Gen. Physiol. Biophys. (2021), **40,** xxx–xxx

doi: 10.4149/gpb_2021022

Transcriptomic signature of Alzheimer's disease tau seed-induced pathology

Peter Szalay[1,2,3,*], Nikoleta Csicsatkova[1,4,*], Katarina Matyasova[1,4], Veronika Mate[1,3], Martin Cente[1,3], Tomas Smolek[1,3,5], Veronika Brezovakova[1,6], Lucie Kawecka[6], Norbert Zilka[1,3] and Santosh Jadhav[1]

[1] *Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia*
[2] *University of Veterinary Medicine and Pharmacy in Kosice, Kosice, Slovakia*
[3] *Axon Neuroscience R&D Services SE, Bratislava, Slovakia*
[4] *Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia*
[5] *Neuroimunology institute, n.p.o., Bratislava, Slovakia*
[6] *BrainTest, s.r.o., Bratislava, Slovakia*

Abstract. Spreading of tau pathology to anatomical distinct regions in Alzheimer's disease (AD) is associated with progression of the disease. Studies in recent decade have strived to understand the processes involved in this characteristic spread. We recently showed that AD-derived insoluble tau seeds are able to initiate neurofibrillary pathology in transgenic rodent model of tauopathy. In the present study, we pursued to identify the molecular changes that govern the induction and propagation of tau pathology on the transcriptomic level. We first show that microglia in vicinity to AD-Tau-induced pathology has phagocytic morphology when compared to PBS-injected group. On transcriptomic level, we observed deregulation of 15 genes 3-month post AD-Tau seeds inoculation. Integrated bioinformatic analysis identified 31 significantly enriched pathways. Amongst these, the inflammatory signalling pathway mediated by cytokine and chemokine networks, along with, toll-like receptor and JAK-STAT signalling were the most dominant. Furthermore, the enriched signalling also involved the regulation of autophagy, mitophagy and endoplasmic reticulum stress pathways. To our best of knowledge, the study is the first to investigate the transcriptomic profile of AD-Tau seed-induced pathology in hippocampus of transgenic model of tauopathy.

Key words: Alzheimer's disease — Tau spreading — Misfolded tau — Gene expression — Microglia

Introduction

Alzheimer's disease (AD) is a progressive age-associated neurodegenerative tauopathy characterized by neurodegeneration and dementia. For decades, studies have attempted to understand the properties that initiate and drive the disease progression, mainly in AD without any known genetic predisposition (late-onset or sporadic form). Besides environmental and immune risk factors, studies implicate genetic susceptibility and metabolic modifications in manifestation of AD (Miech et al. 2002; Cacabelos et al. 2005). In addition, genome-wide meta-analysis studies (GWAS)

* These authors contributed equally to this work.

Electronic supplementary material. The online version of this article (doi: 10.4149/gpb_2021022) contains Supplementary material.

Correspondence to: Santosh Jadhav, Institute of Neuroimmunology, Slovak Academy of Sciences, Dubravska cesta 9, 845 10 Bratislava, Slovakia
E-mail: santosh.jadhav@savba.sk

implicate a number of genes involved in immune system and tau metabolism as potential risk factors for sporadic late-onset AD (Ridge et al. 2016; Jansen et al. 2019). Moreover, we recently demonstrated the importance of genetic background in immune-system modulation, *via* microglia, as a crucial factor on the propagation of tau pathology (Smolek et al. 2019a).

In AD and few other tauopathies there is the distinct spread of tau pathology in the brain (Braak and Braak 1991; Delacourte et al. 1999). The pathology manifests in the hippocampus and spread to anatomically connected regions. Several studies in recent years have strived to mimic the characteristic spread in transgenic rodent models of AD or tauopathy, using inoculates of insoluble tau from AD brain (Smolek et al. 2019a, 2019b), protein isolates from transgenic rodent models of tauopathy (Levarska et al. 2013), or recombinant forms of tau (Iba et al. 2013; Reyes et al. 2013). Interestingly, tau species from different tauopathies induce pathology idiosyncratic to the specific disease (Clavaguera et al. 2013; Boluda et al. 2015), exhibiting a strain-dependent role of tau protein in disease manifestation (Levarska et al. 2013). Moreover, different tau forms, such as oligomers or fibrils, also differ in their seeding potency. In addition, the mode of transmission such as secretion, mechanism of uptake is progressively unearthed (Saman et al. 2012; Yamada et al. 2014; Tang et al. 2015; Kang et al. 2019; Morozova et al. 2019; Brunello et al. 2020).

Studies have shown the differential expression of genes in rodent model of AD, in late stages of the disease (Annese et al. 2018; Rothman et al. 2018; Lau et al. 2020). However, very little information is known about the early molecular changes in brain microenvironment following initiation and propagation of tau pathology *in-vivo*. Therefore, in this study, we performed intracerebral injections of human AD-derived tau seeds to identify primary changes in the hippocampus of sporadic rat model of tauopathy. Our transcriptomic analysis revealed deregulation of 15 genes linked to different pathways involved in neurons and glial cells. To our best of knowledge, the study is the first to investigate the transcriptomic profile of tau seed-induced pathology in hippocampus of transgenic model of tauopathy.

Materials and Methods

Transgenic model

Transgenic rat model (TG) expressing human truncated tau aa151-391,4Repeat was used in the study. The line has been previously characterized (Koson et al. 2008), and used for analysis of tau-induced spreading and propagation of pathology *via* intracranial application of insoluble tau from AD brain (Smolek et al. 2019b). All animals were housed under specific pathogen free facility with access to water and food *ad libitum*, and were kept under diurnal lighting conditions. Experiments were performed with the approval of the Institute's Ethical Committee, and the study was approved by the State Veterinary and Food Administration of the Slovak Republic (approval #Ro-3748/2020-220).

Isolation of sarkosyl insoluble tau from human brain

Braak stage 5 AD brain was purchased from the University of Geneva brain collection, Switzerland, in accordance with the material transfer agreement. Isolation of sarkosyl insoluble tau was performed as previously described (Jadhav et al. 2015). Briefly, Parietal cortex was homogenized in a buffer containing 20 mM Tris, 0.8 M NaCl, 1 mM EGTA, 1 mM EDTA, and 10% sucrose, supplemented with protease and phosphatase inhibitors. After centrifugation at 20,000 × *g* for 20 min, the supernatant (S1) was collected, and a small fraction was saved as the total protein fraction. N-lauroylsarcosine (sarkosyl) in concentration of 40% w/v in water was added to the S1 to a final concentration of 1% and stirred for 1 h at room temperature. The sample was then centrifuged at 100,000 × *g* for 1 h at 25°C and resulting pellet (P2) was washed and re-suspended in phosphate-buffered saline (PBS) to 1/50 volume of the S1 fraction, and sonicated briefly. 20 µg w/v of the P2 fraction (AD-Tau) corresponding to the S1 fraction, was used for the SDS-PAGE analysis. Blots were developed using pan-tau antibody DC25 (Axon Neuroscience R&D Services, Bratislava, Slovakia). Intensity of bands were quantified using AIDA Biopackage (Advanced Image Data Analyzer software; Raytest, Germany), and concentration of insoluble tau fraction was estimated using a standard curve with reference intensities of known concentrations of recombinant human tau 2N4R (Tau40) as previously described (Smolek et al. 2019b).

Stereotaxic surgery

Male transgenic rats (age 3 months) were anesthetized *via* intraperitoneal injection of a cocktail containing Zoletil (30 mg/kg) and Xylariem (10 mg/kg). Animals were fixed to a stereotaxic apparatus and an UltraMicroPump III Microsyringe injector and Micro4 Controller (World Precision Instruments, FL, United States) were used for intracranial applications. Stereotaxic coordinates for the injection were A/P: −3.6 mm, L: ± 2.0 mm, D/V: 3.3 from bregma (Paxinos and Watson 1996). Animals received bilateral injections of 1500 ng (concentration 500 ng/µl) of sarkosyl-insoluble tau ($n = 6$) or PBS ($n = 5$) at a rate of 1.25 µl/min, and the needle was positioned for 5 min before slow withdrawal to prevent leakage of the infused liquid. After 3 months, animals were anesthetized, perfused transcardially with 1×PBS-Heparin

and sacrificed. Hippocampi from left hemispheres were frozen for transcriptomic analysis, and whole right hemispheres were used for histological assessment.

Immunohistochemistry

The right hemispheres were fixed in sucrose solutions (15, 25, and 30% for 24 h each) followed by freezing in 2-methyl butane. Frozen tissues were serially cut into 40-µm-thick sagittal sections using a cryomicrotome (Leica CM1850, Leica Biosystems). The sections were blocked with Aptum Section block (Aptum Biologics Ltd., Oxford, UK) followed by incubation with antibodies AT8 (Mouse monoclonal, Thermo-Scientific, IL, USA), GFAP (Rabbit polyclonal, Abcam, Bratislava, Slovakia), or Iba-1 (Rabbit polyclonal, Wako, Japan) overnight at 4°C. After washing, the sections were incubated for 1 h with respective Alexa conjugated secondary antibodies (Invitrogen, Eugene, Oregon, USA). After washing, the sections were mounted onto slides using Vectashield mounting medium (Vector laboratories, USA), and examined with laser scanning confocal microscope LSM 710 (Carl Zeiss, Jena, Germany).

Gene expression profiling by real-time PCR

Left hemispheres were used for transcriptomic analysis from PBS- and AD-Tau-injected transgenic rats. Total RNA was extracted using the TRI Reagent according to manufacturer's instructions (Sigma-Aldrich, USA, Cat#. T9424). Resulting RNA was briefly air-dried and suspended in 100 µl of RNAse-free water (Qiagen, Germany, Cat#. 129112). The integrity of isolated RNA samples was determined using Agilent 2100 Bioanalyzer (Agilent Technologies, Germany, Cat #. 5067-1511). For transcriptomic analysis, only high-quality RNA samples were used, RNA integrity number for PBS- or AD-Tau-injected groups were 8.7 ± 0.14 standard deviation (SD) and 8.8 ± 0.15 SD, respectively.

Profiling of gene expression was performed using the Rat Inflammatory Cytokines and Receptors PCR array (Qiagen, Germany, Cat #. PARN-011Z), Rat Phagocytosis PCR array (Qiagen, Germany, Cat #. PARN-173Z) and Rat Autophagy PCR array (Qiagen, Germany, Cat #. PARN-084Z), evaluating a total of 238 genes.

Total RNA was reversely transcribed into cDNA by RT2 first strand kit (Qiagen, Germany, Cat #. 330401), and 100 ng of resulting cDNA was used as a template for each qPCR reaction. Components of 25 µl qPCR reaction were as follows: 12.5 µl 2×RT2 SYBRGreen/ROX mastermix; 12 µl RNase-free water and 0.5 µl of cDNA (200 ng/µl). Cycling conditions included an initial denaturation at 95°C for 10 min, and 42 cycles of 95°C for 15 s cycle denaturation, together with amplification at 60°C for 1 min. PCR specificity was checked by melting curve analysis.

Fold change of target genes expression in each PBS- and AD-Tau-injected animal was compared to the average of control PBS-injected group using $2^{-\Delta\Delta CT}$ method with Ribosomal protein lateral stalk subunit P1 (Rplp1) as endogenous reference. The Rplp1 was identified as the most stable gene across all samples, evaluated with the Endogenous control pipeline using ExpressionSuite software v.1.1 (Applied Biosystems, Foster City, USA). Comprehensive list of analysed genes together with calculated fold change and statistical evaluation is included in the Supplementary Material (Table S1–S4).

Statistical evaluation of gene expression

Statistical analysis was performed using software R, version 4.0.3. The hypotheses were tested at a significance level of 0.05. Gene expression measures in PBS- and AD-Tau-injected groups were tested for outliers, and observations that were more than three times the interquartile range from the first and third quartile were eliminated. Then, for each gene, the null hypothesis H_0: $\mu_{controls} - \mu_{Tau} = 0$ was tested against H_1: $\mu_{controls} - \mu_{Tau} = 0$, where $\mu_{controls}$ is mean of control PBS group and μ_{tau} is mean of AD-Tau group.

The analysis was performed using a bootstrap version of two-sample Student *t*-test with Welch degrees of freedom with 1000 replications using the *boot.t.test* from the *simpleboot* library.

The complete results from statistical analysis including sample size, means and SD of both groups, means difference, 95% confidence intervals of a mean difference and *p*-values are reported in Supplementary Material (Table S1-S4). The direction describes the sign of the difference between the means $\mu_{controls} - \mu_{Tau}$.

Pathway enrichment analysis

To enable the pathway enrichment analysis (PEA), we employed PathDIP. PathDIP is an annotated database of signaling cascades that integrates pathways with physical protein-protein interactions to predict significant physical associations between proteins and curated pathways (http://ophid.utoronto.ca/pathDIP). In this study, the identified rat genes were annotated for their human orthologs. Human orthologs for all 15 dysregulated rat genes were used to query pathDIP version 4.0.21.4 (Database version 4.0.7.0) (Rahmati et al. 2020) to identify significantly enriched pathways, with *q*-value <0.05 (false discovery rate: Benjamini-Hochberg method). We used all pathway sources, and only literature curated (core) pathway memberships. Pathway annotations from 22 different pathway sources were tested for enrichment and gene-pathway matrix was generated to highlight dysregulated genes in enriched pathways. Raw data, search results, and evaluation are included in Supplementary Material (Table S1–S4).

Results

Insoluble tau from AD-induced morphological changes on microglia in hippocampus of transgenic rodent model

In the present study, we performed bilateral injections of insoluble tau isolated from human AD brain (AD-Tau; 900 ng) (Fig. 1A) or PBS in the hippocampus of 3 months old TG rodent model of tauopathy. Using immunohistochemistry, we observed the presence of tau pathology in the hippocampus of AD-tau-injected rodents but not in PBS-injected groups, as previously reported (Smolek et al. 2019a). We were interested to know whether AD-Tau-induced pathology activates glial cells, specifically microglia and astrocytes. Therefore, we performed co-immunostaining using phospho-tau antibody AT8 (pathological tau marker) with either Iba-1 (microglia

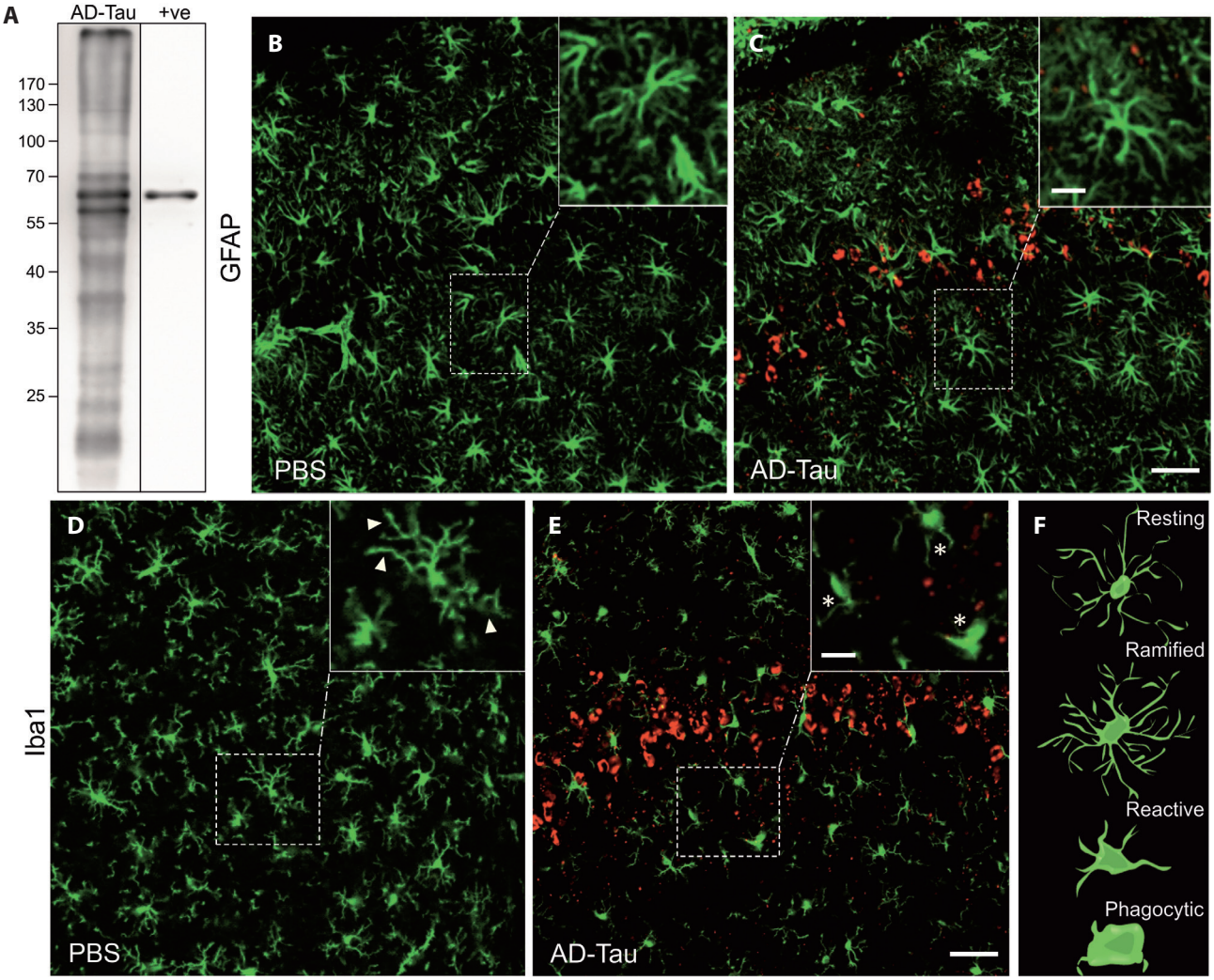

Figure 1. Histological assessment of hippocampus of PBS- and AD-Tau-injected rodents. **A**. Immunoblot using pan-tau antibody DC25 shows the presence of insoluble tau from AD brain. Recombinant human Tau 40 was used as positive control (+ve). Representative confocal images showing co-immunostaining using astrocyte marker-GFAP (green) and tau marker-AT8 (red) in PBS-injected (**B**) and AD-Tau-injected (**C**) groups. Insets showing higher magnification of the area highlighted in B and C, respectively. No prominent difference in astrocyte morphology between the two groups was observed. Representative confocal images showing co-staining using microglia marker Iba1 (green) and AT8 (red) in PBS-injected (**D**) and AD-Tau-injected (**E**) groups. The microglia in PBS injected groups have numerous processes (arrow heads in inset of D); whereas, in AD-Tau-injected group the microglia show increased cell body size with deramification of the processes (phagocytic morphology) (asterisks in inset of E). Scale bar: 50 μm (inset 20 μm). **F**. Illustration depicting the stages involved in activation of microglia in response to stimuli. In brief, resting microglia becomes ramified with more processes. In later stages, it becomes reactive with increased body size but with reduced number of processes. The microglia finally attain phagocytic morphology with few or no processes. AD, Alzheimer's disease; PBS, phosphate-buffered saline.

marker) or GFAP (astrocyte marker). Interestingly, we did not observe any significant morphological changes in astroglia between the two groups (Fig. 1B,C). However, we observed changes in morphology of microglia in vicinity to AT8 positive neurofibrillary structures in hippocampus of AD-Tau-injected rodents (Fig. 1E). Activation of microglia is characterized by progressive transformation of cells from reactive (small cell body and numerous processes) to phagocytic morphology, i.e. with larger cell body and few to no processes (Fig. 1F). Interestingly, microglia (~97%) in vicinity to AT8 positive structures, in AD-Tau-injected rodents were phagocytic (*in inset Fig. 1E). In contrast, the microglias in the PBS-injected group were either resting or ramified with numerous processes (arrowheads in inset Fig. 1D).

Insoluble tau from human AD induced differential expression of genes associated with inflammation

We were then interested to identify the molecular changes associated with insoluble tau-induced pathology in transgenic rodent model of tauopathy. We performed transcriptomic profiling of 238 different genes in the hippocampus of PBS- and AD-Tau-injected TG rodents using three different PCR arrays kits (Supplementary Material, Table S1-S4). Using quantitative PCR, we detected an altered expression of 15 genes after 3 months following the AD-Tau injection. In the identified group, 11 genes were up-regulated (*Mbl2, Il17f, Cxcl11, Ccl4, Osm, Tnfα, Ccl7, Tgm2, Ccr10, Wnt5* and *Gabarap*), and 4 genes displayed reduced expression (*Bnip3*, *Hprt1*, *Il5r* and *Tnfsf13b*) (Table 1).

To reveal functional annotation of signalling pathways associated with spreading of tau pathology we performed integrated bioinformatic analysis. Human orthologs of dysregulated genes from the rat model were further analysed using pathDIP portal to identify specific pathways associated with involved genes and to highlight the significantly enriched pathways. We identified 31 significantly enriched pathways with the inflammatory signalling mediated by cytokine and chemokine network, along with TLRs and JAK-STAT signalling were the most dominant. Moreover, the enriched signalling also involved the regulation of autophagy, mitophagy and endoplasmic reticulum stress pathways (Table 2). Evaluation of the gene pathway matrix revealed that *Ccl4* and *Tnfα* represent the top most abundant genes shared among the significantly enriched pathways suggesting their important role in the molecular response mechanisms involved in the spreading of pathological tau aggregates in the brain Supplementary Material (Table S1–S4).

Discussion

The mechanism/s involved in initiation and propagation of tau pathology *in-vivo* has gained wider attention in recent years. Several research groups, including us, have identified and documented processes involved in the characteristic spread of tau (Clavaguera et al. 2013; Boluda et al. 2015; Smolek et al. 2019a, 2019b). In the present study, we extend our previous observation and explore the changes on molecular level in brain microenvironment post inoculation of insoluble tau seeds. We employed a sporadic rat model expressing human truncated tau aa151-391 that develops robust neurofibrillary pathology, akin to tauopathies. Despite retaining the expression of misfolded tau aa151-391,

Table 1. Differentially expressed genes in rats injected with AD-Tau compared to animals injected with PBS (control)

Gene symbol	Gene name	Fold change	*p*-value
Mbl2	Mannose binding lectin 2	33.15	0.016
Il17f	Interleukin 17F	3.37	0.044
Cxcl11	C-X-C motif chemokine ligand 11	1.95	0.048
Ccl4	C-C motif chemokine ligand 4	1.93	0.01
Osm	Oncostatin M	1.79	0.002
Tnf	Tumor necrosis factor	1.59	0.012
Ccl7	C-C motif chemokine ligand 7	1.55	0.038
Tgm2	Transglutaminase 2	1.5	0.006
Ccr10	C-C motif chemokine receptor 10	1.35	0.026
Wnt5	Wnt family member 5A	1.26	0.032
Gabarap	GABA type A receptor-associated protein	1.05	0.016
Hprt1	Hypoxanthine phosphoribosyltransferase 1	−1.18	0.02
Bnip3	BCL2 interacting protein 3	−1.25	0.032
Tnfsf13b	TNF superfamily member 13b	−1.32	0.004
Il5ra	Interleukin 5 receptor subunit alpha	−2.78	0.042

Table 2. Enriched pathways associated with spreading of AD-Tau aggregates

Signaling	Pathway name	Pathway source	*q*-value*
Cytokines	Cytokine Signaling in immune system	REACTOME	3.50609E−03
	Cytokine-cytokine receptor interaction	KEGG	2.35584E−09
	Signaling by interleukins	REACTOME	5.56810E−03
	Interleukin-4 and Interleukin-13 Signaling	REACTOME	6.78072E−03
	Interleukin-10 Signaling	REACTOME	2.37526E−02
	IL-17 Signaling	KEGG	6.02465E−03
	IL23-mediated Signaling events	PID	2.01809E−02
Chemokines	Chemokine Signaling	WikiPathways	2.95941E−03
	Chemokine Signaling	KEGG	3.43831E−03
	Chemokine receptors bind chemokines	REACTOME	2.26209E−02
	Cytokines chemokines production	ACSN2	1.08907E−03
Immune cells and inflammation	Inflammatory Signaling	ACSN2	2.01809E−02
	Recruitment of immune cells	ACSN2	1.98639E−02
	Inflammation mediated by chemokine and cytokine Signaling	Panther Pathway	2.32315E−02
	miRNAs involvement in the immune response in sepsis	WikiPathways	2.01809E−02
	NF-kappa B Signaling	KEGG	5.65365E−03
	Intestinal immune network for IgA production	KEGG	2.36651E−02
	Inflammatory bowel disease (IBD)	KEGG	3.41348E−02
	Thioguanine metabolism pathway	SMPDB	2.31433E−02
	Legionellosis	KEGG	2.84571E−02
	Lung fibrosis	WikiPathways	3.44696E−02
Toll-like receptor signaling	Toll-like receptor Signaling	WikiPathways	6.16538E−03
	Toll-like receptor Signaling	KEGG	6.45435E−03
	Regulation of toll-like receptor Signaling	WikiPathways	2.05721E−03
JAK-STAT signaling	JAK STAT pathway in postconditioning ischemia	IPAVS	3.87602E−03
	GP130_JAK_STAT	IPAVS	5.40987E−03
Purine nucleotide salvage	Guanine and guanosine salvage	HumanCyc	3.55866E−02
	Adenine and adenosine salvage III	HumanCyc	4.47457E−02
Mitophagy	Mitophagy – animal	KEGG	3.41348E−02
Autophagy	Autophagy	Spike	3.42884E−02
ER stress	ER Stress Map	IPAVS	6.07290E−03

*false discovery rate: Benjamini-Hochberg method (FDR: BH-method). ER, endoplasmic reticulum.

the transgenic rodents do not develop pathology in the hippocampus. However, using intracerebral injections of AD-derived insoluble tau seeds we were able to induce tau pathology in hippocampus of these animals (Smolek et al. 2019a, 2019b). Moreover, the AT8 positive structures were also observed in synaptically connecting regions adjacent to the site of inoculation. In addition, the exogenous AD-Tau seeds recruited endogenous rat tau in the neurofibrillary inclusions. Therefore, we used a similar approach to investigate tau-induced changes in hippocampal microenvironment employing PCR array profiling.

Neuroinflammation is cardinal hallmark of AD characterized by activation of the innate immune system, the trigger for which is yet uncertain. GWAS suggest that genes associated with inflammation are a risk factor in onset and progression of AD (Castanho et al. 2020; Li and De Muynck 2021). In line, differential expression of genes associated with inflammation is observed in AD (Wang et al. 2018; Chew and Petretto 2019; Kim et al. 2019). Moreover, a recent study also implicates the deregulation of genes associated with microglia in AD (Li and De Muynck 2021; Sobue et al. 2021). Therefore, we investigated the transcriptomic profile associated with the seeding and initiation of tau pathology in rodent model of tauopathy using three different PCR arrays, which extensively covers genes involved in key aspects of neuroinflammation (cytokines and their receptors, autophagy (ER stress, oxidative stress), and phagocytosis). We identified 15 genes that are differentially expressed in response to exogenous AD-derived tau seeds. Integrated bioinformatic analysis revealed significant enrichment of

pathways associated with neuroinflammation, recruitment of immune cells, regulation of autophagy, and cell organelles stress signalling. At the molecular level, our data suggest the initiation of signalling cascades, mainly in microglia, in response to tau-induced pathology. In particular, elevated expression of *Tnfα*, *Ccl4* and *Ccl7*, factors that are chemotactic to phagocytic cells, indicate activation and recruitment of these cells during tau seeding and propagation. Likewise, the notable upregulation of *Cxcl11* may relate to the effector function of microglia, mediated *via* receptor protein Cxcr3, which is crucial for their recruitment (Rappert et al. 2004; Koper et al. 2018). This correlates well with the phagocytic morphology of microglia in proximity to AT8 positive structures in the hippocampus of tau-seeded rodents.

Furthermore, the gene *Mbl2,* which is highly upregulated following the seeding of tau aggregates is a member of lectin pathway of complement system. It acts as opsonin and is involved in the regulation of innate immunity and removal of senescent and apoptotic cells by macrophages (Turner 1998).

Among other genes, we observed upregulation of oncostatin M, a pleiotropic cytokine of the IL-6 family involved in cell communication and signalling in the immune system, and exerts a direct neuroprotective activity in the CNS (Houben et al. 2019).

In addition to the inflammatory signalling by cytokines, chemokines and TLRs, we identified the enrichment of autophagy, mitophagy and endoplasmic reticulum pathways associated genes in hippocampus of AD-Tau-seeded rodents. This molecular association indicates the clearance of tau aggregates through lysosomal pathway and chaperone-mediated autophagy (Wang et al. 2009; Abisambra et al. 2013). Furthermore, it highlights the degradation pathways associated with neurodegeneration directing towards establishment of neuro-proteostasis in the brain (Opattova et al. 2015).

Neuroinflammation is inevitably associated with activation of microglia (Zotova et al. 2010). It is reported that tau oligomers and fibrils induce activation of microglia and evoke their morphological alterations (Morales et al. 2013). However, it is also shown that neutralization of AD-Tau seeds by microglia is compromised since the cells released inefficiently degraded tau back to extracellular space, thereby contributing to spreading of tau pathology (Hopp et al. 2018). Moreover, tau hyperphosphorylation affects microglia-dependent tau degradation (Perea et al. 2018). These phenomena more likely relates to dual activity of microglia in tau spreading cascades, and can be explained by diverse molecular pathways linked to tau-induced neurodegeneration.

One of the limitations, in the study, is that we employed male rodents in order to avoid heterogeneity; therefore, sex specific response to tau-induced pathology in females may vary. Secondly, we used bulk hippocampal tissues for transcriptomic analysis, and microglial activation was observed only in the vicinity of the AT8 positive structures. Despite this limitation, we observed transcriptional changes; however, the actual number of deregulated genes may be greater than observed.

Overall, for the first time, our results suggests, that exogenous tau seeding in transgenic rodent model of tauopathy induce specific activation of pro-inflammatory signalling and participation of phagocytic cells, including microglia, that are involved in the manifestation of tau-induced pathology and spreading.

Acknowledgement. The authors thank Jozef Vegh for his technical assistance. The work was supported by research grants APVV-17-0642, APVV-18-0515, APVV-14-0872, JPsustaiND 681043, VEGA 2/0118/19 and VEGA 2/0110/20. Bioinformatic analyses were in part supported by the Ministry of Education of the Slovak Republic, awarded to BrainTest, s.r.o. within the research scheme "Stimuli for research and development".

Conflict of interest. The authors declare no conflict of interest.

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Received: May 6, 2021
Final version accepted: July 1, 2021

doi: 10.4149/gpb_2021022

Supplementary Material

Transcriptomic signature of Alzheimer's disease tau seed-induced pathology

Peter Szalay[1,2,3,*], Nikoleta Csicsatkova[1,4,*], Katarina Matyasova[1,4], Veronika Mate[1,3], Martin Cente[1,3], Tomas Smolek[1,3,5], Veronika Brezovakova[1,6], Lucie Kawecka[6], Norbert Zilka[1,3] and Santosh Jadhav[1]

[1] *Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia*
[2] *University of Veterinary Medicine and Pharmacy in Kosice, Kosice, Slovakia*
[3] *Axon Neuroscience R&D Services SE, Bratislava, Slovakia*
[4] *Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia*
[5] *Neuroimunology institute, n.p.o., Bratislava, Slovakia*
[6] *BrainTest, s.r.o., Bratislava, Slovakia*

Table S1. "Genes" spreadsheet: List of 238 profiled genes using the PCR arrays including the calculated Fold change and bootstrap statistics (*p* value)

Table S2. "Pathway annotations" spreadsheet: Pathway annotations for the dysregulated genes via pathDIP: Annotated database of signaling cascades (http://ophid.utoronto.ca/pathDIP/)

Table S3. "PEA" spreadsheet: Pathway enrichment analysis of identified known pathways via pathDIP: Annotated database of signaling cascades (http://ophid.utoronto.ca/pathDIP/)

Table S4. "Genes pathway matrix" spreadsheet: Membership of dysregulated genes in significant pathways as analyzed *via* pathDIP

Table S1-S4.xlsx