Characterization of the Human Analogue of a Scrapie-responsive Gene*

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We recently described a novel mRNA denominated ScRG-1, the level of which is increased in the brains of Scnagnie-infected mice (Dandy-Dron, F., Guillol, F., Benboudjema, L., Deslys, J.-P., Lasmézas, C., Dormont, D., Tovey, M. G., and Dron, M. (1998) J. Biol. Chem. 273, 7691–7697). The increase in ScRG-1 mRNA in the brain follows the accumulation of PrPSc*, the proteinase K-resistant form of the prion protein (PrP), and precedes the widespread neuronal death that occurs in late stage disease. In the present study, we have isolated a cDNA encoding the human counterpart of ScRG-1. Comparison of the human and mouse transcripts firmly established that both sequences encode a highly conserved protein of 98 amino acids that contains a signal peptide, suggesting that the protein may be secreted. Examination of the distribution of human ScRG-1 mRNA in adult and fetal tissues revealed that the gene was expressed primarily in the central nervous system as a 0.7-kilobase message and was under strict developmental control.

The transmissible spongiform encephalopathies (TSE) are in a group of progressive neurodegenerative diseases that includes human pathologies such as Creutzfeldt-Jakob disease (CJD), Gerstman-Strãüssler-Scheinker syndrome and Kuru, and animal diseases such as scrapie and bovine spongiform encephalopathy (1).

To identify the genes the altered expression of which is associated with or may even be responsible for the neurodegenerative changes observed in TSE, we have systematically analyzed modifications of gene expression in scrapie-infected mouse brain using "mRNA differential display" (2). This approach has led to the detection of an increased level of expression of eight cellular genes. One of these genes, denominated scrapie-responsive gene 1 (ScRG-1), previously unrecognized, is expressed principally in the brain. Enhanced expression of ScRG-1 in the brain of scrapie-infected mice occurs concomitantly with increased expression of GFAP mRNA, a marker of astrocytosis (3). Moreover, ScRG-1 mRNA was found to be preferentially expressed in cells of glial origin and to encode a protein with a putative signal peptide (2). These observations suggest that ScRG-1 may play a role in the host response to prion-associated infections. Previous reports have suggested that certain molecules enhanced in TSE may be detrimental to neurone survival (4–6). However, the role of overexpressed proteins (7–9), including ScRG-1, in the pathogenesis of TSE remains to be determined. We report herein the nucleotide sequence, size characterization, and tissue distribution of human ScRG-1 mRNA.

EXPERIMENTAL PROCEDURES

RNA Extraction and Northern Blot Hybridization—RNA was extracted from the brains of either mock infected C57Bl/6 mice or mice infected with the C506M strain of scrapie, as described previously (2). Total RNA was extracted by the method of Chirgwin et al. (10) from the frontal cortex obtained at autopsy from a patient free from any neurological disease (patient 941005, 46 years old) and from a patient with typical neuropathological findings of sporadic Creutzfeldt-Jakob disease (patient 93005, 59 years old). The diagnosis was confirmed by the presence of the proteinase K-resistant form of PrP (data not shown). Samples of normal human brain and of the brain from a patient diagnosed with CJD were obtained by informed consent, under the auspices of the Program de Recherche sur les Encéphalopathies Spongiformes Sub-aiguës Transmissibles and les Prions (CNRS, France). Human poly(A)† mRNA was obtained as described previously (2). Northern blots were performed using glyoxal denaturation, and the blotted membranes were hybridized using probes radiolabeled to a specific activity of at least 1 × 10⁶ cpm/mg, as described previously (2). The blots were first exposed to autoradiography and then quantified using a PhosphorImager (Molecular Dynamics). The multiple human tissue Northern blot and the membrane containing the RNA dots from different human tissues were from CLONTECH laboratories.

Cloning and Sequencing of the Human cDNA—1 µg of poly(A)† mRNA from the human control sample was primed with oligo(dT) and converted into double strand cDNA using standard procedures. One-twentieth of the cDNA synthesized was amplified by polymerase chain reaction using specific forward (5’-TAAGGGAAAATCACGCTGTG-3’) and reverse (5’-CTTATTTACTACTGTGTTTAACACT-3’) primers and Taq DNA polymerase. The amplified product was purified, sequenced using the Thermo Sequenase cycle sequencing kit (Amersham Pharmacia Biotech), and further cloned in the pCR2.1 Toto plasmid vector from Invitrogen.

Cloning and Sequencing of Mouse ScRG-1 cDNA—Mouse ScRG-1 cDNA was isolated by screening a library of whole BALB/c adult brain cDNA cloned in Agt11, with the ScRG-1 cDNA clone 24 previously isolated by RACE (2) used as a probe. The cDNA inserted in the selected lambda (clone 1) was isolated and further sequenced.

RESULTS AND DISCUSSION

Isolation of Human ScRG-1 cDNA—To isolate the human counterpart of the mouse ScRG-1 cDNA, the murine sequence (10) from the "Laboratoire de Recherche sur les Encephalopathies Spongiformes Sub-aiguës Transmissibles et les Prions, Action Concertée Coordonnée Number 2), from INSERM, and from the Association Nouvelles Recherches Biomédicales. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AJ223206 and AJ224677. § Recipient of a fellowship from the Association Recherche et Partage.
Isolation of the Human ScRG-1 mRNA

The human ORF was found to encode a protein of 98 amino acids, which is 51 amino acids more than previously predicted for the murine protein (2). Comparison of human and murine cDNA sequences indicated that introduction of a one-nucleotide gap in the murine ORF would generate an open reading frame corresponding to a protein of 98 amino acids the sequence of which would be almost identical to that encoded by the human ScRG-1 cDNA. This prompted us to clone a new mouse brain ScRG-1 cDNA from a lambda library, using as a probe the ScRG-1 cDNA clone 24 previously isolated by RACE (2). The sequence of clone 1 obtained from the library was then determined (Fig. 1A). The nucleotide sequences of the different cDNAs previously obtained by RACE (2) were also re-examined. The mouse ORF, which was reported to contain two guanosines at positions 345 and 346, was found in fact to contain three guanosines at positions 345, 346, and 347, immediately following a sequence consisting of a succession of nucleotides repeated twice, thereby constructing an imperfect palindrome. The additional guanosine signal (position 345) was very weak in intensity and abnormally close to the following nucleotide. The presence of the “missing G” was confirmed by sequencing a recently isolated murine genomic DNA clone of ScRG-1 (data not shown). The corrected nucleotide sequence of the mouse brain ScRG-1 cDNA and derived amino acid sequence (Fig. 1, A and B) have been reported in the data bases.

Comparison of the human and mouse ScRG-1 coding sequences showed that 82.5% of the amino acids of the two predicted proteins are identical, with a stretch of 40 identical contiguous amino acids in the carboxyl-terminal region of the protein (Fig. 1B). The ScRG-1 proteins exhibit no apparent homology with other known proteins. Both the human and murine proteins contain a cleavable signal peptide of 20 amino acids in length. Furthermore, the probability that ScRG-1 is external to the plasma membrane is 56% for the murine protein and 48% for human protein according to the PSORT II program of protein localization site prediction. The predicted molecular mass of the mature protein for the two species is approximately 9 kDa, which is in the range of the molecular weight of most cytokines and neurotransmitters. A N-glycosylation site was detected at positions 72–75 of the protein for both species so that the molecular weight of the ScRG-1 protein could be substantially higher in vivo. A tyrosine kinase phosphorylation site was also detected at positions 63–70 in both proteins.

Characterization of Human Brain ScRG-1 mRNA

Northern blot analysis was carried out using RNA from both human and mouse brain to determine the size of the transcripts in the two species. 14 individual RNA species were used as molecular weight markers. The blot was split in two parts to separate the mouse and human samples, and each part was hybridized under stringent conditions with a radiolabeled probe derived from murine and human ScRG-1 cDNA, respectively (Fig. 2, A and B). One band corresponding to 0.7 kb in size was detected in the human samples and as expected, two bands of 2.6 and 0.7 kb were detected in the murine samples. A very faint band of 2.6 kb was also revealed upon overexposure of the autoradiograms of the human blot (data not shown). The faster migrating band was relatively broad, with an estimated size of between 0.66 and 0.82 kb (mean size of 0.74 kb) in both species. Quantification by PhosphorImager indicated that the 0.7-kb message represented at least 75–80% of the ScRG-1 transcripts in murine brain and about 98% of the transcripts in human brain. The relative abundance of the ScRG-1 mRNA in this organ was about 40 times less than the level of β-actin mRNA in both species.

We have reported previously that the 0.7-kb ScRG-1 transcript is overexpressed 2–3-fold in scrapie-infected mouse brain (Ref. 2 and Fig. 2). As shown in Fig. 2B, the level of the 2.6-kb transcript is also increased 2–3-fold in this experimental model of TSE disease. It was of interest to determine whether the level of human ScRG-1 mRNA is increased in samples of brain...
from patients with CJD. Poly(A)$^+$ mRNA was isolated from normal human brain and from the brain (frontal cortex) of a patient diagnosed with CJD. The expression of ScRG-1 mRNA was examined by Northern blot analysis using human ScRG-1 cDNA as a probe (Fig. 2A). ScRG-1 mRNA was found to be 3-fold more abundant in the brain of a patient with CJD than in the brain of a normal individual. In contrast, β-actin mRNA levels were similar in both samples (Fig. 2A). As expected an increased expression of GFAP transcripts was also detected in the CJD mRNA, indicating that a glial reaction had occurred in the brain tissue examined (Fig. 2A), and the presence of proteinase K-resistant PrP$^{	ext{Sc}}$ was also clearly detected in this sample (data not shown). Although these results are highly suggestive, only two individuals have been compared and any definitive conclusion concerning the expression of the ScRG-1 gene will have to await more extensive studies, particularly those employing the techniques of in situ hybridization and/or immunocytochemistry in addition to Northern blot analysis. It is quite possible that the increased expression observed results from the activation of a particular subset of cells in the brain. Thus, the increase in ScRG-1 mRNA detected using Northern blot analysis may underestimate an increased expression in a particular cell population, because all brain cells contribute to the mRNA analyzed by this method. Interestingly, the level of cathepsin S transcripts was also found to be higher in the CJD RNA sample examined (Fig. 2A) in agreement with the 3–8-fold increase in the cathepsin S mRNA, which has been reported in scrapie-infected mouse brain (2). The gliosis that precedes the spongiosis and neuronal death in TSE consists of several areas of brain investigated, and in spinal cord but is poorly or not expressed in at all in fetal brain, indicating marked developmental regulation of the gene. A high level of ScRG-1 mRNA may be expressed preferentially in brain tissue, testis, aorta, and pregnant uterus. It was of interest therefore to determine the specificity of expression of the gene in different tissues, using a membrane to which poly(A)$^+$ RNA from 50 human tissues had been immobilized, by Northern blotting, mRNA from various human tissues, heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas for the presence of ScRG-1 mRNA (Fig. 2C). The ScRG-1 message was highly expressed in the brain as expected and also to a 6-fold lesser extent in the heart. A faint signal was also detected upon overexposure of the blot in almost all the organs examined, indicating that the low level of hybridization observed in most of the human organs and shown in Fig. 2C corresponds to a real albeit low level of expression (data not shown).

**Tissue Distribution and Developmental Expression of Human ScRG-1 mRNA**—Although mouse ScRG-1 mRNA was found to be expressed preferentially in brain tissue (2), the expressed sequence tags related to ScRG-1 and used to define primers to isolate the human cDNA (see above) were recovered from various tissues including brain, testis, aorta, and pregnant uterus.

**Isolation of the Human ScRG-1 mRNA**

Fig. 2. Northern blot analysis of human and mouse ScRG-1 mRNA. A Northern blot containing 2 μg of poly(A)$^+$ RNA from the frontal cortex of patient 941005 (normal) and patient 930005 (CJD) (A) were transferred to a membrane that was hybridized under stringent conditions with radiolabeled human ScRG-1 cDNA. The blot was further dehybridized and rehybridized successively with probes derived from cDNA of the human cathepsin S, hamster GFAP, and murine β-actin. Similarly, a Northern blot (B) containing 10 μg of total brain RNA from C57Bl/6 mice, mock infected or infected with the C506M3 strain of scrapie and sacrificed 170 days post inoculation, was hybridized with the murine ScRG-1 cDNA clone 24 isolated by RACE (2). The third part of figure (C) shows the autoradiogram of a membrane containing 2 μg of poly(A)$^+$ mRNA from different adult human tissues, probed with the human ScRG-1 cDNA. The different ScRG-1 transcripts shown in the three panels are indicated by arrows, and the corresponding sizes are stated.
man and mouse, the presence of a cleavable signal peptide indicating that the ScRG-1 protein is secreted outside the cell, and its high level of expression in the central nervous system. The potential importance of the gene is also emphasized by the observation that its expression is under developmental regulation.

REFERENCES

1. Prusiner, S. B. (1997) Science 278, 245–251
2. Dandroy-Dron, F., Guillo, F., Benheadjema, L., Deslys, J.-P., Lasmezas, C., Dormont, D., Tovey, M. G., and Dron, M. (1998) J. Biol. Chem. 273, 7691–7697
3. Gomi, H., Yokoyama, T., Fujimoto, K., Ikeda, T., Kateh, A., Itoh, T., and Itohara, S. (1996) Neuron 14, 29–41
4. Brown, D. R., Schmidt, B., and Kretzschmar, H. A. (1996) Nature 380, 345–347
5. Williams, A., Van Dam, A. M., Ritchie, D., Eikelenboom, P., and Fraser, H. (1997) Brain Res. 754, 171–180
6. Raeb, A. J., Race, R. E., Brander, S., Priola, S. A., Sailer, A., Bessen, R. A., Mucke, L., Manso, J., Aguzz, A., Oldstone, M. B. A., Weissmann, C., and Chesebro, B. (1997) EMBO J. 16, 1057–1065
7. Duguid, J., and Trzepacz, C. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 114–117
8. Campbell, I. L., Eddleston, M., Kemper, P., Oldstone, M. B. A., and Hobbs, M. V. (1994) J. Virol. 68, 2383–2387
9. Diedrich, J. F., Minoian, H., Carp, R. I., Whitaker, J. N., Race, B., Frey, W., III, and Haase, A. T. (1991) J. Virol. 65, 4759–4768
10. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) Biochemistry 18, 5294–5299
11. Lemere, C. A., Munger, J. S., Shi, G. P., Natkin, L., Haass, C., Chapman, H. A., and Selkoe, D. J. (1995) Am. J. Pathol. 146, 848–860
12. Rigier, R., Edenhofer, F., Lasmezas, C. I., and Weiss, S. (1997) Nat. Med. 3, 1383–1388