Neurobiology of Aging 69 (2018) 151–166

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Loss of Trem2 in microglia leads to widespread disruption of cell coexpression networks in mouse brain

Guillermo Carbajosa [a,*], Karim Malki [b], Nathan Lawless [b], Hong Wang [c], John W. Ryder [c], Eva Wozniak [d], Kristie Wood [d], Charles A. Mein [d], Richard J.B. Dobson [a,e,f], David A. Collier [b], Michael J. O'Neill [b], Angela K. Hodges [g], Stephen J. Newhouse [a,e,f]

[a] Department of Biostatistics and Health Informatics, Institute of Psychiatry Psychology and Neuroscience, King's College London, London, UK
[b] Eli Lilly and Company, Windlesham, UK
[c] Eli Lilly and Company, Indianapolis, IN, USA
[d] Barts and the London Genome Centre, John Vane Science Centre, Barts and the London School of Medicine and Dentistry, London, UK
[e] NIHR Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London, London, UK
[f] Farr Institute of Health Informatics Research, UCL Institute of Health Informatics, University College London, London, UK
[g] Maurice Wohl Clinical Neuroscience Institute James Black Centre Institute of Psychiatry, Psychology and Neuroscience (IoPPN), King's College London, London, UK

ARTICLE INFO

Article history:
Received 18 January 2018
Received in revised form 26 March 2018
Accepted 28 April 2018
Available online 17 May 2018

Keywords:
TREM2
Alzheimer's disease
Weighted gene coexpression network analysis
RNA-Seq
Knockout mouse model
Endothelial cells

ABSTRACT

Rare heterozygous coding variants in the triggering receptor expressed in myeloid cells 2 (TREM2) gene, conferring increased risk of developing late-onset Alzheimer's disease, have been identified. We examined the transcriptional consequences of the loss of Trem2 in mouse brain to better understand its role in disease using differential expression and coexpression network analysis of Trem2 knockout and wild-type mice. We generated RNA-Seq data from cortex and hippocampus sampled at 4 and 8 months. Using brain cell-type markers and ontology enrichment, we found subnetworks with cell type and/or functional identity. We primarily discovered changes in an endothelial gene-enriched subnetwork at 4 months, including a shift toward a more central role for the amyloid precursor protein gene, coupled with widespread disruption of other cell-type subnetworks, including a subnetwork with neuronal identity. We reveal an unexpected potential role of Trem2 in the homeostasis of endothelial cells that goes beyond its known functions as a microglial receptor and signaling hub, suggesting an underlying link between immune response and vascular disease in dementia.

1. Introduction

Genome-wide association studies and genome sequencing have identified more than 25 Alzheimer's disease (AD) risk loci, including common, low-risk variants and rare moderate risk variants, in addition to the classical risk variants in the apolipoprotein E (APOE) gene (Guerreiro et al., 2013b; Hollingworth et al., 2011; Jonsson et al., 2013; Lambert et al., 2013; Naj et al., 2011; Pimenova et al., 2018; Steinberg et al., 2015). Although the identity of many of the associated disease genes and the mechanisms by which they increase risk remain unclear, there is evidence that they cluster around the immune system, protein and lipid metabolism, especially inflammatory response, endocytosis, and amyloid precursor protein (App) metabolism (Hardy et al., 2014; Naj and Schellenberg, 2017; Villegas-Llerena et al., 2016). Many implicated genes encode proteins that are highly expressed in microglia (ABI3, PLCG3, TREM2, SPI1, BIN1, CD33, INPP5D, MS4A6A) and/or have a role in the innate immune system in the brain (Huang et al., 2017; Pimenova et al., 2018). One of these, TREM2 (triggering receptor expressed on myeloid cells 2), has an AD-associated risk allele (R47H) with an effect size in AD similar to that of ApoE4 (odds ratio 2.90–5.05) (Guerreiro et al., 2013b; Jonsson et al., 2013).

TREM2 function can be compromised as a result of rare non-synonymous variants that cause Nasu-Hakola disease when both alleles are affected (Numasawa et al., 2011; Ridha et al., 2004; Soragna, 2003) or significantly increase the risk of developing AD (Guerreiro et al., 2013b; Jonsson et al., 2013; Sims et al., 2017), behavioral variant frontotemporal dementia (Guerreiro et al.,

* Corresponding author at: SGDP Centre, IoPPN, Box PO 80, De Crespigny Park, Denmark Hill, London SE5 8AF, UK. Tel.: +44 207 848 0969; fax: +44 207 848 0866.
E-mail address: guillermo.carbajosa_antona@kcl.ac.uk (G. Carbajosa).

0197-4580/
https://doi.org/10.1016/j.neurobiolaging.2018.04.019

2013a; Le Ber et al., 2013), semantic variant of primary progressive aphasia or occasionally Parkinson's disease (PD) (Borroni et al., 2014; Liu et al., 2016; Rayaprolu et al., 2013) when 1 allele is affected. Evidence suggests TREM2 may be important for normal brain remodeling, which peaks around adolescence (Chertoff et al., 2013) and in later life in response to age-related damage or pathologies. It is not clear whether the same signals or brain regions are affected by TREM2 activity throughout life. In people where TREM2 is compromised by complete loss of function, symptoms begin in adolescence and tend to implicate frontal lobe dysfunction, whereas in people with preservation of some normal TREM2 activity, symptoms appear much later in life and implicate the hippocampus (Giraldo et al., 2013). Although in both cases, progressive white matter changes in the brain and dementia symptoms occur.

TREM2 is a receptor highly expressed on macrophages, including microglia in the brain (Butovsky et al., 2014; Hickman et al., 2013; Paloneva et al., 2002; Schmid et al., 2002). It appears to be an important damage sensing receptor. It can respond to lipid and lipoprotein species such as phosphatidyl serine (Cannon et al., 2012; Wang et al., 2015), clusterin, and apolipoproteins, including APOE (Atagi et al., 2015; Poliani et al., 2015; Yeh et al., 2016) and nucleotides and anionic species such as heparin sulphate, proteoglycans, or other negatively charged carbohydrates (Daws et al., 2003; Kawabori et al., 2015; Kober et al., 2016), and is required for efficient bacterial clearance (N'Diaye et al., 2009). TREM2 signaling is propagated through the adaptor protein DAP12, which activates a number of pathways including Syk, P13 K, and mitogen-activated protein kinase, which culminate in increased phagocytosis and expression of an anti-inflammatory phenotype in microglia (Kleinberger et al., 2014; Neumann and Takahashi, 2007; Peng et al., 2010; Poliani et al., 2015; Takahashi et al., 2007). Loss of Trem2 function culminates in a decrease in the number and activation of microglia in mouse models of AD or in mice treated with cuprizone to damage myelin (Cantoni et al., 2015; Ulrich et al., 2014; Wang et al., 2015). Trem2-deficient dendritic cells secrete more TNF-α, IL-6, and IL-12 compared with wild-type (WT) cells, particularly when activated with lipopolysaccharides suggesting there may be a shift toward cells expressing a proinflammatory and potentially more damaging phenotype in the absence of Trem2 (Hamerman et al., 2006; Turnbull et al., 2006). However, not all findings are consistent. A recent report demonstrated reduced AD pathology in an amyloid mouse crossed with a Trem2 knockout (KO) mouse (Jay et al., 2015).

The main AD-associated TREM2 variant R47H has been shown to alter glycosylation and trafficking of the TREM2 protein between the golgi and endoplasmic reticulum resulting in fewer functional TREM2 receptors in the cell membrane and thus loss of TREM2 function (Park et al., 2015). The presence of this variant also reduces the cleavage of full-length TREM2 to a soluble extracellular fragment and, in both TREM2 risk variant carriers and in people with AD, less soluble TREM2 is present in cerebrospinal fluid (Kleinberger et al., 2014) suggesting TREM2 dysfunction may be a common feature in AD and not just in those AD patients carrying a loss of function variant. Notwithstanding this, recent findings suggest that soluble form of the innate immune receptor TREM2 levels may be reduced or increased depending on the stage of AD and variant (Brendel et al., 2017).

TREM2 and other late-onset AD susceptibility genes MS4A4A/4 E/6A, CD33, HLA-DRB5/DRB1, and INPP5D are all part of a distinctive brain coexpression module, which also contains the signaling partner for TREM2, TYROBP, or DAP12 (Forabosco et al., 2013; Hawrylycz et al., 2012; Zhang et al., 2013). This module appears to represent a biological network active in microglial cells with an innate immune function. It is significantly perturbed in AD brain (Forabosco et al., 2013; Hawrylycz et al., 2012; Zhang et al., 2013) and remarkably contains fewer than 150 genes. This module shares identity with peripheral macrophages (Forabosco et al., 2013), and many of the genes in the module are also altered in AD blood cells (Lunnon et al., 2012). Elevated expression of many of these genes, particularly TREM2 appears to be associated with the emergence of amyloid rather than Tau pathology in AD mouse models (Jiang et al., 2014; Matarin et al., 2015). Both TREM2 and Tyrobp have also been identified as major hubs in human APOE-expressing mice following traumatic brain injury (Castranio et al., 2017).

Prominent voices in the field of AD research are proposing that to fully understand the etiology of AD, we have to go beyond reductionist approaches and the amyloid cascade linearity and that there is a need for studies that address the complex cellular context of the disease, which involves interactions between different cell types as the disease progresses across time and tissue (De Strooper and Karran, 2016). It is suggested that temporal resolution can be obtained from cohorts of mice at different stages of the disease. Previous studies using a systems level approach on TREM2 have lacked the aforementioned temporal resolution. Furthermore, the use of microarrays to measure gene expression could have meant that subtle effects are not detected. In our study, we tried to address these concerns by profiling Trem2 KO mice gene expression using RNA-Seq at 2 time points and tissues. We used brain cell markers to infer cell type specificity and detected gene coexpresion dysruption affecting a module with endothelial identity at an early stage that causes widespread disruption of other cell-type subnetworks, including a subnetwork with neuronal identity.

2. Methods

2.1. Design

Brain tissue samples were obtained from male Trem2 KO and WT control mice at 2 time points: 4 months and 8 months. These time points span the onset and late disease stages in well-established AD mouse models (Matarin et al., 2015). Hippocampus and cortex were selected because they represent tissues affected in AD at early and late stages, respectively (Mastrangelo and Bowers, 2008; Matarin et al., 2015). RNA-Seq was used to profile the transcriptomes for each sample. Two technical replicates were obtained for each sample. Expression data analyzed in this study are available at Gene Expression Omnibus data repository from the National Center for Biotechnology through accession number GSE104381.

Differential expression (DE) analysis allowed us to detect changes in expression between time points and tissues. Coexpression analysis was performed to detect higher-level disturbances in gene expression networks. Enrichment analysis of the results allowed us to detect functions and pathways more altered in the absence of Trem2. Finally, the integration of cell type markers enabled us to go further and not only detect time and tissue-specific changes but also uncover how the interactions between different cell types were affected and at which time point and tissue these changes were occurring. Fig. 1A displays an overview of the experimental and analytical workflow.

2.2. Generation of Trem2-/- mice

The Trem2-/- mouse model (Trem2tm1(KOMP)Vlcg) was generated by knocking a LacZ reporter cassette into the endogenous Trem2 locus in place of exons 2 and 3 and most of exon 4, resulting in a loss of Trem2 function and expression of the LacZ reporter under the control of the Trem2 promoter, as described previously (Jay et al., 2015). The mouse line was originally generated by the trans-NIH KnockOut Mouse Project (KOMP). Frozen sperms were obtained from the UC Davis KOMP repository, and a colony of mice was established at Taconic, Cambridge City, MA, USA. Mice were