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Meta-analysis of mouse transcriptomic studies supports a context-dependent astrocyte reaction in acute CNS injury versus neurodegeneration

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Abstract

Background: Neuronal damage in acute CNS injuries and chronic neurodegenerative diseases is invariably accompanied by an astrocyte reaction in both mice and humans. However, whether and how the nature of the CNS insult—acute versus chronic—influences the astrocyte response, and whether astrocyte transcriptomic changes in these mouse models faithfully recapitulate the astrocyte reaction in human diseases remains to be elucidated. We hypothesized that astrocytes set off different transcriptomic programs in response to acute versus chronic insults, besides a shared "pan-injury" signature common to both types of conditions, and investigated the presence of these mouse astrocyte signatures in transcriptomic studies from human neurodegenerative diseases.

Methods: We performed a meta-analysis of 15 published astrocyte transcriptomic datasets from mouse models of acute injury (n = 6) and chronic neurodegeneration (n = 9) and identified pan-injury, acute, and chronic signatures, with both upregulated (UP) and downregulated (DOWN) genes. Next, we investigated these signatures in 7 transcriptomic datasets from various human neurodegenerative diseases.

Results: In mouse models, the number of UP/DOWN genes per signature was 64/21 for pan-injury and 109/79 for acute injury, whereas only 13/27 for chronic neurodegeneration. The pan-injury-UP signature was represented by the classic cytoskeletal hallmarks of astrocyte reaction (*Gfap* and *Vim*), plus extracellular matrix (i.e., *Cd44*, *Lgals1*, *Lgals3*, *Timp1*), and immune response (i.e., *C3*, *Serping1*, *Fas*, *Stat1*, *Stat2*, *Stat3*). The acute injury-UP signature was enriched in protein synthesis and degradation (both ubiquitin-proteasome and autophagy systems), intracellular trafficking, and anti-oxidant defense genes, whereas the acute injury-DOWN signature included genes that regulate chromatin structure and transcriptional activity, many of which are transcriptional repressors. The chronic neurodegeneration-UP signature was further enriched in astrocyte-secreted extracellular matrix proteins (*Lama4*, *Cyr61*, *Thbs4*), while the DOWN signature included relevant genes such as *Agl* (glycogenolysis), *S1pr1* (immune modulation), and *Sod2* (anti-oxidant). Only the pan-injury-UP mouse signature was clearly present in some human
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neurodegenerative transcriptomic datasets.

Conclusions: Acute and chronic CNS injuries lead to distinct astrocyte gene expression programs beyond their common astrocyte reaction signature. However, caution should be taken when extrapolating astrocyte transcriptomic findings from mouse models to human diseases.

Keywords: Acute CNS injury, Astrocyte reaction, Meta-analysis, Neurodegenerative diseases, Transcriptomics

Background

Neuronal loss in central nervous system (CNS) acute injuries and chronic neurodegenerative diseases is invariably accompanied by an astrocyte reaction, which has been traditionally depicted by an increased glial fibrillary acidic protein (GFAP) immunoreactivity. However, thanks to the improvement of cell purification protocols and the development of transcriptomic methodologies (microarray and RNA-seq), the complexity and heterogeneity of this astrocyte reaction is emerging above and beyond this increased GFAP immunoreactivity. One area of uncertainty is whether there is any disease-specificity in astrocyte reaction or, conversely, this is just a non-specific response of astrocytes to any kind of neuronal injury. More specifically, whether reactive astrocytes could be neurotoxic in certain pathological conditions and neuroprotective in others is an area of active research. For example, it has been proposed that reactive astrocytes are neurotoxic (so called A1 astrocytes) in the lipopolysaccharide (LPS)-induced sepsis mouse model, but neuroprotective (termed A2 astrocytes) in the middle cerebral artery occlusion (MCAO) stroke mouse model [1, 2]. Another controversial question is whether astrocyte proliferation is a universal feature of astrocyte reaction. For example, astrocyte proliferation has been demonstrated in acute injury conditions such as traumatic brain injury (TBI) and stroke [3–5] but appears more limited in the context of chronic neurodegenerative diseases [5–9].

We hypothesized that astrocytes set off different transcriptomic programs in response to acute versus chronic disease, but that a common pan-injury signature underlies both types of conditions. To test this hypothesis, we took advantage of publicly available microarray and RNA-seq mouse astrocyte transcriptomic datasets from acute lesions [LPS-induced sepsis, MCAO stroke, spinal cord injury (SCI), and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)] and chronic neurodegenerative disease models [amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD)], and applied bioinformatics tools [gene set enrichment analysis (GSEA) and meta-analysis]. Specifically, we asked whether (1) the previously reported A1, A2, and pan-reactive signatures can be extrapolated to conditions beyond LPS-induced sepsis and MCAO stroke; (2) distinct acute, chronic, and pan-injury (common to acute and chronic conditions) signatures can be identified; (3) reactive astrocytes in these conditions activate proliferative pathways as much as inflammatory cascades; and (4) astrocyte transcriptomic signatures obtained from mouse models correlate with changes in astrocyte gene expression levels in the corresponding human diseases.

Methods

All analyses were conducted using R version 3.5.1 [10]

Mouse gene expression analysis

Publicly available transcriptomic datasets of mouse astrocytes were identified either by using search terms in Gene Expression Omnibus (GEO) [11] or from relevant literature (Table 1). Microarray data were processed and normalized using RMA package in R. For RNA-seq datasets, we used fragments per kilobase of transcript per million mapped reads (FPKM) values whenever available; when only the raw sequencing fastq files were available, we used the *salmon* package to process and estimate transcript abundance [24]. Differentially expressed genes (DEGs) were identified using *limma* [25] and *voom+ limma* [26] as genes with a statistically significant difference in expression level ($p < 0.05$) between the diseased (i.e., transgenic) and control (i.e., wild-type) mice.

Meta-analysis of mouse transcriptomic studies

Meta-analyses were conducted using the *sumz* function in the metap package in R, which first converts the *P* values into *Z* scores, and then computes the composite *Z* score [27]. Square root of the sample sizes was used as weights in the *sumz* procedure to control for the number of samples in each study. The *p* values were corrected for multiple comparisons using a false discovery rate (FDR) < 5%. We conducted meta-analyses within acute injury and neurodegenerative mouse studies separately. We identified the pan-injury signature as those genes that met two criteria: (1) had a statistically significant multiple-comparison-corrected meta *p* value (meta $p < 0.05$) and (2) had a statistically significant ($p < 0.05$) differential expression in at least 33% of both acute injury and neurodegenerative mouse datasets. The chronic neurodegeneration-specific signature was defined as those genes with a statistically significant adjusted meta

Table 1 Summary of publicly available mouse astrocyte transcriptomic datasets used in this study

Label	Injury type	Comparison	N	Sex (*n* M/F)	Age	CNS region	Astrocyte isolation method	RNA method	Accession #	Ref.
LPS	Acute	LPS vs placebo	5/4	5M/4M	30–35 days	ctx+cc	FACS of Aldh1L1-eGFP+ cells	Microarray	GSE35338	[1]
MCAO-1	Acute	MCAO vs sham surgery	3/3	3M/3M	30–35 days	ctx+cc+hipp+str	FACS of Aldh1L1-eGFP+ cells	Microarray	GSE35338	[1]
MCAO-2	Acute	Transient MCAO ipsi- vs contra and control (no TMX)	3/3	NA	3–5 months	Hemibrain	Cx43-Cre-ERT/RiboTag	RNA-seq	GSE103783	[12]
SCI-1	Acute	SCI at T10 vs non-injured	4/4	4F/4F	2–4 months	Spinal cord	mGFAP-Cre-RiboTag	RNA-seq	GSE76097	[13]
SCI-2	Acute	SCI at T9 (hemi- and full transection vs non-injured)	9/3	9M/3M	12 weeks	Spinal cord	FACS of Aldh1L1-eGFP+ cells	RNA-seq	GSE96054	[14]
MPTP-24 h	Acute	MPTP vs vehicle (sac 24 h post-single i.p. inj.)	3/3	3F/3F	4–7 months	str	TRAP of Aldh1L1-eGFP-L10a	Microarray	https://doi.org/10.17632/ktgcp4mtk2.1	[15]
SOD1-G93A-1	Chronic	G93A SOD1 vs wt	4/3	NA	90 days	Spinal cord	FACS Aldh1L1-eGFP/G93A SOD1	Microarray	GSE111031	[16]
SOD1-G93A-2	Chronic	G93A SOD1 vs wt	3/3	3M/3M	120 days	Spinal cord	LCM of Aldh1L1+ cells	Microarray	GSE69166	[17]
SOD1-G37R	Chronic	G37R SOD1 vs wt	4/6	4M/6M	8 months	Spinal cord	TRAP of Aldh1L1-eGFP-L10a	RNA-seq	GSE74724	[18]
APPPS1-1	Chronic	APPswePS1dE9 vs wt	4/4	NA	15–18 months	Whole ctx	FACS with Glt-1 Ab	Microarray	GSE74615	[19]
APPPS1-2	Chronic	APPswePS1dE9 vs wt	4/4	NA	15–18 months	Whole ctx	FACS of GFAP-GFP cells	Microarray	GSE74614	[20]
APPPS1-3	Chronic	APPswePS1dE9-GFP vs wt-GFP	4/7	4M/7M	9 months	CA1 hipp	FACS of eGFP+ cells	RNA-seq	GSE108520	[21]
PS2APP-1	Chronic	PS2APP vs wt	4/5	2M2F/1M4F	13 months	Whole brain	FACS with GFAP Ab	RNA-seq	GSE75431	[22]
PS2APP-2	Chronic	PS2APP vs wt	5/5	NA	11.5 months	Whole brain	FACS with GFAP Ab	RNA-seq	GSE129770	[23]
P301S-tau	Chronic	hMAPT-P301S tau vs wt	5/6	5M/6M	6 months	Whole ctx	FACS with GFAP Ab	RNA-seq	GSE129797	[23]

Abbreviations: *Ab* antibody, *Aldh1L1* aldehyde dehydrogenase 1 L1, *APP* amyloid-β precursor protein, *CA1* cornus ammonis 1, *cc* corpus callosum, *CNS* central nervous system, *ctx* cortex, *Cx43* connexin-43, *eGFP* enhanced green fluorescent protein, *FACS* fluorescence-activated cell sorting, *GFAP* glial fibrillary acidic protein, *Glt1* glutamate transporter 1, *i.p.* intra-peritoneal, *LCM* laser capture microdissection, *LPS* lipopolysaccharide, *MAPT* microtubule-associated protein tau, *MCAO* middle cerebral artery occlusion, *MPTP* 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, *PS1/2* presenilin 1/2, *sac* sacrifice, *SCI* spinal cord injury, *SOD1* superoxide dismutase 1, *TRAP* translating ribosome affinity purification

p value (meta $p < 0.05$) in the neurodegenerative meta-analysis, which had a statistically significant differential expression ($p < 0.05$) in 33% or more neurodegenerative studies but none of the acute injury datasets. Similarly, the acute injury-specific signature was defined as those genes with a statistically significant adjusted meta p value (meta $p < 0.05$) in the acute injury meta-analysis, which had a statistically significant differential expression ($p < 0.05$) in 33% or more acute injury studies but none of the chronic neurodegeneration datasets. DEGs were considered upregulated (UP) if the logarithm of the fold change (logFC) was >0 and downregulated (DOWN) if the logFC was <0. Although some of the datasets analyzed had some contamination from microglial genes [17, 18, 21], this analytic approach minimized the probability of including these microglial genes from a single dataset in the final meta-analytic signatures. Moreover, we confirmed the expression of the resulting genes by astrocytes in a previously published RNA-seq study of cell subpopulations isolated from the mouse brain [28].

Gene set enrichment analysis (GSEA)

To identify signaling pathways, we searched for the terms "KAPPA", "NFAT", "MAPK", "JAK/STAT", "WNT/BETA-CATENIN", and "SONIC HEDGEHOG" within the KEGG, REACTOME, BIOCARTA, PID, and GO pathways compiled in the MSigDB version 6.2 [29] and obtained a total of 86 gene sets. Next, we performed gene set enrichment analysis (GSEA) [30, 31] to determine the enrichment of each of these gene sets in each of the 15 mouse astrocyte transcriptomic datasets and

generated a heatmap with the normalized enrichment scores (NES) and another heatmap with the $-\log_{10}$ of the p values. Next, we conducted a meta-analysis of the resulting GSEA p values and ranked the gene sets in descending adjusted meta p value, representing the relative relevance of these signaling pathways in the astrocytes from each of the mouse models.

Validation of mouse astrocyte signatures in human transcriptomic datasets

To validate the meta-analytic mouse transcriptomic signatures, we investigated the differential expression of each of the signature genes in human microarray and RNA-seq bulk tissue and astrocyte-specific datasets from AD, Parkinson's disease (PD), and ALS. Table 2 depicts the human datasets used in these analyses, with sample size, CNS region of interest, transcriptomic method, accession number, and reference. The AD datasets were analyzed by comparing individuals with Braak NFT V/VI (AD) versus Braak NFT 0/I/II (controls), as described previously [37]. The differential expression of diseased versus control individuals for the PD and ALS datasets was analyzed using *limma*. Violin plots were generated using ggplot2 version 3.2.1 [38] to represent the logFC of each signature gene in diseased versus control individuals.

Results

Compilation of acute injury and neurodegenerative astrocyte transcriptomic datasets

A total of 15 transcriptomic datasets from astrocytes isolated from mouse models were obtained from GEO. Dataset category (acute injury versus neurodegeneration), mouse model, mouse age and sex, CNS region, method of astrocyte isolation, GEO accession number, and literature reference for these datasets can be found in Table 1. Mouse acute injury datasets (n = 6) included LPS-induced sepsis (n = 1) [1], MCAO stroke (n = 2) [1, 12], SCI (n = 2) [13, 14], and acute toxic parkinsonism (MPTP, n = 1) [15]. For one of the stroke studies [1], only the dataset from 3 days after MCAO was analyzed, because gene expression changes were maximum 3 days and attenuated by 7 days after MCAO surgery. The MPTP dataset [15] was classified as acute injury rather than chronic neurodegeneration because the authors examined the changes in astrocyte transcriptome after an acute neurotoxic injury to the substantia nigra dopaminergic neurons (i.e., 12 h, 24 h, and 48 h after a single MPTP intra-peritoneal injection), rather than after chronic MPTP administration through a subcutaneous osmotic pump [39]. Since the three acute MPTP time points are not independent datasets, only the 24 h dataset was used for the meta-analysis [15]. Mouse neurodegenerative datasets (n = 9) included ALS (various SOD1 mutants, n = 3) [16–18] and AD models of both brain β-amyloidosis (n = 5) (APPswe/PSEN1deltaE9 n = 3 and PS2APP n = 2) [19–23], and tauopathy (P301S-MAPT, n = 1) [23]. Whenever more than one age group was available, only the older age group was analyzed.

A1 and A2 astrocyte transcriptomic signatures are not CNS disease-specific

Recently, an A1 neurotoxic signature and an A2 neuroprotective signature have been defined based on the DEGs from two acute injury mouse models: LPS-induced sepsis and MCAO stroke, respectively, with a third "pan-reactive" signature representing the common overlap between sepsis- and stroke-induced transcriptomic changes [1, 2]. Moreover, it has been suggested that, in neurodegenerative diseases, astrocytes also upregulate the A1 neurotoxic signature and downregulate

Table 2 Summary of publicly available human neurodegenerative transcriptomic datasets used in this study

Label	Comparison	N	Sample type	RNA method	CNS region	Accession #	Ref.
AD-DLPC	AD (Braak V/VI) vs CTRL (Braak 0/I/II)	140/112	Bulk tissue	RNA-seq	Dorsolateral prefrontal cortex	syn3505720	[32]
AD-PHG	AD (Braak V/VI) vs CTRL (Braak 0/I/II)	62/79	Bulk tissue	RNA-seq	Parahippocampal gyrus	syn5898488	[33]
AD-astro1	AD (Braak V/VI) vs CTRL (Braak 0/I/II)	6/6	LCM of GFAP+ astrocytes	Microarray	Lateral temporal cortex	GDS4135	[34]
AD-astro2	AD (Braak V/VI) vs CTRL (Braak 0/I/II)	7/12	FACS with GFAP Ab	RNA-seq	Superior frontal gyrus	GSE125050	Friedman, Hansen (unpublished)
PD-SN	PD vs CTRL	3/3	Bulk tissue	Microarray	Substantia nigra	GSE54282	[35]
PD-Str	PD vs CTRL	6/6	Bulk tissue	Microarray	Striatum	GSE54282	[35]
ALS-SC	SALS vs CTRL	5/4	Bulk tissue	Microarray	Spinal cord gray matter	GSE833	[36]

Abbreviations: *Ab* antibody, *AD* Alzheimer's disease, *ALS* amyotrophic lateral sclerosis, *CNS* central nervous system, *CTRL* control, *DLPC* dorsolateral prefrontal cortex, *FACS* fluorescence-activated cell sorting, *GFAP* glial fibrillary acidic protein, *LCM* laser capture microdissection, *PD* Parkinson's disease, *PHG* parahippocampal gyrus, *SALS* sporadic ALS, *SC* spinal cord, *SN* substantia nigra, *Str* striatum

the A2-neuroprotective signature [2]. To test the hypothesis that these A1, A2, and pan-reactive signatures can be extrapolated to other acute injuries as well as neurodegenerative diseases, we examined the differential expression of the genes comprising these signatures in the 15 mouse datasets described above. The heatmaps in Fig. 1 show the fold change of the expression of the genes comprising these three signatures and the *p* values of these comparisons. Surprisingly, overall the A1, A2, and pan-reactive genes were upregulated in astrocytes across most acute injury and neurodegeneration mouse models. Only MPTP appeared to induce a differential response, with an upregulation of the A2 and pan-reactive signatures and a downregulation of the A1 signature. Of note, astrocytes from acute injury mouse models tended to exhibit higher level of expression of all three gene cassettes than those from neurodegeneration mouse models. Overall, these analyses demonstrate that the previously described A1 and A2 signatures do not discern between CNS conditions and, in fact, are not mutually exclusive, but part of a broader pan-injury program that is common to both acute injury and chronic neurodegenerative responses.

Meta-analysis of mouse astrocyte transcriptomic studies reveals distinct signatures of astrocyte reaction in acute CNS injuries versus chronic neurodegenerative diseases

Since the previously defined A1 and A2 signatures did not discriminate between injuries of very different nature, we next sought to describe new acute, chronic, and pan-injury astrocyte transcriptomic signatures. To this end, we conducted a meta-analysis of the DEGs, both upregulated (UP) and downregulated (DOWN), from the mouse acute and chronic datasets separately (Fig. 2a). We defined the pan-injury signature as genes with a statistically significant multiple-comparison-corrected

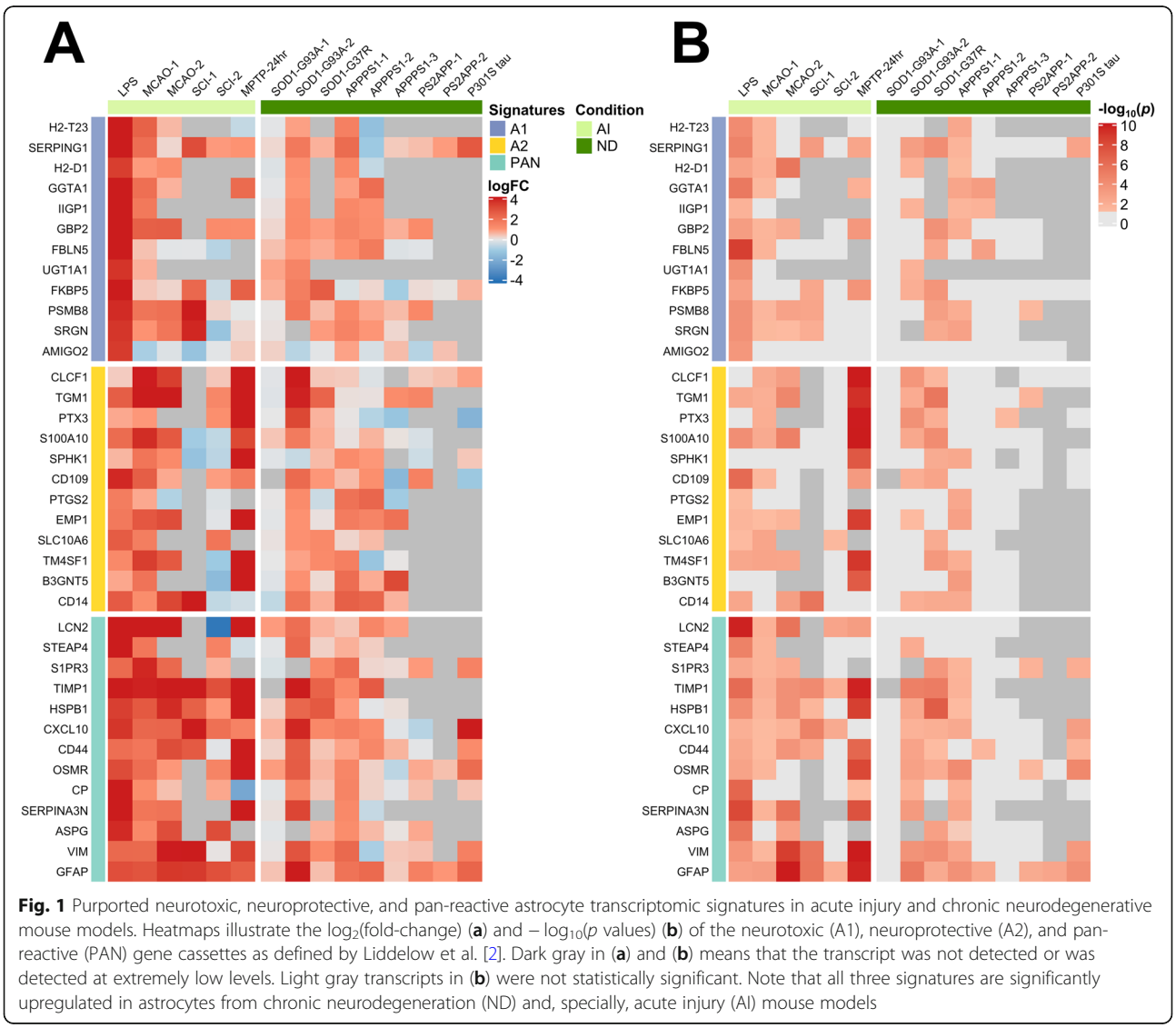

Fig. 1 Purported neurotoxic, neuroprotective, and pan-reactive astrocyte transcriptomic signatures in acute injury and chronic neurodegenerative mouse models. Heatmaps illustrate the $\log_2$(fold-change) (**a**) and $-\log_{10}(p$ values) (**b**) of the neurotoxic (A1), neuroprotective (A2), and pan-reactive (PAN) gene cassettes as defined by Liddelow et al. [2]. Dark gray in (**a**) and (**b**) means that the transcript was not detected or was detected at extremely low levels. Light gray transcripts in (**b**) were not statistically significant. Note that all three signatures are significantly upregulated in astrocytes from chronic neurodegeneration (ND) and, specially, acute injury (AI) mouse models

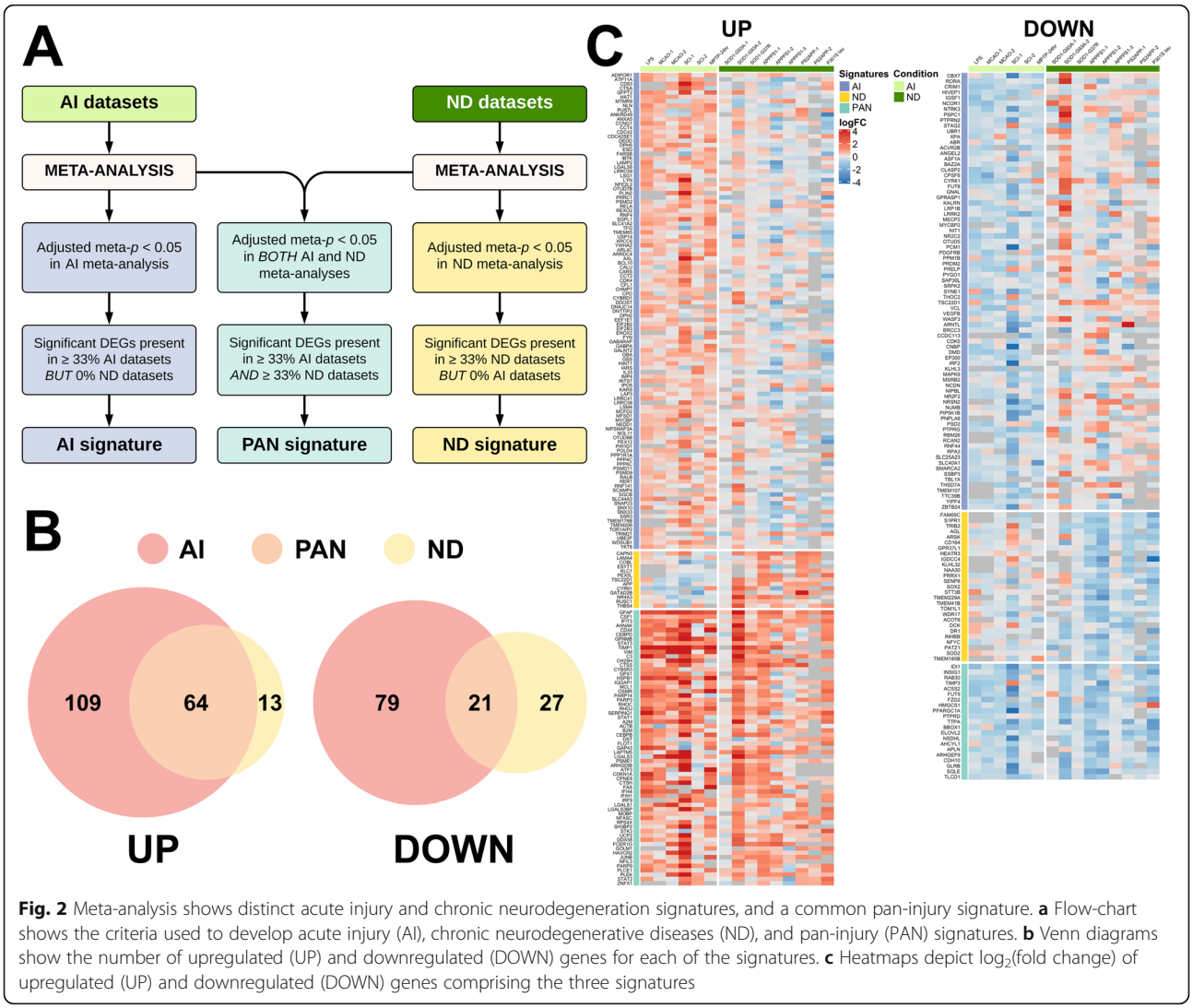

Fig. 2 Meta-analysis shows distinct acute injury and chronic neurodegeneration signatures, and a common pan-injury signature. **a** Flow-chart shows the criteria used to develop acute injury (AI), chronic neurodegenerative diseases (ND), and pan-injury (PAN) signatures. **b** Venn diagrams show the number of upregulated (UP) and downregulated (DOWN) genes for each of the signatures. **c** Heatmaps depict $\log_2$(fold change) of upregulated (UP) and downregulated (DOWN) genes comprising the three signatures

meta-analysis p value ($p < 0.05$) in both acute and chronic datasets and which, as an additional filter, also reached statistical significance in at least 33% of both acute and chronic datasets (see the meta p value of each signature gene and the number of acute and chronic datasets in which it was significant in Supplemental Table 1). The chronic neurodegeneration-specific signature was defined as DEGs that had an adjusted meta p value < 0.05 in the meta-analysis of chronic neurodegeneration datasets, were present in 33% or more of the neurodegeneration datasets, and absent in all acute injury datasets. Similarly, the acute injury-specific signature was defined by DEGs that had an adjusted meta p value < 0.05 in the meta-analysis of acute injury datasets, were present in 33% or more of all the acute injury datasets, and absent in all chronic neurodegeneration datasets.

Using these criteria, we obtained an acute injury-specific signature with 109 upregulated and 79 downregulated genes; a chronic neurodegeneration-specific signature with 13 upregulated and 27 downregulated genes, and a pan-injury signature with 64 upregulated genes and 21 downregulated genes. The Venn diagrams in Fig. 2b and the heatmaps in Fig. 2c and Supplemental Figure 1 depict the results of the meta-analysis for the UP and DOWN signatures, respectively. To characterize these signatures, we next interrogated curated databases such as Gene Ontology (GO biological processes, cellular components, and molecular functions) and Kyoto Encyclopedia of Genes and Genomes (KEGG) and generated heatmaps of relevant gene cassettes.

Pan-injury astrocyte transcriptomic signature

The pan-injury-UP signature corresponded to biological processes such as cytokine receptor and interferon signaling and to cellular components such as secretory vesicles, cell surface, and extracellular matrix. Specifically, this signature was represented by genes related to the cytoskeleton (*Actb,*

Dst, *Gfap*, *Vim*), extracellular matrix (*Cd44*, *Lgals1*, *Lgals3*, *Lgals3bp*, *Timp1*), and immune response (*C3*, *Csf1*, *Fas*, *Fcer1g*, *Havcr2*, *Ifi44*, *Ifih1*, *Ifit3*, *Irf9*, *Osmr*, *Serping1*, *Stat1*, *Stat2*, *Stat3*), whereas the pan-injury-DOWN signature was comprised of genes with very diverse functions, although a lipid metabolism gene cassette (*Acss2*, *Bbox1*, *Elovl2*, *Fzd2*, *Hmgcs1*, *Sqle*) could be identified (Fig. 3).

Acute injury-specific astrocyte transcriptomic signature

The acute injury-UP signature corresponded to biological processes related to protein synthesis and degradation (proteasome and autophagy), response to cytokine and extracellular stimulus, and innate immune system, and to cell components such as endoplasmic reticulum, Golgi apparatus, and secretory granules and vesicles. Specifically, this signature includes gene cassettes related to protein synthesis (*Calu*, *Cars*, *Dph2*, *Dph5*, *Eef1e1*, *Eif2b2*, *Eif2b3*, *Farsb*, *Iars*, *Kars*), ER-Golgi trafficking (*Ipo5*, *Lsg1*, *Nipsnap3a*, *Ralb*, *Rer1*, *Scamp4*, *Snap23*, *Snx10*, *Snx33*, *Ssr3*, *Ykt6*), the ubiquitin-proteasome system (*Cct2*, *Cct4*, *Dnajc14*, *Otud6b*, *Otud7b*, *Psmd2*, *Psmd9*, *Psmd11*, *Ube2f*, *Usp14*), autophagy (*Chmp7*, *Cpd*, *Ctsa*, *Cd63*, *Gba*, *Lamp2*, *Lap3*), immune response (*Bcl10*, *Fyn*, *Rela*, *Lgals8*, *Ppp6c*), and anti-oxidant defense (*Cybrd1*, *Enox2*, *Gss*, *Nfe2l2*, *Pex12*) (Fig. 4). By contrast, the acute injury-DOWN signature was related to transcription and chromatin remodeling processes, localized to chromatin and cell nucleus, and was mainly comprised of genes that regulate chromatin structure and transcriptional activity (*Arntl*, *Asf1a*, *Baz2a*, *Cbx7*, *Cnbp*, *Cpsf6*, *Ep300*, *Hivep1*, *Ncor1*, *Nipbl*, *Nr2c2*, *Nr2f2*, *Prdm2*, *Pspc1*, *Pygo1*, *Rnf44*, *Rora*, *Rpa3*, *Smarca2*, *Ssbp3*, *Stag2*, *Tsc22d1*, *Xpa*, and *Zbtb24*) (Fig. 5). Of note, many of these transcripts encode transcriptional repressors (i.e., *Baz2a*, *Cbx7*, *Cnbp*, *Ncor1*, *Zbtb24*), therefore their downregulation

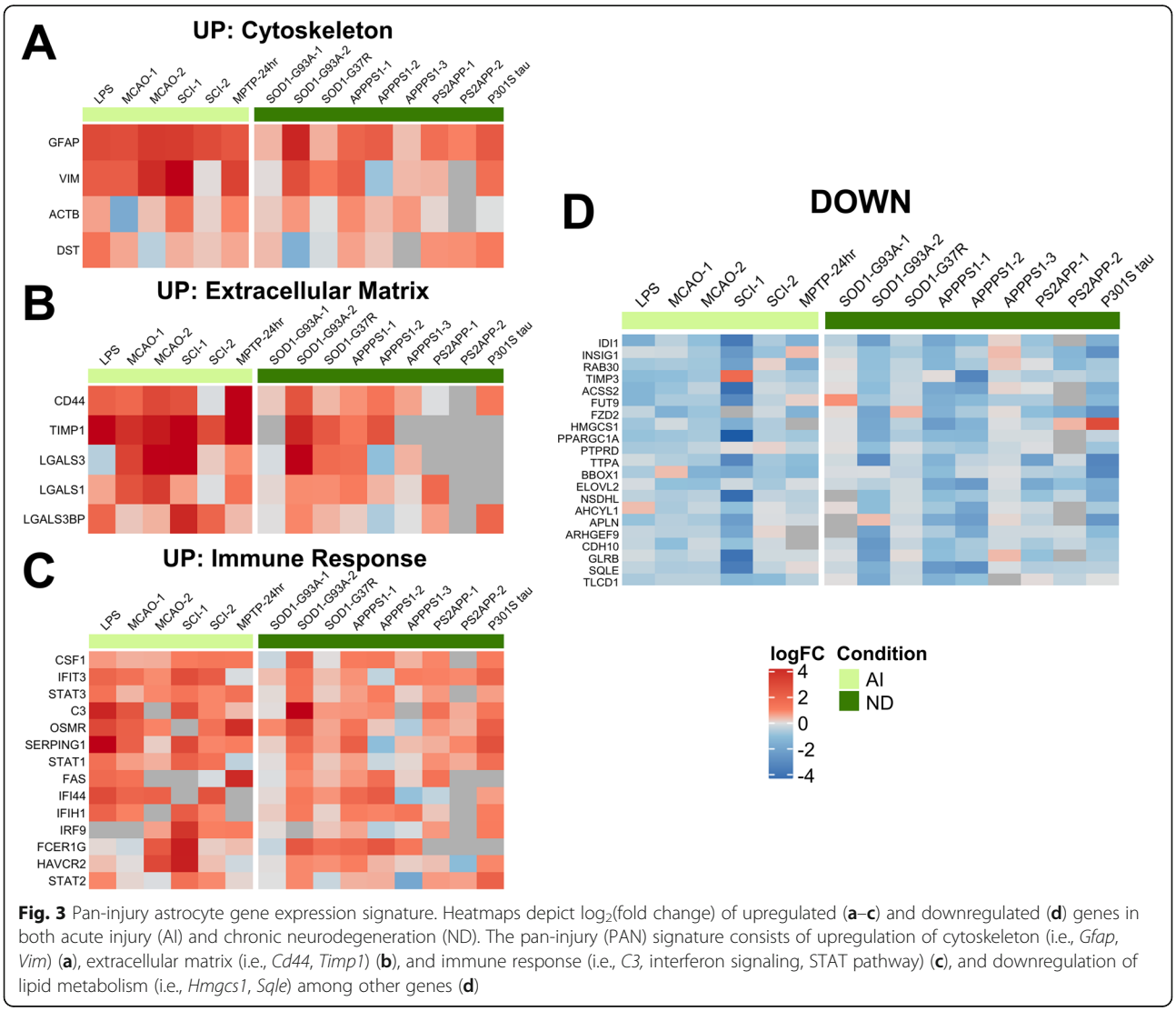

Fig. 3 Pan-injury astrocyte gene expression signature. Heatmaps depict $\log_2$(fold change) of upregulated (**a**–**c**) and downregulated (**d**) genes in both acute injury (AI) and chronic neurodegeneration (ND). The pan-injury (PAN) signature consists of upregulation of cytoskeleton (i.e., *Gfap*, *Vim*) (**a**), extracellular matrix (i.e., *Cd44*, *Timp1*) (**b**), and immune response (i.e., *C3*, interferon signaling, STAT pathway) (**c**), and downregulation of lipid metabolism (i.e., *Hmgcs1*, *Sqle*) among other genes (**d**)

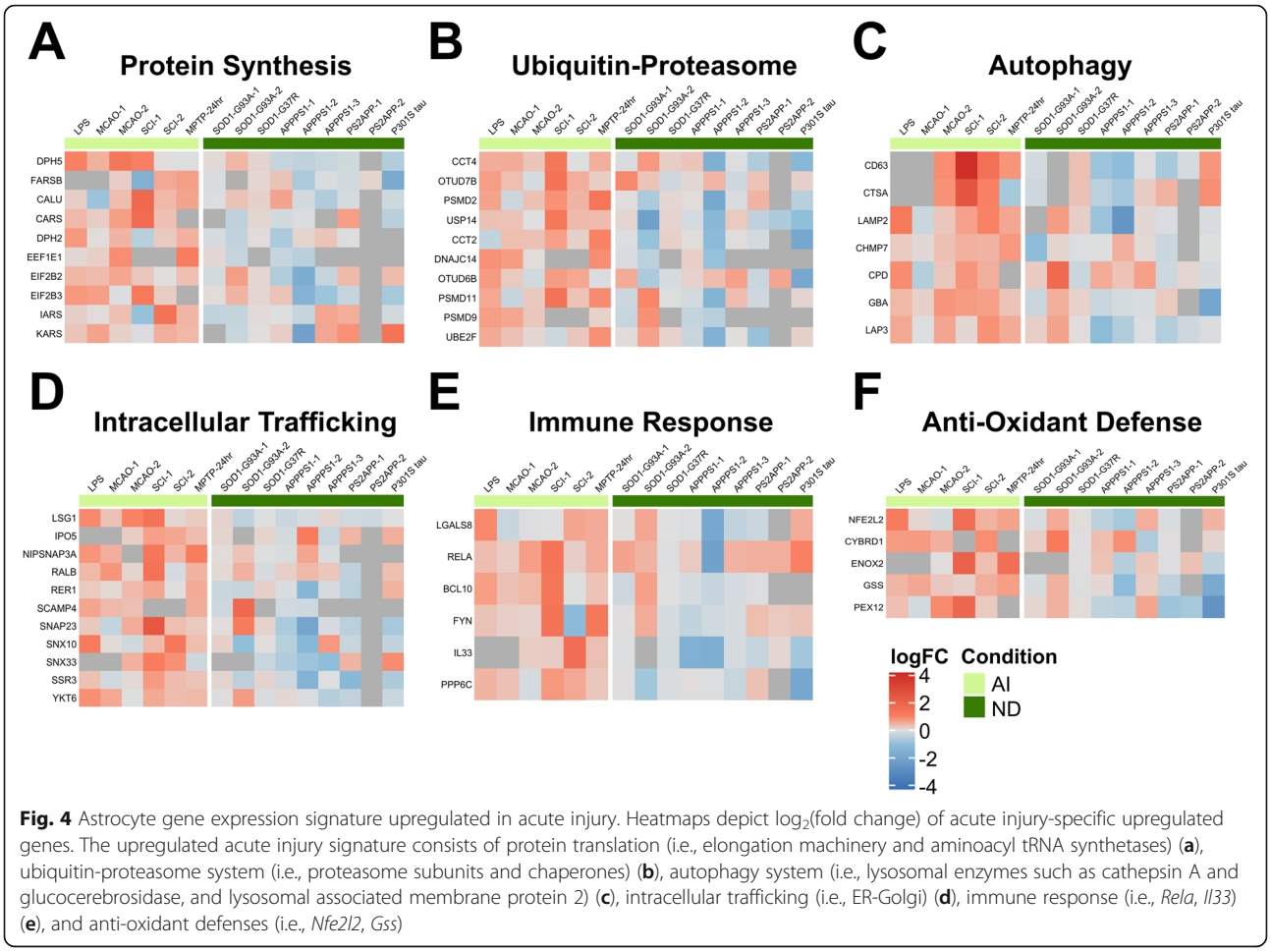

Fig. 4 Astrocyte gene expression signature upregulated in acute injury. Heatmaps depict $\log_2$(fold change) of acute injury-specific upregulated genes. The upregulated acute injury signature consists of protein translation (i.e., elongation machinery and aminoacyl tRNA synthetases) (**a**), ubiquitin-proteasome system (i.e., proteasome subunits and chaperones) (**b**), autophagy system (i.e., lysosomal enzymes such as cathepsin A and glucocerebrosidase, and lysosomal associated membrane protein 2) (**c**), intracellular trafficking (i.e., ER-Golgi) (**d**), immune response (i.e., *Rela*, *Il33*) (**e**), and anti-oxidant defenses (i.e., *Nfe2l2*, *Gss*)

would lead to an enhanced transcriptional activity in the astrocytes. Other downregulated gene cassettes of interest in this signature included immune response (*Igsf1*, *Irf2*, *Mapk9*, *Rcan2*) and trophic factors (*Acvr2b*, *Ntrk3*, *Pdgf3rb*, *Vegfb*).

Chronic neurodegeneration-specific astrocyte transcriptomic signature

The chronic neurodegeneration-specific signature was notably the smallest in number of genes, and these turned out to be functionally very heterogeneous. The chronic neurodegeneration-UP signature (n = 13) included three genes encoding extracellular matrix proteins that are known to be secreted by astrocytes (*Cyr61*, *Lama4*, and *Thbs4*). Other chronic neurodegeneration-UP genes were *App*, *Capn3*, *Cobl*, *Esyt1*, *Gatad2b*, *Klc1*, *Nr4a3*, *Pex5l*, *Rusc1*, and *Tsc22d1* (Fig. 6a). The chronic neurodegeneration-DOWN signature (n = 27) included a metabolic gene cassette (*Acot6*, *Agl*, *Arsk*, *Naa30*, *Stt3b*) and a transcription/chromatin remodeling gene cassette (*Dr1*, *Nfyc*, *Patz1*, *Prrx1*) together with relevant genes such as *Cd164*, *Heatr3*, *S1pr1*, and *Sod2* (Fig. 6b).

Astrocyte reaction involves upregulation of NFκB, MAPK, JAK-STAT, and CaN-NFAT signaling pathways, but not Wnt/β-Catenin and Sonic hedgehog proliferative pathways

We next investigated which molecular signaling pathways are involved in astrocyte reaction in each condition and whether astrocyte proliferation is part of astrocyte reaction. Specifically, we hypothesized that acute CNS injuries would trigger astrocyte proliferation to create a scar and limit neuronal damage, whereas this proliferative potential of astrocytes could be exhausted in chronic neurodegenerative diseases. A literature review revealed four main signaling pathways involved in astrocyte reaction: nuclear factor kappa B (NFκB) [40], calcineurin-nuclear factor activating of T-cells (CaN-NFAT) [41–44], Janus Kinases/Signal Transducer and Activator of Transcription (JAK/STAT) [21, 45–47], and mitogen-activated protein kinase (MAPK) [34, 48–51], and two main pathways involved in astrocyte proliferation: Wnt/β-catenin [52, 53] and Sonic hedgehog [5, 54].

To investigate whether these pathways are upregulated in reactive astrocytes in a context-dependent fashion, we performed GSEA [30] with the gene sets comprising these six pathways (see the "Methods" section) on the 6

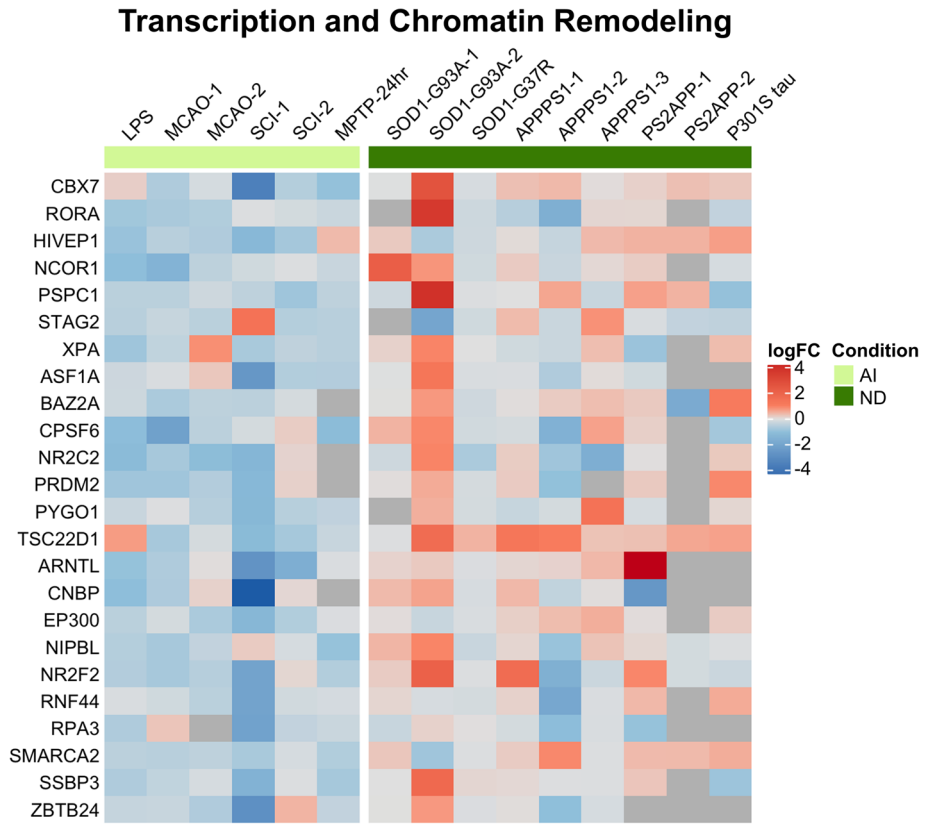

Fig. 5 Astrocyte gene expression signature downregulated in acute injury. Downregulated genes in acute injury were mainly related to chromatin remodeling and organization and transcription, including many transcriptional repressors

acute injury and 9 chronic neurodegeneration astrocyte transcriptomic datasets, followed by a meta-analysis of the resulting GSEA *p* values. We identified a total of 86 gene sets related to one of these six signaling pathways with the following distribution: Wnt/β-catenin $n = 28$, MAPK $n = 21$, NFκB $n = 19$, JAK/STAT $n = 9$, NFAT $n = 6$, and Sonic hedgehog $n = 3$. Figure 7 shows a heatmap with the NES resulting from the GSEA ranked by descending adjusted meta-analytic *p* value, whereas Supplemental Figure 2 heatmap depicts the $-\log_{10}(p \text{ values})$ from this GSEA. The NFκB, JAK/STAT, and MAPK signaling pathways encompassed the top gene sets and were upregulated in most datasets from both acute injury and chronic neurodegeneration categories. By contrast, the Wnt/β-catenin and Sonic hedgehog gene sets were predominantly downregulated and did not reach the meta-analytic statistical significance in most instances. Of note, the astrocyte pan-injury signature described above included *Stat1*, *Stat2*, and *Stat3*, whereas the acute injury-UP signature included *Rela*, which encodes for the effector subunit of NFκB p65, and the acute injury-DOWN signature included *Rcan2*, which is the main repressor of Ca-N/NFAT signaling, suggesting that the JAK/STAT signaling pathway is active in all astrocyte reactions, whereas NFκB and NFAT signaling pathways are primarily turned on in response to acute injuries.

Validation of mouse astrocyte signatures in human neurodegenerative diseases

Lastly, we sought to validate the acute injury, chronic neurodegeneration, and pan-injury mouse astrocyte signatures in human brain transcriptomic (microarray and RNA-seq) datasets from neurodegenerative disease-relevant CNS regions. These included two large AD datasets, the Religious Orders Study and Memory and Aging Project (ROSMAP, dorsolateral prefrontal cortex) [32] and the Mount Sinai Brain Bank (MSBB, parahippocampal gyrus) [33], two PD datasets (substantia nigra and striatum) [35], and one sporadic ALS dataset (spinal cord gray matter) [36]. In addition to these bulk tissue transcriptomic datasets, we analyzed two astrocyte-specific AD datasets: one in which GFAP+ astrocytes were laser capture microdissected from lateral temporal cortex frozen sections [34], and another in which superior frontal gyrus astrocytes were labeled with a GFAP antibody and sorted from other cell types in the suspension through fluorescence-activated cell sorting (FACS)

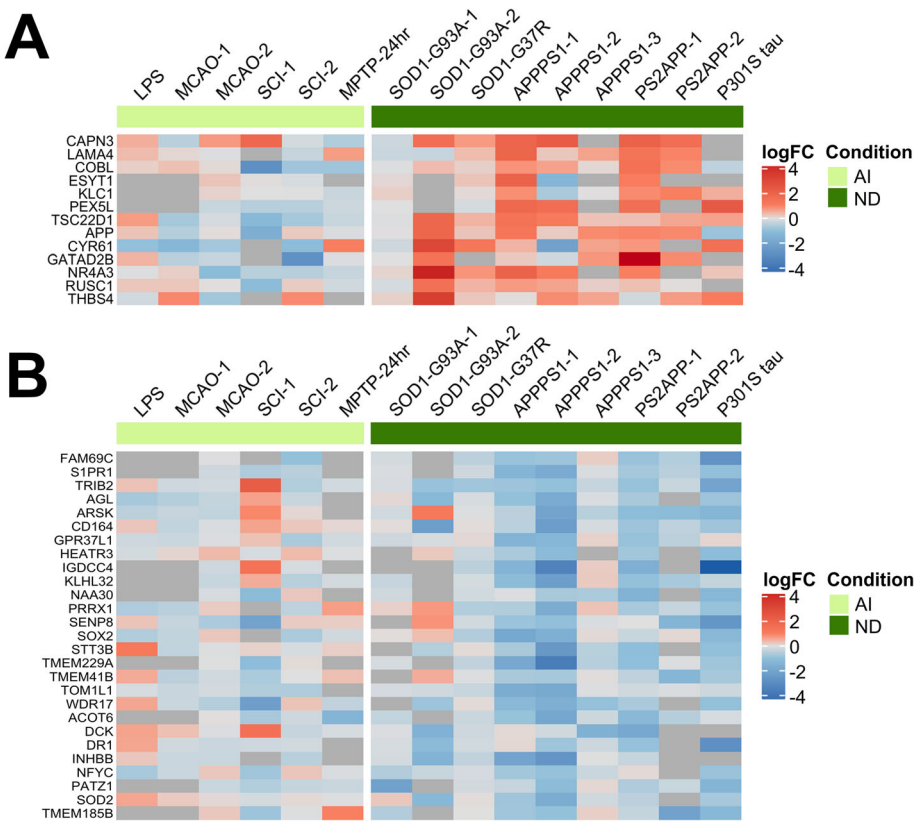

Fig. 6 Astrocyte gene expression signature in chronic neurodegeneration. The chronic neurodegeneration signature is characterized by upregulation (**a**) of amyloid precursor protein, calpain-3, and secreted matricellular proteins (i.e., *Cyr61*, *Lama4*, *Thbs4*), and downregulation (**b**) of metabolic processes (i.e., *Acot6*, *Agl*, *Naa30*)

(Friedman and Hansen, unpublished). Statistically significant DEGs ($p < 0.05$) between diseased and control individuals are illustrated with violin plots in Fig. 8, whereas violin plots with all the mouse signature genes are shown in Supplemental Figure 3. Inspection of these violin plots indicates that most genes from the pan-injury-UP signature are also upregulated in the two AD bulk tissue RNA-seq datasets from ROSMAP and MSBB. In contrast, the mouse chronic neurodegenerative-UP and all the DOWN signatures were not reflected in these large AD datasets. Similarly, none of the three mouse astrocyte signatures was clearly present in the two astrocyte-specific AD datasets, nor could they be validated in the two human PD and the ALS datasets.

Discussion

The main finding of this study is that, while sharing a common (pan-injury) astrocyte gene expression signature, mouse models of acute CNS injuries and neurodegenerative diseases are associated with very distinct astrocyte transcriptomic responses. On the one hand, astrocyte response to acute injury is remarkably characterized by an increased protein synthesis and degradation. Protein synthesis is unleashed by both upregulating the translational machinery of elongation factors (*Eef1e1*, *Eif2b2*, *Eif2b3*) and aminoacyl tRNA synthetases (*Cars*, *Iars*, *Kars*) and downregulating transcriptional repressors. Proteins involved in trafficking between the ER and the Golgi apparatus are also upregulated, likely reflecting the increasing demand for the folding, maturation, and intracellular transport of newly synthetized proteins. This massive protein synthesis is paralleled by an increased activity of protein degradation systems including the ubiquitin-proteasome system (i.e., upregulation of chaperones, ubiquitin ligases, and proteasome subunits) and autophagy (i.e., upregulation of lysosomal membrane proteins and enzymes), which probably serves the elimination of phagocytosed apoptotic and necrotic neurons from the injury area. In addition, the acute injury astrocyte signature consists of an immune response involving the upregulation of *Rela* (encoding for the NFκB effector subunit p65) and *Il33* (encoding for interleukin-33) and the downregulation of *Rcan2* (encoding for the regulator of calcineurin 2), among other genes. Of note, interleukin-33 is a nuclear alarmin expressed by astrocytes that induces microglial

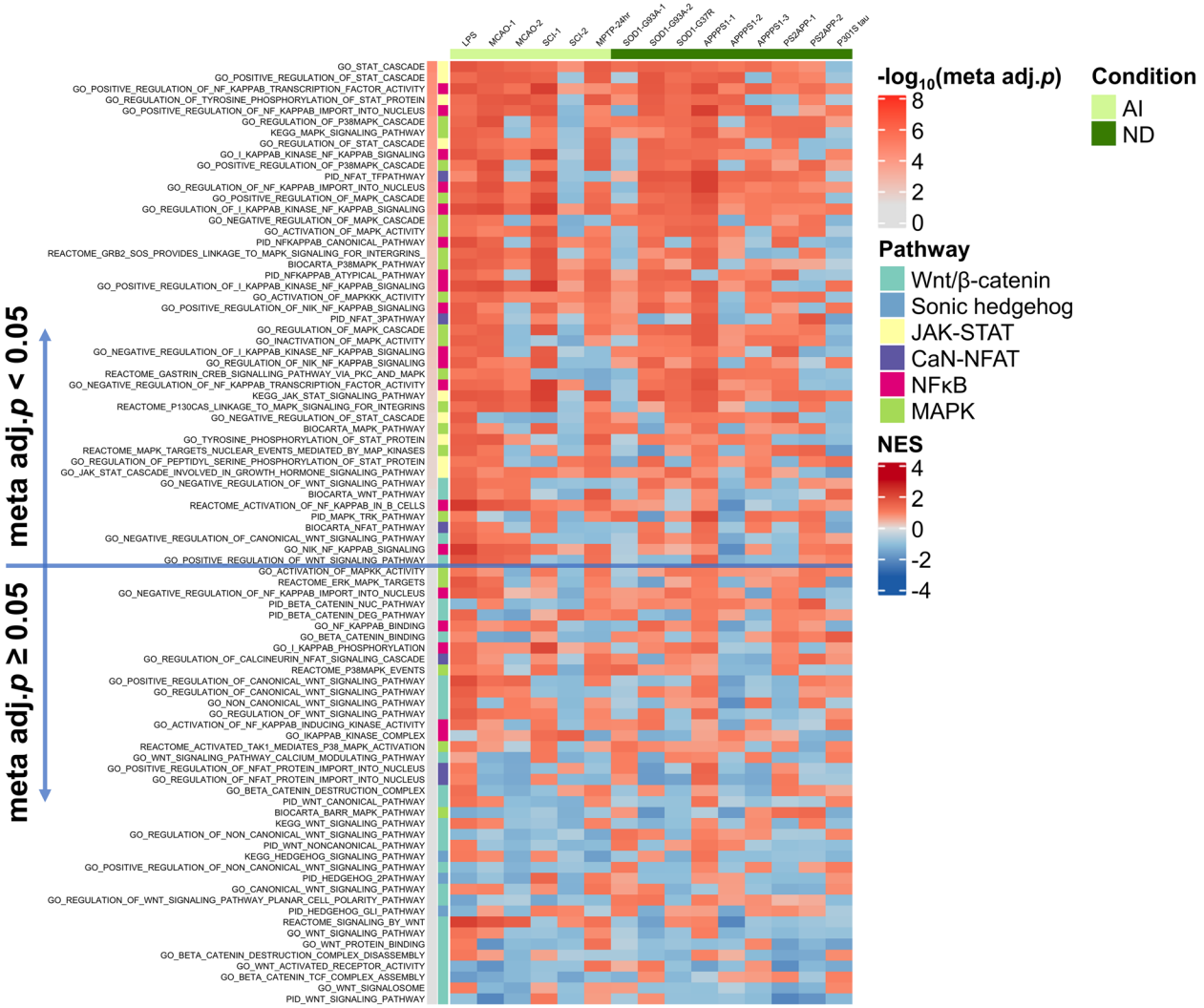

Fig. 7 Inflammatory but not proliferation signaling pathways are upregulated in acute injury and chronic neurodegeneration. Heatmap shows the normalized enrichment score (NES) of pro-inflammatory (NFκB, calcineurin/NFAT, MAPK, and JAK/STAT) and proliferative (Wnt/β-catenin and sonic hedgehog) gene sets in the astrocyte transcriptomic datasets analyzed. The gene sets are color-coded by pathway and ordered by descending meta-analytic *p* value [− log10(Meta adj. *p*)]. Note that NFκB, MAPK, and JAK/STAT gene sets are more significantly upregulated than Wnt/β-catenin and sonic hedgehog

engulfment of synapses during development [55, 56], and is neuroprotective in multiple models of acute CNS injury by shifting microglial phenotype towards a phagocytic one [57–62]. Lastly, the acute injury signature included an upregulation of anti-oxidant defenses exemplified by both *Nfe2l2* (encoding the nuclear factor erythroid-derived 2-like 2 or NRF2), which is a major regulator of cellular anti-oxidant defenses and confers neuroprotection in acute CNS injuries [63–66] and neurodegenerative disease models [67, 68], and *Gss,* an NRF2 target gene encoding for the anti-oxidant enzyme glutathione synthetase.

In sharp contrast, the chronic reaction signature seen in neurodegenerative disease mouse models was much smaller, with the downregulated genes exceeding the number of upregulated ones. This could be partially explained by the fact that the median number of DEGs contributing to the meta-analysis was smaller for the chronic neurodegeneration datasets compared to acute injury datasets (UP, 823 vs 1402; DOWN, 775 vs 1468). An alternative explanation could be that our meta-analysis combined datasets obtained from very different models, CNS regions, and age groups, with disparate methodologies of astrocyte isolation or astrocyte RNA purification, and different transcriptomic methods (microarray versus RNA-seq). There are regional differences in mouse astrocyte transcriptome, and aging has been associated with region-specific transcriptomic

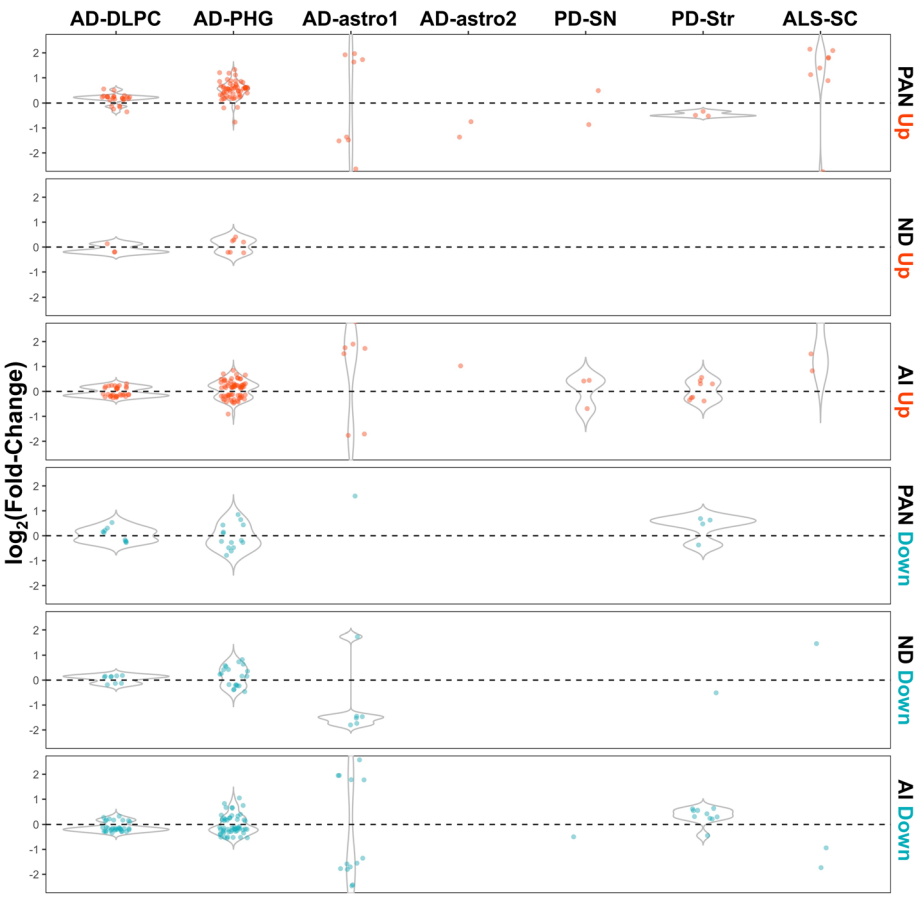

Fig. 8 Validation of mouse astrocyte transcriptomic signatures in human neurodegenerative transcriptomic datasets. Violin plots represent the logFC of statistically significant DEGs ($p < 0.05$) from the acute injury (AI), chronic neurodegenerative (ND), and pan-injury (PAN) mouse astrocyte signatures in diseased versus control human subjects. Note that only the two AD bulk tissue datasets (AD-DLPC and AD-PHG) showed some concordance with the pan-injury-UP mouse astrocyte signatures, but not with any of the others. Abbreviations: AD-DLPC, AD dorsolateral prefrontal cortex from ROSMAP; AD-PHG, AD parahippocampal gyrus from MSBB; AD-astro1, GFAP+ astrocytes laser capture microdissected from lateral temporal cortex; AD-astro2, GFAP+ astrocytes sorted from superior frontal cortex; ALS-SC, amyotrophic lateral sclerosis spinal cord gray matter; PD-SN, Parkinson's disease substantia nigra; PD-Str, Parkinson's disease striatum

changes in mouse astrocytes [69–72]. Moreover, technological heterogeneity could be another contributor: for example, RNA-seq has several advantages over microarrays such as improved sensitivity, wider dynamic range, and the ability to detect novel and non-coding transcripts. This heterogeneity could have limited our ability to identify a common signature across disease mouse models and may have had a greater impact in the meta-analysis of chronic neurodegeneration datasets than in the meta-analysis of acute injury datasets. However, inspection of Table 1 reveals a similar degree of heterogeneity across acute injury and chronic neurodegeneration mouse studies, which did not preclude obtaining a sizable acute injury signature after meta-analysis. Thus, the findings of fewer DEGs in the chronic neurodegeneration datasets and a smaller chronic neurodegeneration signature compared to the acute injury datasets and signature, respectively, support the idea that mouse reactive astrocytes mount a less vigorous response in chronic relative to acute injuries.

A closer inspection of the list of chronic neurodegeneration-UP genes revealed that three of these genes are secreted extracellular matrix proteins: *Cyr61* (CCN1 or cellular communication network factor 1), *Lama4* (laminin α4), and *Thbs4* (thrombospondin 4). Cyr61/CCN1 is a secreted matricellular protein that has been involved in cell-cell adhesion, angiogenesis, and arborization of dendrites of hippocampal neurons [73–76]. Immunohistochemical studies have revealed an upregulation of several laminins in reactive astrocytes in AD, Down's syndrome, and ALS [77–79]. Thrombospondins are astrocyte-secreted matricellular proteins involved in

synaptogenesis [80, 81]. Since Aβ peptide can lead to synapse loss by inhibiting the secretion of thrombospondin 1 (TSP-1) by astrocytes [82, 83], and the P301S tauopathy mouse model exhibits decreased levels of TSP-1 in the brain [84], this upregulation of *Thbs4* in neurodegenerative disease models could be compensatory to a decrease in TSP-1 levels. Three other transcripts upregulated in chronic neurodegeneration models encode for transcription factors: *Gatad2b*, *Nr4a3*, and *Tsc22d1*. Remarkably, GATA zinc finger domain containing 2B (GATAD2B) is a transcriptional repressor whose loss of function mutations have been associated with intellectual disability and synapse loss [85]. Nuclear receptor subfamily 4 group A member 3 has been involved in Lewy body disease and multiple system atrophy [86]. TSC22D1 is a pro-apoptotic tumor suppressor transcription factor induced by transforming growth factor β (TGFβ), that was found as putatively involved in AD pathophysiology in an unbiased network analysis of transcription factors and their targets [87] and was also present in our acute injury-DOWN signature. The presence of *App* (encoding the amyloid β precursor protein or AβPP) in the neurodegeneration-UP signature is intriguing and cannot just be explained by a leakage of the *APP* transgene expression to astrocytes in AβPP-overexpressing AD mouse models [88], because it was also notably upregulated in other mouse models such as SOD1-G93A ALS mice. Increased AβPP expression has been reported in reactive astrocytes under certain conditions, both in vitro [89] and in vivo [90–92].

Among the chronic neurodegeneration-DOWN genes, *Agl*, *S1pr1,* and *Sod2* stand out. α-Glycosidase (also called α-amylase), encoded by *Agl*, is the rate-limiting enzyme of glycogenolysis and its downregulation in neurodegenerative mouse models implies an impairment of the energy supply to neurons through the astrocyte-neuron lactate shuttle. However, while *Agl* mRNA levels were found to be reduced in the hippocampal formation of AD patients compared to non-demented controls, an increased AGL immunoreactivity has been reported in AD reactive astrocytes [93, 94]. The sphingosine phosphate receptors 1 and 3 (S1PR1 and S1PR3) are upregulated by astrocytes in MS lesions [95–97], and their modulation by the FDA-approved MS drug and sphingosine analog fingolimod has beneficial anti-inflammatory and neurotrophic effects not only in MS but also in AD mouse models [98–101]. Deficiency of the anti-oxidant enzyme superoxide dismutase 2 (SOD2) in astrocytes leads to astrocyte oxidative stress [102], and a recent single nuclei RNA-seq study has found a downregulation of *Sod2* in astrocytes from human AD brains [103]. On the other hand, post-mortem neuropathological studies have reported an enhanced SOD2 immunoreactivity in astrocytes in several neurodegenerative diseases such as ALS [104, 105] and FTLD-tau and TDP-43 [106], suggesting the existence of a mismatch either between mRNA and protein levels or between human disease and mouse models.

Besides these acute and chronic injury-specific signatures, our meta-analysis rendered a pan-injury signature with genes upregulated and downregulated in both types of CNS conditions. Our pan-injury-UP signature included 5 of the 13 genes proposed by Liddelow et al. as pan-reactive: *Cd44*, *Gfap*, *Osmr*, *Timp1*, and *Vim*. In addition, the putative A1 (neurotoxic) genes *C3* and *Serping1* were part of our pan-injury-UP signature [2]. Other pan-injury upregulated genes were *Lgals1*, *Lgals3,* and *Lgals3bp* encoding galectins 1 and 3 and galectin 3 binding protein, respectively. Astrocyte galectin-1 has been associated with worsening neurodegeneration of motor neurons in an ALS mouse model [107], but with neurotrophic and anti-inflammatory effects in models of MS [108] and brain ischemia [109, 110]. The pan-injury-DOWN signature included genes involved in lipid metabolism (*Acss2*, *Bbox1*, *Elovl2*, *Fzd2*, *Hmgcs1*, *Sqle*), and other relevant genes such as *Apln, Insig1*, *Ppargc1a,* and *Ttpa*. *Apln* encodes for apelin, a secreted peptide that has been shown to be neuroprotective in multiple acute injury and chronic neurodegenerative models [111–115]. *Insig1* encodes for insulin induced gene 1 and its downregulation suggests a globally reduced insulin signaling [116]. *Ppargc1a* encodes for the peroxisome proliferator activator receptor γ coactivator 1α, and it is noteworthy that PPARγ agonists have been proposed as anti-inflammatory drugs for many CNS conditions [117]. *Ttpa* encodes for α-tocopherol (vitamin E) transfer protein and, thus, has an anti-oxidant function; indeed, its deficiency leads to oxidative stress and enhanced Aβ deposition in transgenic AD mice [118, 119].

While BrDU incorporation experiments and immunohistochemistry for proliferative markers (i.e., Ki67) are better suited to examine astrocyte proliferation in mouse models of CNS injury, our GSEA of relevant gene sets suggests that pro-inflammatory signaling pathways (JAK/STAT, MAPK, NFκB, calcineurin/NFAT) are preponderant over proliferative signaling pathways (Wnt/β-catenin and Sonic hedgehog) in both acute and chronic conditions, supporting the idea that astrocyte reaction represents mainly a phenotypic change of existing astrocytes rather than proliferation of glial progenitors [8, 120].

Finally, our exploratory validation of these mouse astrocyte signatures in human neurodegenerative transcriptomic datasets revealed little concordance, arguing for caution when extrapolating the findings of astrocyte mouse transcriptomic studies to the human disease scenario. These mouse versus human discrepancies can be explained by a combination of reasons: (1) there are inherent genomic, transcriptomic, and lifespan/aging differences between mice and humans [121]; (2) mouse

models are unlikely to recapitulate the high degree of heterogeneity and complexity of human diseases; (3) most human transcriptomic datasets available were obtained from bulk tissue, which by definition dilutes the astrocyte transcripts among those from all the other cell types and reduces the sensitivity to detect astrocyte-specific changes; (4) the isolation of astrocytes in the two human astrocyte-specific AD datasets was based on their GFAP immunoreactivity, likely biasing the astrocyte population towards a reactive phenotype in both AD and control individuals, and thus limiting the ability to find differences between both groups; and (5) there is a notable overlap in transcriptome between astrocytes and other cell types, singularly microglia. While recent single nuclei RNA-seq in AD and control human brains have produced quantitatively limited astrocyte datasets [103, 122, 123], future astrocyte-enriched single nuclei RNA-seq studies from various human diseases will warrant a similar methodological approach and allow a more definite validation of the present findings from mouse models.

Conclusions

In summary, our meta-analysis of publicly available astrocyte transcriptomic datasets from multiple acute and chronic CNS injury mouse models highlights the heterogeneous nature of astrocyte reaction and provides important clues about its context dependence.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12974-020-01898-y.

Additional file 1: Figure S1. Meta-analysis shows distinct acute injury and chronic neurodegeneration signatures, and a common pan-injury signature. Heatmaps depict $-\log_{10}$(p value) of up-regulated (UP) and down-regulated (DOWN) genes comprising the acute injury (AI), chronic neurodegeneration (ND) and pan-injury (PAN) signatures.

Additional file 2: Figure S2. Inflammatory but not proliferation signaling pathways are up-regulated in acute injury and chronic neurodegeneration. Heatmap shows the $-\log_{10}$(p value) of pro-inflammatory (NFκB, calcineurin/NFAT, MAPK, and JAK/STAT) and proliferative (Wnt/β-catenin and sonic hedgehog) gene sets in the astrocyte transcriptomic datasets analyzed, corresponding to the GSEA in Figure 7. The gene sets are color-coded by pathway and ordered by descending meta-analytic *p* value [− log10(Meta adj. p)]. Note that NFκB, MAPK and JAK/STAT gene sets are statistically more significant than Wnt/β-catenin and sonic hedgehog.

Additional file 3: Figure S3. Validation of mouse astrocyte transcriptomic signatures in human neurodegenerative transcriptomic datasets. Violin plots represent the logFC of all genes (both statistically significant and non-significant) from the acute injury (AI), chronic neurodegenerative (ND) and pan-injury (PAN) mouse astrocyte signatures in diseased versus control human subjects. Abbreviations: AD-DLPC = AD dorsolateral prefrontal cortex from ROSMAP; AD-PHG = AD parahippocampal gyrus from MSBB; AD-astro1 = GFAP+ astrocytes laser capture microdissected from lateral temporal cortex; AD-astro2 = GFAP+ astrocytes sorted from superior frontal cortex; ALS-SC = amyotrophic lateral sclerosis spinal cord gray matter; PD-SN = Parkinson's disease *substantia nigra*; PD-Str = Parkinson's disease *striatum*.

Additional file 4: Table S1.

Abbreviations

Ab: Antibody; Aβ: Amyloid β peptide; AβPP: Amyloid-β precursor protein; AD: Alzheimer's disease; AI: Acute injury; Aldh1L1: Aldehyde dehydrogenase 1 L1; AGL: α-Glycosidase; ALS: Amyotrophic lateral sclerosis; CA1: Cornus ammonis 1; CaN-NFAT: Calcineurin-nuclear factor activating of T-cells; cc: Corpus callosum; CCN1: Cellular communication network factor 1; CNS: Central nervous system; CTRL: Control; ctx: Cortex; Cx43: Connexin-43; DEGs: Differentially expressed genes; DLPC: Dorsolateral prefrontal cortex; DOWN: Downregulated; eGFP: Enhanced green fluorescent protein; ER: Endoplasmic reticulum; FACS: Fluorescence-activated cell sorting; FDA: Food and Drug Administration; FDR: False discovery rate; FKPM: Fragments per kilobase of transcript per million mapped reads; FTLD: Frontotemporal lobar degeneration; GATAD2B: GATA zinc finger domain containing 2B; GFAP: Glial fibrillary acidic protein; Glt1: Glutamate transporter 1; GO: Gene Ontology; GSEA: Gene set enrichment analysis; i.p.: Intra-peritoneal; JAK/STAT: Janus Kinases/Signal Transducer and Activator of Transcription; KEGG: Kyoto Encyclopedia of Genes and Genomes; LCM: Laser capture microdissection; logFC: Logarithm of fold-change; LPS: Lipopolysaccharide; MAPK: Mitogen-activated protein kinase; MAPT: Microtubule-associated protein tau; MCAO: Middle cerebral artery occlusion; MPTP: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MS: Multiple sclerosis; MSBB: Mount Sinai Brain Bank; ND: Neurodegeneration; NES: Normalized enrichment score; NFκB: Nuclear factor kappa B; NFT: Neurofibrillary tangle; NRF2: Nuclear factor erythroid-derived 2-like 2; PAN: Pan-injury; PD: Parkinson's disease; PHG: Parahippocampal gyrus; PPARγ: Peroxisome proliferator activator receptor γ; PS1/2: Presenilin 1/2; RNA-seq: Ribonucleic acid sequencing; ROSMAP: Religious Orders Study and Memory and Aging Project; S1PR1/3: Sphingosine phosphate receptors 1/3; sac: Sacrifice; SALS: Sporadic ALS; SC: Spinal cord; SCI: Spinal cord injury; SN: Substantia nigra; SOD1/2: Superoxide dismutase 1/2; Str: Striatum; TBI: Traumatic brain injury; TDP-43: Tar DNA-binding protein 43 kDa; TGFβ: Transforming growth factor β; TRAP: Translating ribosome affinity purification; TSP-1: Thrombospondin 1; UP: Upregulated

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Authors' contributions

SD designed the analyses, analyzed some of the data, interpreted the results, and edited the manuscript. ZL wrote the R code and analyzed most of the data. AN analyzed some of the data. BTH provided intellectual input and edited the manuscript. AS-P conceived the idea, designed the analyses, interpreted the results, and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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