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The effect of PrP^{Sc} accumulation on inflammatory gene expression within sheep peripheral lymphoid tissue

Anton G. Gossner, John Hopkins*

The Roslin Institute & R(D)SVS, University of Edinburgh, Easter Bush, Midlothian EH25 9RG, UK

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ABSTRACT

Accumulation of the misfolded prion protein, PrP^{Sc} in the central nervous system (CNS) is strongly linked to progressive neurodegenerative disease. For many transmissible spongiform encephalopathies (TSEs), peripheral lymphoid tissue is an important site of PrP^{Sc} amplification but without gross immunological consequence. Susceptible VRQ homozygous New Zealand Cheviot sheep were infected with SSBP/1 scrapie by inoculation in the drainage area of the prescapular lymph nodes. The earliest time that PrP^{Sc} was consistently detected by immunohistology in these nodes was D50 post infection. This transcriptomic study of lymph node taken before (D10) and after (D50) the detection of PrP^{Sc}, aimed to identify the genes and physiological pathways affected by disease progression within the nodes as assessed by PrP^{Sc} detection. Affymetrix Ovine Gene arrays identified 75 and 80 genes as differentially-expressed at D10 and D50, respectively, in comparison with control sheep inoculated with uninfected brain homogenate. Approximately 70% of these were repressed at each time point. RT-qPCR analysis of seven genes showed statistically significant correlation with the array data, although the results for *IL1RN* and *TGIF* were different between the two technologies. The ingenuity pathway analysis (IPA) and general low level of repression of gene expression in lymphoid tissue, including many inflammatory genes, contrasts with the pro-inflammatory and pro-apoptotic events that occur within the CNS at equivalent stages of disease progression as assessed by PrP^{Sc} accumulation.

1. Introduction

Sheep scrapie is a transmissible spongiform encephalopathy (TSE), a group of fatal neurodegenerative diseases of the central nervous system (CNS). A key feature of TSEs is the conversion of the host-encoded prion protein PrP^{C} to disease-associated PrP^{Sc} (Prusiner, 1982); the replication of pathological PrP^{Sc} from physiological PrP^{C} is a critical component of the disease (Prusiner et al., 1999). The essential role of PrP^{C} in TSE disease is confirmed by the resistance of PrP^{null} mice to disease (Bueler et al., 1993); by the reciprocal relationship of PrP gene (*PRNP*) copy number and incubation period (Bueler et al., 1993; Manson et al., 1994), and by the fact that resistance to sheep scrapie is influenced by polymorphisms of *PRNP* at codons 136 (V or A), 154 (R or H) and 171 (R or Q) (Goldmann et al., 1994). With SSBP/1 scrapie in Cheviot sheep, VRQ homozygotes have the shortest incubation period (Houston et al., 2002).

The CNS is the major target organ for TSE disease and neurodegeneration is associated with the accumulation of PrP^{Sc} within neurons (Mallucci et al., 2003). Many TSE agents, including natural sheep scrapie, are associated with replication of infectivity in peripheral lymphoid tissue prior to the invasion of the CNS (Mabbott and Bruce, 2003). PrP^{Sc} replicates in follicular dendritic cells (FDC) in spleen and lymph node germinal centres (Jeffrey et al., 2000; McCulloch et al., 2011) and interference of this prolongs the incubation period. However, in contrast to neurons, PrP^{Sc} replication by FDC does not lead to their degeneration or the inhibition of gross immunological functions (Heikenwalder et al., 2005).

The effects of PrP^{Sc} accumulation on the CNS transcriptome has been investigated in several different species, including mice (Xiang et al., 2004), cattle (Almeida et al., 2011), sheep (Filali et al., 2012; Gossner and Hopkins, 2014) and humans (Tian et al., 2013) with the aim of identifying genes associated with TSE disease progression. Similar analysis of secondary lymphoid tissues is so far limited to two sheep studies; an investigation on mesenteric lymph node in natural scrapie (Filali et al., 2014) and our preliminary study (Gossner et al., 2011b) on SSBP/1 scrapie. The earliest time that PrP^{Sc} was consistently detected by

* Corresponding author at: The Roslin Institute & R(D)SVS, University of Edinburgh, Easter Bush, Midlothian EH25 9RG, UK. Fax: +44 131 651 9107
E-mail address: john.hopkins@ed.ac.uk (J. Hopkins).

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immunohistochemistry was at 50 days post infection (D50), in the prescapular lymph node (PSLN) draining the site of scrapie inoculation; and microarray analysis of PSLN and spleen at D75 linked repression of inflammation with the accumulation of PrP^{Sc}. This current study exploits the same model to compare, using the new Affymetrix Ovine Gene 1.1 ST whole-genome expression array and by RT-qPCR, the effects of scrapie infection on the transcriptome of the PSLN early after infection (D10) and after the immunohistochemical detection of PrP^{Sc} (D50). In this way we aim to identify how scrapie infection and/or PrP^{Sc} affect the molecular physiology of secondary lymphoid tissue; and to compare the events in this tissue to the CNS at equivalent stages of disease progression as assessed by PrP^{Sc} accumulation.

2. Materials and methods

2.1. Animals and experimental design

Animals, infections and tissues have been described in detail previously (Gossner et al., 2011a,b). Briefly, Cheviot sheep with *PRNP* homozygous genotype VRQ/VRQ (Houston et al., 2002) were inoculated subcutaneously in the drainage area of the PSLNs with either SSBP/1 brain homogenate (infected) or similarly prepared scrapie-negative brain homogenate; both brain homogenates contained PrP of both VRQ and ARQ genotypes. Three infected and two uninfected controls were killed at 10 days (D10) and 50 days (D50) post infection. Animal experiments were approved by BBSRC Institute for Animal Health Ethical Review Committee and conducted under an Animals (Scientific Procedures) Act 1986 Project Licence.

2.2. Sample collection and total RNA isolation

Tissues were removed post-mortem, dissected into blocks and submerged in RNA*later*® (Ambion) incubated at 4 °C overnight and stored at −80 °C. Total RNA was isolated using the RiboPure™ RNA Purification Kit (Ambion, Huntingdon, UK) with DNase I digestion. RNA quality and integrity was assessed using the Agilent RNA 6000 Nano kit on the Agilent 2100 Bioanalyzer and quantified with a NanoDrop ND-1000 spectrophotometer.

2.3. RNA amplification and microarray hybridization

Transcriptome analysis was by Affymetrix Ovine Gene 1.1 ST arrays, which consist of 508,538 oligomers (25 mer) covering 22,047 genes. These are complementary to approximately 635 bases per gene and cover all exons of each annotated transcript of the Oar v2 sheep genome assembly. Sense-strand cDNA was generated from 0.5 μg of total RNA and subjected to two rounds of amplification using the Ambion® WT Expression Kit. The cDNA was biotin labelled and fragmented using the Affymetrix GeneChip® WT Terminal Labelling and Hybridization kit. Biotin-labelled fragments of cDNA (5.5 μg) were hybridized to the array plates using the appropriate Hyb-Wash-Scan protocol with reagents from the Affymetrix Gene Titan Hyb Wash Stain kit. After hybridization the plates were washed, stained and scanned by the Imaging Station of the GeneTitan System. The Affymetrix® GeneChip® Command Console® Software (v3.0.1) was used to generate array images and the resulting Affymetrix intensity files (CEL files), along with the initial QC analysis.

2.4. Microarray data analysis

The CEL files were imported into Partek Genomics Suite ® software, version 6. 13.0213 (Copyright© 2014; Partek Inc., St. Louis, MO, USA.) and data were analyzed at the gene-level using the mean expression of all exons of a gene. Background correction was performed using the robust multiarray average (RMA) algorithm, with quantile normalization, median polish probe summarization, and $\log_2$ probe transformation. Differentially-expressed genes were identified by analysis of variance (ANOVA), genes with a fold change >1.5 or <−1.5, and p value > 0.05 were retained. Hierarchical clustering was performed on significant genes, with the data normalized to a mean of zero and scaled to standard deviation of one using Partek. Significant genes were annotated based on similarity scores in BLASTN comparisons of Affymetrix Transcript cluster sequences against mRNA sequences in GenBank. The array data have been deposited in ArrayExpress database (www.ebi.ac.uk/arrayexpress) accession number E-MTAB-2327.

Table 1
Significantly increased differentially-expressed genes at D10.

Gene	Gene name	p value	FC
IFI6	Interferon, alpha-inducible protein 6	0.0422	2.62
ADM	Adrenomedullin	0.0482	2.30
ZNF347	Zinc finger protein 347	0.0282	2.04
XAF1	XIAP associated factor 1	0.0143	1.97
OR5S1P	Olfactory receptor, family 5, subfamily S, member 1 pseudogene	0.0225	1.87
GCSH	Glycine cleavage system protein H (aminomethyl carrier)	0.0496	1.85
MOGAT3	Monoacylglycerol *O*-acyltransferase 3	0.0309	1.78
ZBTB16	Zinc finger and BTB domain containing 16	0.0366	1.76
FKBP14	FK506 binding protein 14, 22 kDa	0.0495	1.73
NUDT15	Nudix (nucleoside diphosphate linked moiety X)-type motif 15	0.0353	1.73
S100A5	S100 calcium binding protein A5	0.0040	1.69
ESRP2	Epithelial splicing regulatory protein 2	0.0449	1.66
FAM171B	Family with sequence similarity 171, member B	0.0279	1.64
OR10H1	Olfactory receptor, family 10, subfamily H, member 1	0.0348	1.63
SERBP1	SERPINE1 mRNA binding protein 1	0.0265	1.62
IER3	Immediate early response 3	0.0151	1.60
RASGRF2	Ras protein-specific guanine nucleotide-releasing factor 2	0.0234	1.59
ZC3H12B	Zinc finger CCCH-type containing 12B	0.0049	1.58
GJA3	Gap junction protein, alpha 3, 46kDa	0.0122	1.57
IGFBPL1	Insulin-like growth factor binding protein-like 1	0.0218	1.57
DMXL2	Dmx-like 2	0.0107	1.56
MTERFD1	MTERF domain containing 1	0.0302	1.54
IL1RN	Interleukin 1 receptor antagonist	0.0337	1.52

Genes with fold change (FC) ≥ 1.5 fold and adjusted p value of ≤0.05.