, our study may shed insights into the pathogenesis and lead to therapeutic interventions to treat this disease. Further studies are required to confirm that such RNA interference and RNA foci are involved in SCA8 pathogenesis.

Acknowledgements

We thank Taiwan Foundation for Rare Disorders for providing thesis scholarship and Confocal Microscope Lab, Instrumentation Center, NTU for technical support. This work was supported by grants NSC-93-3112-B-003-001 from the National Science Council, Executive Yuan and ORD93-2 from National Taiwan Normal University.

References

Benzow KA and Koob MD. 2002. The *KLHL1-antisense* transcript (*KLHL1AS*) is evolutionarily conserved. Mamm. Genome 13:134-141.

Carango P, Noble JE, Marks HG and Funanage VL. 1993. Absence of myotonic dystrophy protein kinase (DMPK) mRNA as a result of a triplet repeat expansion in myotonic dystrophy. Genomics 18:340-348.

Cellini E, Nacmias B, Forleo P, Piacentini S, Guarnieri BM, Serio A, Calabro A, Renzi D and Sorbi S. 2001. Genetic and clinical analysis of spinocerebellar ataxia type 8 repeat expansion in Italy. Arch. Neurol. 58:1856-1859.

Chung S, Andersson T, Sonntag KC, Bjorklund L, Isacson O and Kim KS. 2002. Analysis of different promoter systems for efficient transgene expression in mouse embryonic stem cell lines. Stem Cells 20:139-145.

Davis BM, McCurrach ME, Taneja KL, Singer RH and Housman DE. 1997. Expansion of a CUG trinucleotide repeat in the 3' untranslated region of myotonic dystrophy protein kinase transcripts results in nuclear retention of transcripts. Proc. Natl. Acad. Sci. USA 94:7388-7393.

Day JW, Schut LJ, Moseley ML, Durand AC and Ranum LP. 2000. Spinocerebellar ataxia type 8: clinical features in a large family. Neurology 55:649-657.

Ebralidze A, Wang Y, Petkova V, Ebralidse K and Junghans RP. 2004. RNA leaching of transcription factors disrupts transcription in myotonic dystrophy. Science 303:383-387.

Fardaei M, Rogers MT, Thorpe HM, Larkin K, Hamshere MG, Harper PS and Brook JD. 2002. Three proteins, MBNL, MBLL and MBXL, co-localize *in vivo* with nuclear foci of expanded-repeat transcripts in DM1 and DM2

cells. Hum. Mol. Genet. 11:805-814.

Hamshere MG and Brook JD. 1996. Myotonic dystrophy, knockouts, warts and all. Trends Genet. 12:332-334.

Ikeda Y, Shizuka M, Watanabe M, Okamoto K and Shoji M. 2000a. Molecular and clinical analyses of spinocerebellar ataxia type 8 in Japan. Neurology 54:950-955.

Ikeda Y, Shizuka-Ikeda M, Watanabe M, Schmitt M, Okamoto K and Shoji M. 2000b. Asymptomatic CTG expansion at the SCA8 locus is associated with cerebellar atrophy on MRI. J. Neurol. Sci. 182:76-79.

Ikeda Y, Dalton JC, Moseley ML, Gardner KL, Bird TD, Ashizawa T, Seltzer WK, Pandolfo M, Milunsky A, Potter NT, Shoji M, Vincent JB, Day JW and Ranum LP. 2004. Spinocerebellar ataxia type 8: molecular genetic comparisons and haplotype analysis of 37 families with ataxia. Am. J. Hum. Genet. 75:3-16.

Izumi Y, Maruyama H, Oda M, Morino H, Okada T, Ito H, Sasaki I, Tanaka H, Komure O, Udaka F, Nakamura S and Kawakami H. 2003. SCA8 repeat expansion: large CTA/CTG repeat alleles are more common in ataxia patients, including those with SCA6. Am. J. Hum. Genet. 72:704-709.

Juvonen V, Hietala M, Paivarinta M, Rantamaki M, Hakamies L, Kaakkola S, Vierimaa O, Penttinen M and Savontaus ML. 2000. Clinical and genetic findings in Finnish ataxia patients with the spinocerebellar ataxia 8 repeat expansion. Ann. Neurol. 48:354-361.

Koch KS and Leffert HL. 1998. Giant hairpins formed by CUG repeats in myotonic dystrophy messenger RNAs might sterically block RNA export through nuclear pores. J. Theor. Biol. 192:505-514.

Koob MD, Moseley ML, Schut LJ, Benzow KA, Bird TD, Day JW and Ranum LP. 1999. An untranslated CTG expansion causes a novel form of spinocerebellar ataxia (SCA8). Nat. Genet. 21:379-384.

Moseley ML, Schut LJ, Bird TD, Koob MD, Day JW and Ranum LP. 2000. SCA8 CTG repeat: *en masse* contractions in sperm and intergenerational sequence changes may play a role in reduced penetrance. Hum. Mol. Genet. 9:2125-2130.

Moseley ML, Zu T, Ikeda Y, Gao W, Mosemiller AK, Daughters RS, Chen G, Weatherspoon MR, Clark HB, Ebner TJ, Day JW, Ranum LP. 2006. Bidirectional expression of CUG and CAG expansion transcripts and intranuclear polyglutamine inclusions in spinocerebellar ataxia type 8. Nat. Genet. 38:758-769.

Mosemiller AK, Dalton JC, Day JW and Ranum LPW. 2003. Molecular genetics of spinocerebellar ataxia type 8 (SCA8). Cytogenet. Genome Res. 100:175-183.

Mutsuddi M, Marshall CM, Benzow KA, Koob MD and Rebay I. 2004. The spinocerebellar ataxia 8 noncoding RNA causes neurodegeneration and associates with staufen in *Drosophila*. Curr. Biol. 14:302-308.

Nemes JP, Benzow KA, Moseley ML, Ranum LP and Koob MD. 2000. The SCA8 transcript is an antisense RNA to a brain-specific transcript encoding a novel actin-binding protein (KLHL1). Hum. Mol. Genet. 9:1543-1551.

Ranum LP and Day JW. 2002. Myotonic dystrophy: clinical and molecular parallels between myotonic dystrophy type 1 and type 2. Curr. Neurol. Neurosci. Rep. 2:465-470.

Ranum LP and Day JW. 2004. Pathogenic RNA repeats: an expanding role in genetic disease. Trends Genet. 20:506-512.

Sasagawa N, Takahashi N, Suzuki K and Ishiura S. 1999. An expanded CTG trinucleotide repeat causes *trans* RNA interference: a new hypothesis for the pathogenesis of myotonic dystrophy. Biochem. Biophys. Res. Commun. 264:76-80.

Schols L, Bauer I, Zuhlke C, Schulte T, Kolmel C, Burk K, Topka H, Bauer P, Przuntek H and Riess O. 2003. Do CTG expansions at the SCA8 locus cause ataxia? Ann. Neurol. 54:110-115.

Silveira I, Alonso I, Guimaraes L, Mendonca P, Santos C, Maciel P, Fidalgo De Matos JM, Costa M, Barbot C, Tuna A, Barros J, Jardim L, Coutinho P and Sequeiros J. 2000. High germinal instability of the (CTG)n at the SCA8 locus of both expanded and normal alleles. Am. J. Hum. Genet. 66:830-840.

Sobrido MJ, Cholfin JA, Perlman S, Pulst SM and Geschwind DH. 2001. SCA8 repeat expansions in ataxia: a controversial association. Neurology 57:1310-1312.

Stevanin G, Herman A, Durr A, Jodice C, Frontali M, Agid Y and Brice A. 2000. Are (CTG)n

expansions at the SCA8 locus rare polymorphisms? Nat. Genet. 24:213.

Sulek A, Hoffman-Zacharska D, Bednarska-Makaruk M, Szirkowiec W and Zaremba J. 2004. Polymorphism of trinucleotide repeats in non-translated regions of SCA8 and SCA12 genes: allele distribution in a Polish control group. J. Appl. Genet. 45:101-105.

Taneja KL, McCurrach M, Schalling M, Housman D and Singer RH. 1995. Foci of trinucleotide repeat transcripts in nuclei of myotonic dystrophy cells and tissues. J. Cell Biol. 128:995-1002.

Tazon B, Badenas C, Jimenez L, Munoz E and Mila M. 2002. SCA8 in the Spanish population including one homozygous patient. Clin. Genet. 62:404-409.

Timchenko NA, Cai ZJ, Welm AL, Reddy S, Ashizawa T and Timchenko LT. 2001. RNA CUG repeats sequester CUGBP1 and alter protein levels and activity of CUGBP1. J. Biol. Chem. 276:7820-7826.

Topisirovic I, Dragasevic N, Savic D, Ristic A, Keckarevic M, Keckarevic D, Culjkovic B, Petrovic I, Romac S and Kostic VS. 2002. Genetic and clinical analysis of spinocerebellar ataxia type 8 repeat expansion in Yugoslavia. Clin. Genet. 62:321-324.

Watanabe T, Takagi A, Sasagawa N, Ishiura S and Nakase H. 2004. Altered expression of CUG binding protein 1 mRNA in myotonic dystrophy 1: possible RNA-RNA interaction. Neurosci. Res. 49:47-54.

Worth PF, Houlden H, Giunti P, Davis MB and Wood NW. 2000. Large, expanded repeats in SCA8 are not confined to patients with cerebellar ataxia. Nat. Genet. 24:214-215.

Wu YR, Lin HY, Chen CM, Gwinn-Hardy K, Ro LS, Wang YC, Li SH, Hwang JC, Fang K, Hsieh-Li HM, Li ML, Tung LC, Su MT, Lu KT and Lee-Chen GJ. 2004. Genetic testing in spinocerebellar ataxia in Taiwan: expansions of trinucleotide repeats in SCA8 and SCA17 are associated with typical Parkinson's disease. Clin. Genet. 65:209-214.

Wullner U. 2003. Genes implicated in the pathogenesis of spinocerebellar ataxias. Drugs Today 39:927-937.

Zeman A, Stone J, Porteous M, Burns E, Barron L and Warner J. 2004. Spinocerebellar ataxia type 8 in Scotland: genetic and clinical features in seven unrelated cases and a review of published reports. J. Neurol. Neurosurg. Psychiatry 75:459-465.

生物學報（2007）42(1): 25-33

SCA8 基因 CTG 擴增的異位 RNA 干擾研究

林玄原 洪葦芩 高士寰 張瑞宏 李銘亮* 李桂楨*
國立臺灣師範大學生命科學系

(收稿日期：2006.11.27，接受日期：2007.4.2)

摘　要

第八型脊髓小腦運動失調症(SCA8)和 *SCA8* 基因 3’端 CTG 三核苷重複擴增相關。*SCA8* 基因的 5’端和緊鄰的 *KLHL1* 基因(actin 結合蛋白)的 5’端互補。為檢視 *SCA8* 突變的異位 RNA 干擾效應，我們選殖、定序了包含 0、23、88、157 個 CTA/CTG 重複的人類 *SCA8* cDNA 及全長的 *KLHL1* cDNA、5’端 254-bp 的 *TBP* cDNA (包含 36 個 CAG 重複)，表現在人類胚胎 293 腎細胞，進行螢光活化細胞分檢(FACS)、RNA 螢光原位雜合(RNA FISH)分析。當 GFP 標記的 *KLHL1* cDNA 與 *SCA8* cDNA 共同表現在 HEK-293 細胞時，螢光活化細胞分檢分析顯示 *SCA8* 可負調節 *KLHL1* 的表現。包含 CAG 重複的 5’端 *TBP* cDNA 與 *SCA8* cDNA 共同表現在 HEK-293 細胞時，*SCA8* 抑制其的表現情形和 *SCA8* 重複長度相關。*SCA8* RNA 對 *KLHL1* 和 CAG 重複 RNA 基因表現的抑制，在不包含 CTA/CTG 重複與包含 157 個 CTA/CTG 重複間有顯著差異。RNA 螢光原位雜合(FISH)實驗顯示，88 及 157 個重複 CUG 重複擴增的 *SCA8* RNA 會形成核糖核酸聚焦。本實驗結果顯示 SCA8 的致病機轉可能和核糖核酸聚焦形成及包含 *KLHL1*、CAG 重複 RNA 基因表現的不正常的調控相關。

關鍵詞：第八型脊髓小腦運動失調症、CTG 重複擴增、核糖核酸聚焦、異位 RNA 干擾

*通訊作者：李桂楨 (Guey-Jen Lee-Chen；E-mail: t43019@ntnu.edu.tw)、李銘亮 (Ming-Liang Li；E-mail: t43010@ntnu.edu.tw)；FAX：886-2-29312904