PROGRESS REPORT OF THE CJD FOUNDATION AWARD

Assessing prion infectivity in the skin of sporadic CJD patients
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1. Background and specific aims

The most common sporadic Creutzfeldt-Jakob disease (sCJD) accounts for approximately 85% of human prion diseases and its etiology is still poorly understood (Gambetti et al., 2003). It has been well-documented that sCJD can be transmitted to other individuals by several known iatrogenic routes such as brain- or cornea-related operation or surgeries, even peripheral injections of contaminated growth hormone (Brown, 2012). Identification of these transmissible routes has significantly helped the prevention of sCJD from iatrogenic transmission. However, whether there are other, as yet unidentified routes that may contribute to the etiology of sCJD remains to be determined.

Using the highly sensitive RT-QuIC assay, in collaboration with Dr. Byron Caughey from the NIH Rocky mountain laboratories, our recent study has provided evidence for the first time that the skin of sCJD patients harbors prion-seeding activity (Orrú et al., 2017). Moreover, in collaboration with Dr. Qingzhong Kong from our department at the Case Western Reserve University, we further observed that all twelve mice from two humanized transgenic (Tg) mouse lines inoculated with sCJD skin homogenates from two sCJD patients succumbed to prion disease within 564 days after inoculation (Orrú et al., 2017). The objective of this proposal is to further validate our new finding by extending our current study using different humanized Tg mice available in our group and examining skin samples from patients with different subtypes of sCJD, especially the most common subtype sCJDMM1 that carries PrP^{Sc} type 1 with methionine/methionine (MM) at PrP residue 129.

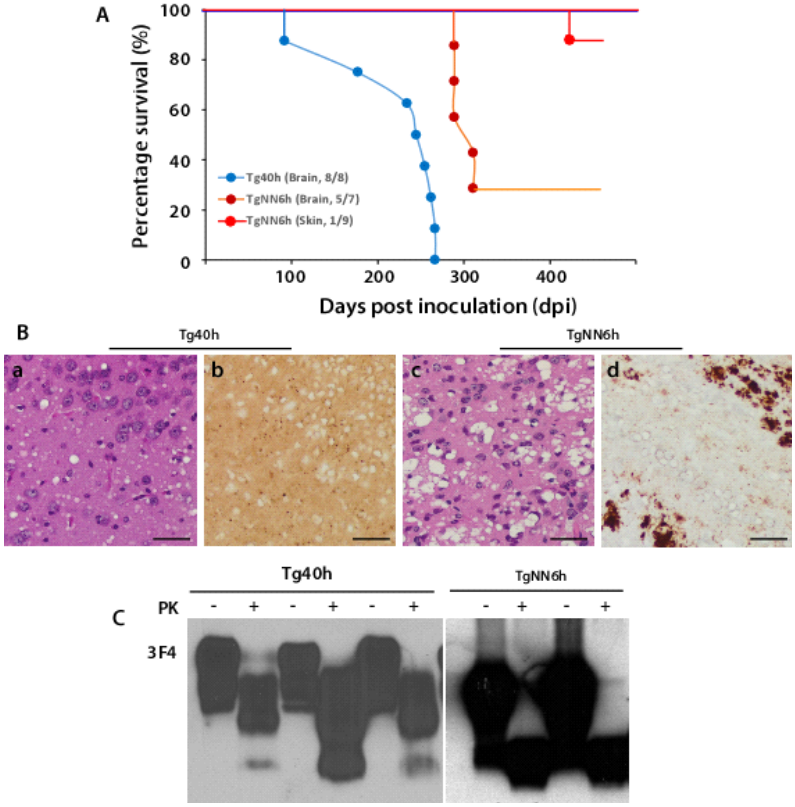

Fig. 1. Bioassay of the brain and skin samples from sCJD patients. (***A***) Percentage survival curves of Tg mice inoculated with brain or skin homogenates from sCJD patients. (***B***) H&E and IHC of brain tissue sections from Tg40h or TgNN6h inoculated with brain homogenate from sCJDMM1 (*a* and *b*) or sCJDMM2 (*c* and *d*). *a* and *c*: H&E; *b* and *d*: IHC with the 3F4 antibody. Scale bars, 50 µM. (***C***) Western blotting (WB) of PrP from brain homogenates of three Tg40h and two TgNN6h mice inoculated with the brain homogenates of sCJDMM1 or sCJD MM2, respectively. Probed with 3F4 (Yuan, Kong and Zou, unpublished data).

2. Experimental results

Since sCJDMM1 accounts for ~60-70% of all sCJD cases (Gambetti et al., 2003), quantifying the infectivity titers of sCJDMM1 skin prions is important for the risk assessment of iatrogenic transmission of sCJD via skin. So, we are determining sCJDMM1 skin infectivity tiers using PrP genotype-matched humanized Tg40h mice expressing human PrP-129M (Kong et al., 2008). In addition, although the skin of patients with sCJDMM2 (carrying PrP^{Sc} type 2 with MM at PrP residue 129) has been found to be infectious in our previous study (Orru et al., 2017), we did not compare its infectivity with the brain infectivity from the same patient. In this study, the brain infectivity from the same patient is also being examined using the humanized TgNN6h mice expressing human PrP-129 with two mutations at the two N-linked glycosylation sites in order to eliminate the two glycans at residue 181 and 197 (Haldiman et al., 2013; Orrú et al., 2017).

1) *Comparison of the infectivity of the brain and skin homogenates from sCJDMM1 with Tg40h mice*—Initially we planned to generate the standard curve for sCJDMM1 brain PrP^{Sc} infectivity in the Tg40h mice but we changed the plan given that there were no sufficient mice within a short time period in order to complete

the titration of serial 10-fold dilutions of a sCJDMM1 brain homogenate during the funding period. Instead, we tried to directly compare the infectivity of skin prions to the brain prions from the same sCJDMM1 patient. Twelve Tg40h mice were inoculated intracerebrally with 30 µl of 5% skin homogenate each whereas eight inoculated with 30 µl of 1% brain homogenate each. So far, all eight Tg mice inoculated with sCJD brain homogenate have succumbed to prion disease at an average of 224±22 (SE) days post-inoculation (dpi) (range, 92 to 267 dpi). PrP^{Sc} and spongiform degeneration were detected in the brain of the inoculated mice with conventional Western blotting and neurohistology including H&E staining and IHC with the 3F4 antibody against human PrP (Fig. 1). In contrast, no clinical signs have been observed in those Tg mice inoculated with the skin homogenates yet. We expect that the mice inoculated with the sCJD skin homogenates will need more time to develop the disease, if the skin of sCJDMM1 is infectious.

2) *Comparison of the infectivity of the brain and skin homogenates from sCJDMM2 with TgNN6h mice*— Twelve TgNN6h mice expressing human PrP with mutated two N-linked glycosylation sites carrying PrP-129M were inoculated intracerebrally with 30 µl of 1% brain homogenate each. So far, five out of seven mice inoculated have developed prion disease at an average of 398±5 dpi, confirmed by the examination of the mouse brain tissues with Western blotting of neurohistology (Fig. 1). In contrast, only one out of nine mice inoculated with sCJD skin homogenates has developed the disease (Fig. 1) while all twelve mice inoculated with non-CJD skin homogenates have exhibited no any clinical signs so far.

3. Future plan

We expect to complete our study within one more year until the end of the normal lifespan of the mice inoculated with non-CJD skin homogenates as negative controls. Once we have all results from the inoculated mice including mice inoculated with sCJD skin or brain homogenates and negative control mice inoculated with non-CJD skin homogenates, we will be able to evaluate the skin prion infectivity by comparing the brain prion infectivity in the same patients with the same lines of humanized Tg mice according to their attack rate and incubation time. We will continue pursuing the quantitation of the exact skin prion infectivity titers by comparing the skin prion infectivity with the standard titration curve of brain prions if our newly applied NIH R01 grant is funded in the future.

4. Publications in part supported by the CJD Foundation grant during the funding period

1) Orrú CD, Yuan J, Appleby BS, Li B, Li Y, Winner D, Wang Z, Zhan YA, Rodgers M, Rarick J, Wyza RE, Joshi T, Wang GX, Cohen ML, Zhang S, Groveman BR, Petersen RB, Ironside JW, Quiñones-Mateu ME, Safar JG, Kong Q, Caughey B, Zou WQ. Prion seeding activity and infectivity in skin samples from patients with sporadic Creutzfeldt-Jakob disease. Sci Transl Med. 2017 Nov 22;9(417). PMCID: PMC5744860

   The study was highlighted by *New York Times*, *Nature Review Neurology*, *The Scientists*, *Neurology Today* etc.

2) Abskharon R, Dang J, Elfarash A, Wang Z, Shen P, Zou LS, Hassan S, Wang F, Fujioka H, Steyaert J, Mulaj M, Surewicz WK, Castilla J, Wohlkonig A, Zou WQ. Soluble polymorphic bank vole prion proteins induced by co-expression of quiescin sulfhydryl oxidase in E. coli and their aggregation behaviors. Microb Cell Fact. 2017 Oct 4;16(1):170. doi: 10.1186/s12934-017-0782-x.

3) Xiao X, Shen P, Wang Z, Dang J, Adornato A, Zou LS, Dong Z, Yuan J, Feng J, Cui L, Zou WQ. Characterization of physiochemical properties of caveolin-1 from normal and prion-infected human brains. Oncotarget. 2017 Jul 21;8(33):53888-53898.

5. References

1. Brown P, Brandel JP, Sato T, Nakamura Y, MacKenzie J, Will RG, Ladogana A, Pocchiari M, Leschek EW, Schonberger LB. Iatrogenic Creutzfeldt-Jakob disease, final assessment. Emerg Infect Dis. 2012 Jun;18(6):901-7. PMCID: PMC3358170
2. Gambetti P, Kong Q, Zou W, Parchi P, Chen SG. Sporadic and familial CJD: classification and characterisation. Br Med Bull 2003;66:213-39
3. Haldiman T, Kim C, Cohen Y, Chen W, Blevins J, Qing L, Cohen ML, Langeveld J, Telling GC, Kong Q, Safar JG. Co-existence of distinct prion types enables conformational evolution of human PrPSc by competitive selection. J Biol Chem. 2013 Oct 11;288(41):29846-61. PMCID:PMC3795283
4. Kong Q, Zheng M, Casalone C, Qing L, Huang S, Chakraborty B, Wang P, Chen F, Cali I, Corona C, Martucci F, Iulini B, Acutis P, Wang L, Liang J, Wang M, Li X, Monaco S, Zanusso G, Zou WQ, Caramelli M, Gambetti P. Evaluation of the human transmission risk of an atypical bovine spongiform encephalopathy prion strain. J Virol. 2008 Apr;82(7):3697-701. PMCID: PMC2268471
5. Orrú CD, Yuan J, Appleby BS, Li B, Li Y, Winner D, Wang Z, Zhan YA, Rodgers M, Rarick J, Wyza RE, Joshi T, Wang GX, Cohen ML, Zhang S, Groveman BR, Petersen RB, Ironside JW, Quiñones-Mateu ME, Safar JG, Kong Q, Caughey B, Zou WQ. Prion seeding activity and infectivity in skin samples from patients with sporadic Creutzfeldt-Jakob disease. Sci Transl Med. 2017 Nov 22;9(417). PMCID: PMC5744860