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TIGAR inclusion pathology is specific for Lewy body diseases.

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Abstract

Background: We previously reported up-regulation of tigarb (the zebrafish orthologue? of human TIGAR, TP53 –Induced Glycolysis and Apoptosis Regulator) in a zebrafish pink1$^{-/-}$ model of Parkinson's disease (PD). Genetic inactivation of tigarb led to the rescue of dopaminergic neurons and mitochondrial function in pink$^{-/-}$ zebrafish. The aim of this study was to determine the relevance of TIGAR for human PD, investigate its disease specificity and identify relevant upstream and downstream mechanisms.

Materials and Methods: TIGAR Immunohistochemistry using a range of antibodies was undertaken for detailed assessment of TIGAR in formalin-fixed, paraffin-embedded tissue from post mortem brains of PD patients and other neurodegenerative disorders (n=10 controls, 10 PD cases, 10 dementia with Lewy bodies, 5 motor neurone disease (MND), 3 multiple system atrophy (MSA) and complemented by immunohistochemistry for p53, hexokinase I (HK-I) and hexokinase II (HK-II; n=4 control, 4 PD, and 4 dementia with Lewy bodies).

Results: TIGAR was detected in Lewy bodies and Lewy neurites in the substantia nigra of sporadic PD and Dementia with Lewy bodies (DLB) patients. Staining of adjacent sections confirmed the presence of TIGAR alongside alpha-synuclein in these LB and Neurites. In contrast, TIGAR-positive aggregates were not seen in cortical Lewy bodies. TIGAR protein was also absent in both TDP-43-positive inclusions in MND and glial cytoplasmic inclusions in MSA. Subsequent investigation of the TIGAR-upstream regulator p53 and the downstream targets HK-I and HK-II in PD brains suggested a possible mild increase in HK-I.

Conclusions: TIGAR protein, is present in SN Lewy bodies of both sporadic PD and DLB. The absence of TIGAR protein in the pathological inclusions of MND or MSA

suggests disease specificity and further raises the possibility that TIGAR may be involved in PD pathogenesis.

1. Introduction

Parkinson's disease (PD) is a common, progressive neurodegenerative disorder. A key pathological hallmark of the disease is loss of dopaminergic neurons in the substantia nigra, resulting in the clinical triad of tremor, akinesia and rigidity. A further diagnostic pathological feature of PD is the Lewy body, primarily located in the substantia nigra, but also found in other brainstem and forebrain locations. Classical substantia nigra Lewy bodies are usually round with a hyaline, eosinophilic core with a paler surrounding halo on haematoxylin and eosin staining. Lewy bodies in the forebrain are paler and lack a halo (Spillantini et al., 1997). Lewy bodies are composed mainly of neurofilaments and ubiquitylated proteins, of which alpha-synuclein has been recognised as the main component (Licker et al., 2009; Spillantini et al., 1997). Immunohistochemistry for alpha-synuclein also reveals neuritic deposits called Lewy neurites (LN). Dementia with Lewy bodies (DLB) has considerable clinical and pathological overlap with Parkinson's disease: It is characterised by Lewy bodies and Lewy neurites both in the midbrain and brainstem as well as the forebrain, with many patients having Parkinsonian symptoms in addition to dementia (Irwin et al., 2017).

Lewy bodies are immunoreactive for alpha-synuclein. However, to date, there are approximately 300 further molecules that have been identified in Lewy bodies (Wakabayashi et al., 2013). Many of these are protein products of genes found to cause PD when mutated, including DJ-1, LRRK2, Parkin and PINK1. In addition, other protein constituents of Lewy bodies are known to have roles in mitochondrial function, the ubiquitin-proteasome system, aggresome formation and autophagy, suggesting a role of these proteins in PD pathogenesis (Velayati et al., 2010; Wakabayashi et al., 2013).

TIGAR (TP53-Induced Glycolysis and Apoptosis Regulator; also known as C12orf5) was identified by Bensaad, et al. 2006. The initial function attributed to this protein is to decrease levels of Fructose-2,6-Biphosphate (Fru-2,P_2), the substrate of Phosphofructokinase-1 (PFK-1), the key enzyme in the glycolytic pathway. By lowering the Fru-2,P_2 levels, TIGAR inhibits the glycolytic pathway and promotes the Pentose Phosphate Pathway (PPP). This, in turn, reduces reactive oxygen species (ROS) by increasing the production of NADPH and reduced glutathione (Bensaad et al., 2006).

TIGAR has also been implicated in apoptosis and autophagy. It has been suggested that TIGAR can promote apoptosis by inhibiting the glycolytic pathway (Bensaad et al., 2006) as well as reacting to raised reactive oxygen species . TIGAR can also modulate reactive oxygen species (Bensaad et al., 2009; Ye et al., 2013): Increased levels of ROS induce p53, which in turn activates transcription of TIGAR, consequently reducing autophagy within the cytoplasm (Cheung et al., 2012).

We previously established a pink1 mutant (pink1$^{-/-}$) zebrafish line with a Stop mutation in the Pink1 kinase domain and abolished kinase activity. This pink$^{-/-}$ zebrafish line was the first vertebrate model of PINK1 deficiency to resemble key features of human PD, namely loss of dopaminergic neurons and marked impairment of mitochondrial function and morphology. Genome-wide, unbiased gene expression analysis identified marked upregulation of tigarb (the zebrafish homologue of TIGAR) mRNA in pink$^{-/-}$ compared to controls. Of note, TigarB knock down normalised mitochondrial function and rescued the dopaminergic neurons (Flinn et al., 2013). This suggested a role for TIGAR in PD pathogenesis. Therefore, we aimed to determine if TIGAR is present in the human substantia nigra, and specific for in PD and DLB-associated pathology.

We also sought to determine whether TIGAR upregulation in human neurodegenerative disorders is due to upregulation of p53 (see above) and whether it may exert its effect in PD via Hexokinase up regulation. (Lee et al., 2015). The Hexokinase family are enzymes that are responsible for the conversion of glucose to glucose-6-phosphate (G-6P). This is a crucial step in the glycolytic pathway and the pentose phosphate pathway. HK-II (hexokinase II) is prevalent in tissues with high energy demand, such as skeletal muscle, heart and adipocytes (John et al., 2011). It also has an important role in regulating cellular survival and autophagy under glucose deprivation conditions or type of cellular damage (Tan and Miyamoto, 2015). Overexpression of mitochondrial HK-II may confer neuronal protection and survival in animal and cellular models of PD and neurodegeneration (Corona et al., 2010; Gimenez-Cassina et al., 2009). Under hypoxic conditions, mitochondrial HK-II is important for TIGAR mitochondrial translocation, where both proteins form a complex. This results in HK-II stabilization and enhanced activity and interaction with TIGAR upon presence of glucose and active HIF-1α. HK-I is present in all cellular types, but especially in the cerebral tissue and blood cells. It has been suggested that HK-I specifically shunts the glycolytic pathway (John et al., 2011; Magrì et al., 2016).

2. Results

2.1 TIGAR in the substantia nigra

Immunohistochemistry for TIGAR was performed in the substantia nigra using antibodies that recognised either the central region (ab62533 and ab10545) or the C-terminal (ab129333, LS-C286858 and PA5-29152) of the protein.

Antibodies that recognise the C-terminus of TIGAR showed neuronal labelling in the cell body and nucleus. Neurons from both controls and Parkinson's disease patients showed a variable degree of cytoplasmic labelling ranging from low to high levels of expression. This variability was seen both within cases and between cases. TIGAR-positive neurites were also seen in the controls as well as PD and DLB cases. Notably, Lewy bodies were positive for TIGAR using all three antisera directed against the C terminus (figure 1 and supplementary figure 1). The presence of TIGAR in Lewy bodies was initially observed in all PD and DLB brains from the Sheffield Brain Tissue Bank (SBTB), and subsequently confirmed in PD and DLB brains from the Queen Square Brain Bank (QSBB). These anti C-terminus antibodies seemed to label a region of the Lewy body that is deep to the 'halo' but does not incorporate the most central region of the structure. The C-terminus antibodies had largely similar staining patterns.

In the substantia nigra, data for the SBTB cohort suggested that there may be a greater proportion of TIGAR-positive neurones in the PD cases compared to controls and more TIGAR-positive neurites in the PD (mean=17.5%, standard deviation=5.8, n=4) and DLB (mean=15.9%, standard deviation=15.1, n=4) cases compared to controls (mean=5.2%, standard deviation=3.06, n=4; $P \leqslant 0.01$). However, these

findings were not replicated in analysis of a larger cohort of cases (controls, PD and DLB n=6 each) from QSBB cases for either the proportion of TIGAR-positive neurones or the number of TIGAR-positive neurites (P≥0.125). The initial finding for the SBTB cases thus most probably represents a spurious result and highlights the need to analyse case series of sufficient size prior to drawing strong conclusions.

Antibodies that only label the central region of TIGAR showed some diffuse and non-specific cytoplasmic neuronal labelling, as well as some cross-reactivity to neuromelanin (supplementary figure 1). There was no difference in the staining pattern between PD cases and controls with no labelling of Lewy bodies. These antibodies showed fairly diffuse, non-specific staining of neurones, neuropil with a strong cross reaction to neuromelanin. There was slightly less background staining for the Abcam antibody, otherwise the two antibodies gave similar staining patterns.

2.2 TIGAR Colocalises with alpha-synuclein

Double-labelling immunohistochemistry for both TIGAR and alpha-synuclein was performed with a combination of TIGAR (visualised by fluorescence or DAB) and alpha-synuclein (visualised in fluorescence) proved to be rather unreliable such that good, interpretable images could only be obtained for 1PD patient for DAB/fluorescence and one PD and one DLB patient with double fluorescence. In these cases, colocalisation of TIGAR and alpha-synuclein was demonstrated (supplementary figure 2).

Therefore, to provide further confirmation that the anti TIGAR C-terminus antibodies were truly labelling Lewy Bodies, adjacent sections of the substantia nigra of four cases of PD were stained for alpha-synuclein and TIGAR. These slides were digitised using a Hamamatsu XR scanner, such that adjacent structures could be manually aligned and the same cells and Lewy bodies could be viewed following both

alpha-synuclein and TIGAR immunohistochemistry. This confirmed that alpha-synuclein positive Lewy bodies were also positive for TIGAR (figure 2).

2.3 p53, HK-I and HK-II in SN

To identify TIGAR-related upstream and downstream mechanisms, we undertook immunohistochemistry for p53 (the most crucial upstream regulator of TIGAR), as well as for Hexokinase I (HK-I) and II (HK-II), both plausible downstream targets of TIGAR. However, there was no detectable p53 expression in PD, DLB or control brains, suggesting that TIGAR activation/aggregation may be triggered by other mechanisms.

Specific immunoreactivity was detected for HK-I, which is well known to be abundant in the brain (Aleshin et al., 1998). HK-I was analysed in the SN of four controls and four PD brains using ImageJ. The immunoreactivity of each image positive for HK-I was somewhat higher for PD cases (mean=67.85%, SD=1.03) than controls and reached marginal significance (mean=61.99%, SD=1.73; P=0.0259, t=2.942, df=6). Only non-specific immunolabelling with HK-II was observed. No intergroup differences or Lewy body labelling was evident (Supplementary figure 3).

2.4 TIGAR-positive Lewy bodies are not detected in the forebrain

TIGAR-positive Lewy bodies were not observed in the hippocampus and mesial temporal lobe, although Lewy pathology had been confirmed in this locus by alpha-synuclein immunohistochemistry. We conclude that hippocampal and cortical Lewy bodies do not contain TIGAR protein.

TIGAR protein again had variable neuronal labelling intensity in the hippocampus in the controls and disease cases with some neurones having strong

immunoreactivity, while others had much lower levels of immunoreactivity. TIGAR-positive neurites were also evident in all groups (figure 3).

2.5 TIGAR expression does not associate with other pathological cellular inclusions

Immunohistochemistry for the C-terminus of TIGAR was negative in the glial cytoplasmic inclusion bodies in the substantia nigra of three cases of multiple system atrophy and in the neuronal cytoplasmic TDP-43-positive inclusions in the ventral horns of 5 cases of amyotrophic lateral sclerosis/MND. We therefore conclude that the incorporation of TIGAR into pathological inclusions is specific for brain stem Lewy bodies in PD and LBD and not a generic feature of all neuropathological proteinaceous inclusions.

3. Discussion

This study assessed TIGAR expression in the post mortem human brain of patients with Parkinson's disease (PD), patients with dementia with Lewy bodies (DLB) as well as in multiple system atrophy (MSA) and amyotrophic lateral sclerosis/motor neurone disease (MND). TIGAR was present in neuronal cell bodies and some neurites in the control and disease cases. Interestingly, three different antibodies directed against the C terminus of the protein specifically labelled brainstem Lewy bodies only. These antibodies did not label cortical Lewy bodies, or the alpha-synuclein-positive glial cytoplasmic inclusions characteristic of MSA. Lewy body labelling was not seen with antibodies against the central portion of TIGAR protein. This may be due to TIGAR cleavage prior to incorporation into Lewy bodies or due to some other posttranslational modification to the central portion of TIGAR, rendering it unavailable for antibody binding.

The TIGAR labelling of substantia nigra Lewy bodies appeared to be deep to the 'halo' region, around but not in the very core of the Lewy body. Assuming that these structures are probably assembled from inside out, it is likely that TIGAR incorporation is a late event in Lewy body formation, as appears to be the case for other proteins (Beyer et al., 2009).

It has been suggested that Lewy body formation could be the result of a cellular self-defence mechanism that disposes of and restricts cytotoxic proteins (Fecchio et al., 2013). It is thus possible that the inclusion of TIGAR in Lewy bodies could be the result of the same cellular mechanism. This suggestion is supported by the finding that TIGAR knock down rescued dopaminergic cells in our zebrafish model of PD (Flinn et al., 2013). However, in other model systems and studies, TIGAR provides

antioxidant defence against ROS, cellular stress and hypoxia via its role in the pentose phosphate pathway (PPP), as well as providing the nucleotides for DNA synthesis (Bensaad et al., 2006) (Bolaños and Heales, 2010).

Previous studies have assessed TIGAR mRNA (by microarray) and protein levels in tissue homogenates of the superior temporal gyrus in post-mortem tissue from the brains of Alzheimer patients and controls (Katsel et al., 2013). Microarray data and protein levels (assessed by Western blotting) showed reduced levels of TIGAR in the AD patient group compared to controls. This study of tissue homogenates is difficult to interpret due to potential intergroup differences in the cellular populations present: It is possible that as the majority of TIGAR protein is neuronal, AD-related neuronal loss and reactive gliosis may explain the apparent loss of TIGAR. Alternatively, the neuronal loss may be mild, but there may be loss of TIGAR expression within neurones in AD.

Within individual brains, we found variable expression of TIGAR expression in neurones such that some neurones were intensely positive, while others showed only background levels of expression. Data from the SBTB cohort of brains suggested a greater proportion of strongly TIGAR-positive neurones in both PD and DLB compared to the controls. However, this was not replicated in QSBB tissue, highlighting the importance of replication studies and larger sample sizes. More accurate methods that would allow TIGAR quantification in specific cell types are required to fully assess TIGAR levels and compare them between patients' brains and controls.

The lack of TIGAR staining seen for both the alpha-synuclein-positive glial cytoplasmic inclusions typical for MSA and the TDP-43-positive neuronal cytoplasmic inclusions observed in MND suggest disease specificity with TIGAR incorporation into pathological inclusions being a specific feature of the neuronal alpha-

synucleinopathies and not pathological proteinaceous inclusions in general. This is in contrast to p62, ubiquitin and many other proteins that are present in the inclusions of a wide range of different neurodegenerative disorders. Furthermore, TIGAR incorporation into Lewy bodies was only seen in brainstem but not cortical Lewy bodies. This phenomenon has also been seen with respect to isopentenyl diphosphate isomerase (Nakamura et al., 2015),. This is unusual, as most Lewy body protein components are present in both brainstem and cortical Lewy bodies (e.g, alpha-synuclein (Baba et al., 1998; Irizarry et al., 1998; Spillantini et al., 1997) , MAP-5 (Gai et al., 1996), HDAC6 (Yasuo et al., 2011), Parkin (Schlossmacher et al., 2002), cyclin dependent kinase 5 (Takahashi et al., 2000), synphilin-1 (Wakabayashi et al., 2002). This, together with the morphological differences between brainstem and cortical Lewy bodies suggests qualitative differences in molecular composition between the two Lewy body types.

We found a near complete absence of demonstrable p53 expression in the midbrain in all groups. This suggests that neither the variable TIGAR expression seen in neuronal cells in control or PD/DLB brains nor its presence in brainstem Lewy bodies is likely to be due to regulation by p53-dependent pathways.

Investigation of the TIGAR downstream targets HK-I and HK-II only revealed a rather modest increase in HK-I expression in the substantia nigra in PD compared to controls that just reached statistical significance ($p=0.02$) and should be interpreted with caution.

4. Conclusion

TIGAR is present in Lewy bodies in the substantia nigra of the alpha-synucleinopathies PD and LBD and co-localises with alpha-synuclein. This suggests

a potential role of TIGAR in the formation of Lewy bodies and possibly also in the pathogenesis of Lewy body disorders more generally. The TIGAR-positive pathological inclusions were only found in the SN in PD and DLB. TIGAR was absent in both TDP-43-positive inclusions in MND, and GCIs in MSA. This suggests TIGAR disease-specificity for Lewy body diseases. Further studies are required to elucidate the role of TIGAR in the pathogenesis of PD and to investigate if there is any relationship to hexokinase expression.

5. Experimental Procedure

5.1 Samples

Post mortem human tissues were obtained from the Sheffield Brain Tissue Bank (SBTB) and the Queen Square Brain Bank for Neurological Disorders (QSBB) with ethical approval provided by the committees of the two brain banks, as sanctioned by the Scotland A Research Ethics Committee (ref 08/MRE00/103; SBTB) and Tissue Bank and NeuroResource REC (Ref. 08/H0718/54+5; QSBB). The SBTB provided tissue from: the midbrain and hippocampus in four cases of PD, four DLB and four matched controls; midbrain from three MSA cases; spinal cord from five MND/amyotrophic lateral sclerosis patients and four controls. The QSBB provided midbrain sections were from six PD, six DLB and six matched controls (Table 2 and 3). PD and DLB were defined on both clinical and pathological criteria.

5.2 Immunohistochemistry

Immunohistochemistry using a standard avidin-biotin complex (ABC) method was performed on 4μm-thick, formalin-fixed, paraffin-embedded sections. Details of the antisera as well as antigen retrieval and immunohistochemistry conditions are

detailed in supplementary table S1. TIGAR Immunostaining was performed with the IntelliPATH FLX Detection Kit and autostainer system (Menarini Diagnostics). For p53, slides were stained on the Dako Omnis Automated Slide Stainer using the Dako Envision Flex High pH (GV80011) kit. The whole slide images were captured using a Hamamatsu NanoZoomer XR slide scanner.

Double labelling immunohistochemistry was attempted to investigate co localisation of TIGAR with alpha-synuclein. Two formats were attempted: 1) anti alpha-synuclein antibody with Alexa Fluor 488 or 555 and TIGAR labelled with DAB or 2) both alpha-synuclein and TIGAR in fluorescence. Both formats involved incubation with Sudan Black to mask auto fluorescent material.

To further confirm immunostaining of TIGAR in Lewy bodies, adjacent sections were immunostained for TIGAR using the PA5-29152 (Thermo Scientific) antibody and alpha-synuclein and digitised. The resultant whole slide images aligned such that the same structures could be assessed in the adjacent sections.

5.3 Quantitative histopathology evaluation

To assess the number of TIGAR-positive and -negative neurones in the substantia nigra in PD, DLB and control cases, the digitised whole slide images were scrutinised on the Hamamatsu NDP view platform by two experienced neuropathologists, one who was blind to diagnosis (JES) and one who was not (JRH). The total number of neurones that were positive and negative for TIGAR were counted and the percentage of neurones that were positive calculated. There was a 90% inter-rater agreement between the two observers. The scores for the two raters were compared to establish inter-rater reliability. Only data obtained from the blinded pathologist (JS) was used for intergroup comparisons. To quantify neuritic pathology

in the substantia nigra, entorhinal cortex and cortex of the occipitotemporal gyrus, six regions of interest per case were randomly captured from the whole slide images at 20x magnification (405 x 721µm). Intensely-stained neurites in the substantia nigra were counted by two blinded researchers (KLRL and LT), and the mean count per case calculated. Inter-rater reliability 99.8% showed agreement between the observers. The same group of controls and patients was used to assess the total number of TIGAR positive neurites for the controls.

HK-I was quantified using image analysis. A total of 6 fields per case were captured from the SN at 40x objective and were analysed with Image J (Schindelin et al., 2012). Each field was divided into 100 equal squares and subjected to colour deconvolution. The Shanbhag threshold (Shanbhag, 1994) was applied to the DAB-only images. Then, the stained area fraction was measured in the fields thresholded. The percentage of each image that was positive for DAB staining was assessed. The mean of the 100 values across the 6 fields was calculated for each case and used in the statistical analysis for each case.

5.4 Statistical analysis

Statistical analysis used for TIGAR included t-Test, Kolmogorov-Smirnov test, Mann U-Withney and Levene's test on Microsoft Excel and SPSS (IBM).

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Figures

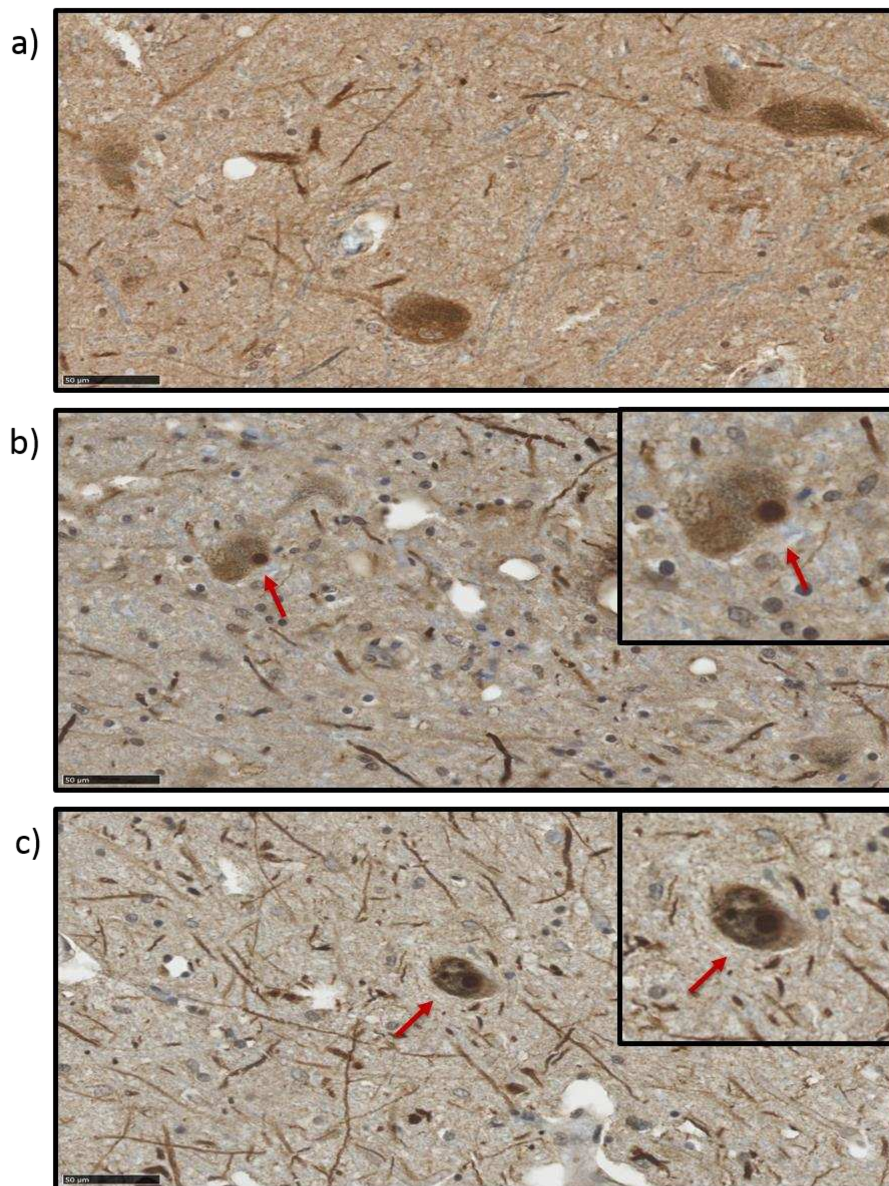


Figure 1: Immunohistochemical staining of the substantia nigra. Immunostaining for TIGAR (DAB – brown; ThermoScientific PA5-29152 antibody) counterstained with haematoxylin (a-c). Representative picture of a control (a), PD (b) and DLB (c) cases. This reveals strong labelling of the neuronal soma in a subset of neurones. Also stained were some neurites and Lewy bodies (red arrows) and neurites. Bar=50μm.

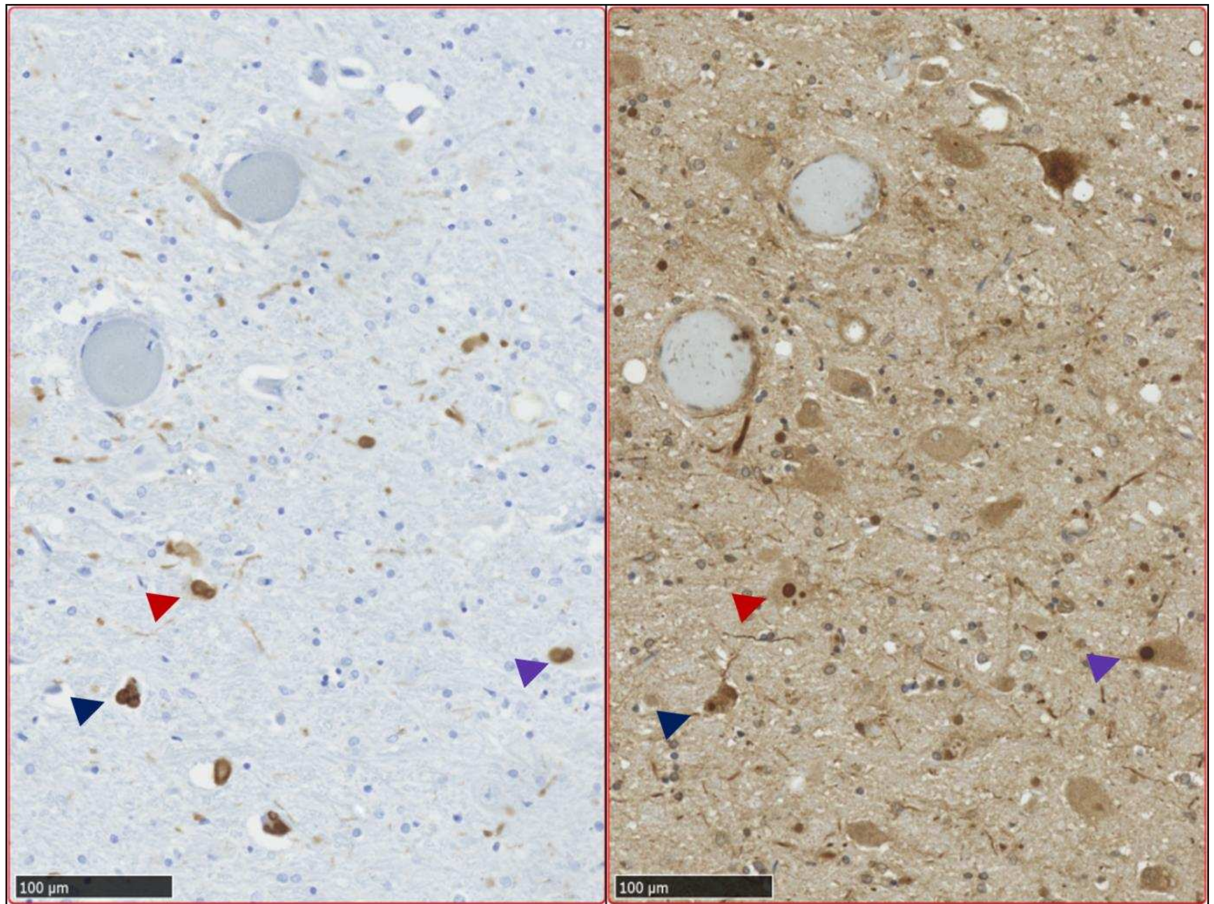


Figure 2. Aligned adjacent sections in the substantia nigra. Representative images of immunohistochemistry for alpha-synuclein (left panel) and TIGAR (right panel; ThermoScientific PA5-29152 antibody) in the SN of a PD case, demonstrating the presence of TIGAR in Lewy bodies (red, blue and purple arrows; bar=100μm).

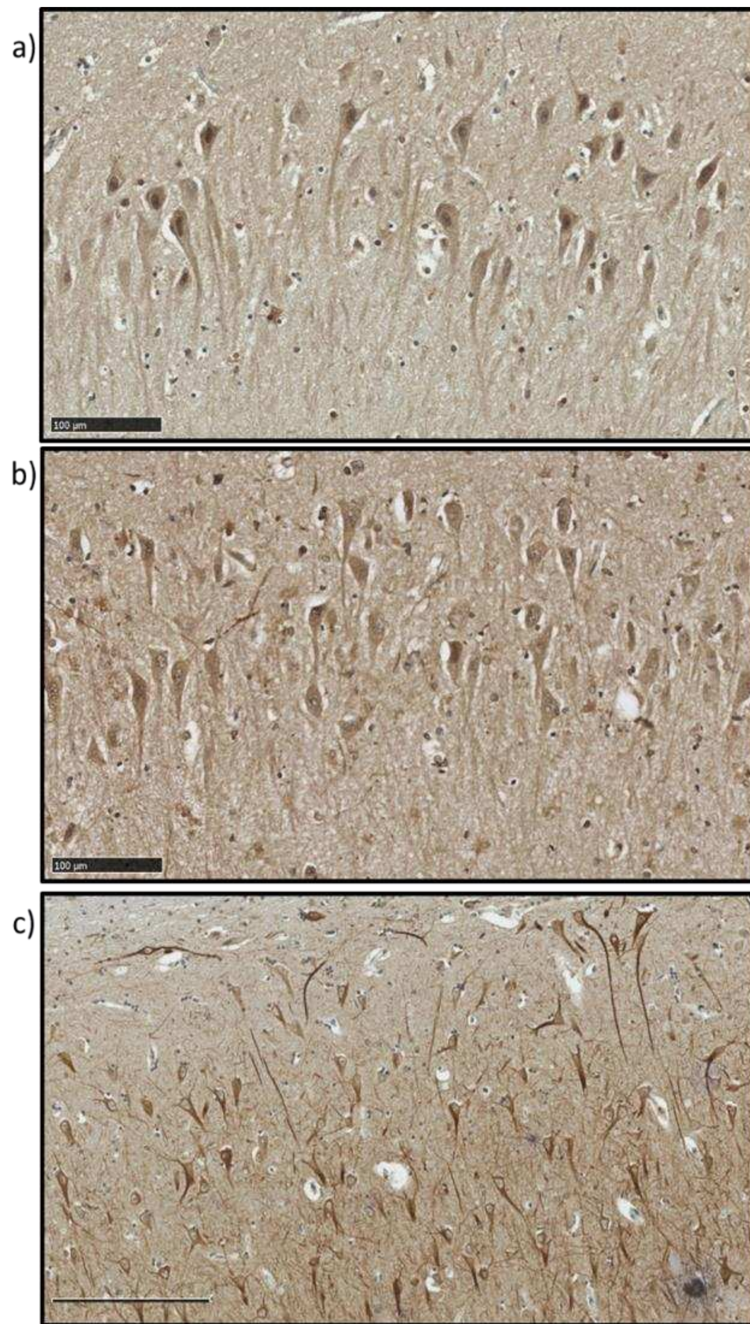
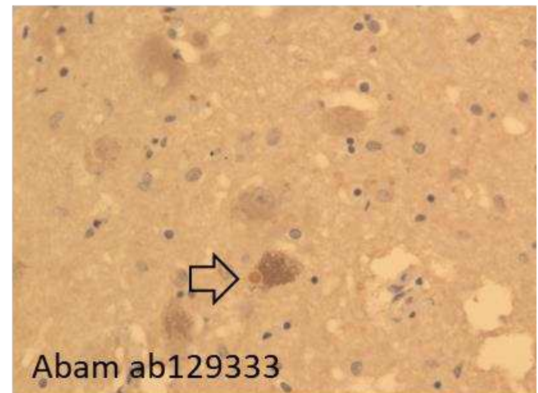
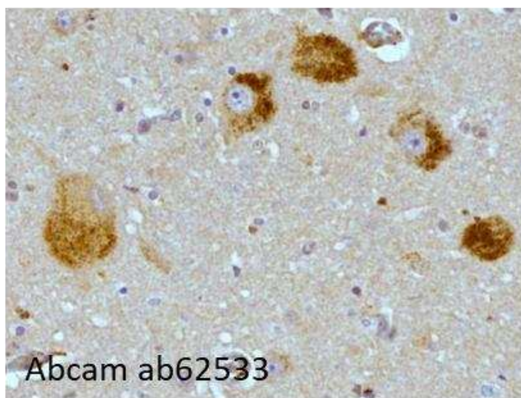
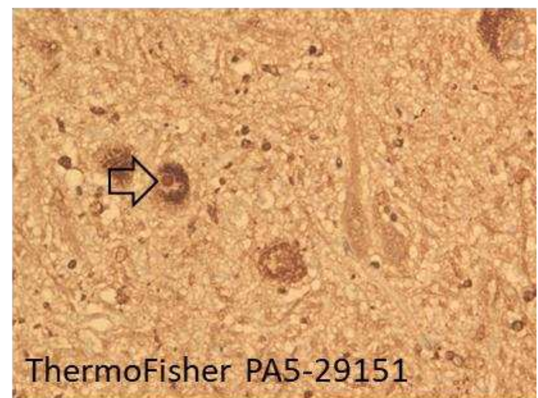


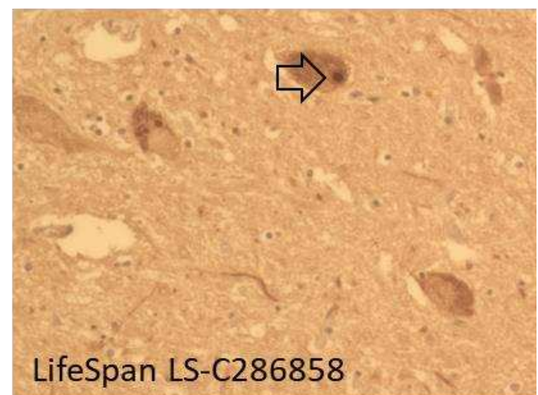
Figure 3. TIGAR staining in hippocampus (ThermoScientific PA5-29152 antibody). Representative image of TIGAR in hippocampal regions CA2 in a control (a), PD (b) and DLB (c). TIGAR expression was variable in the pyramidal cells in all cases, but no difference was seen between control and disease cases. Bar=100μm.

C terminus antibodies

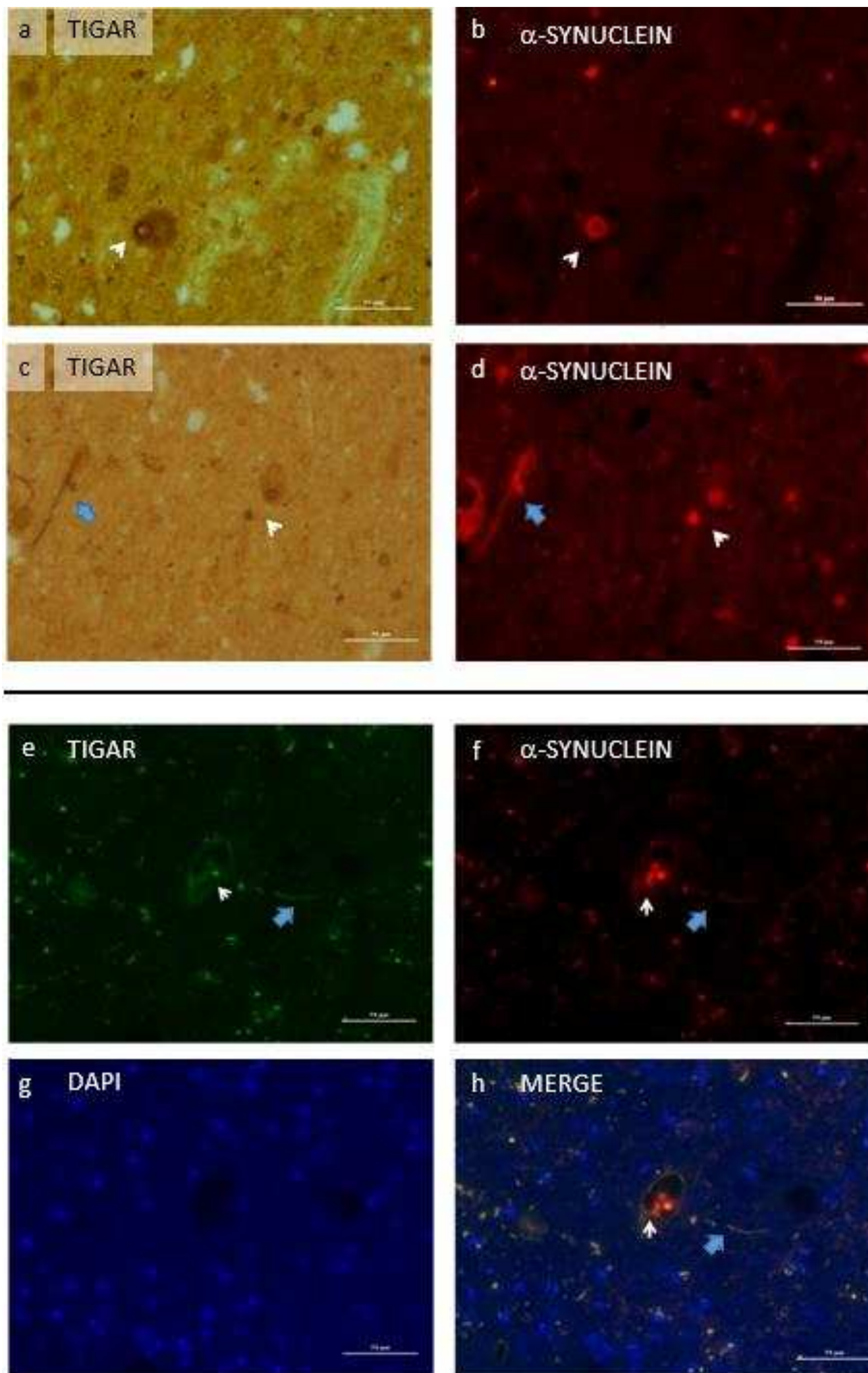




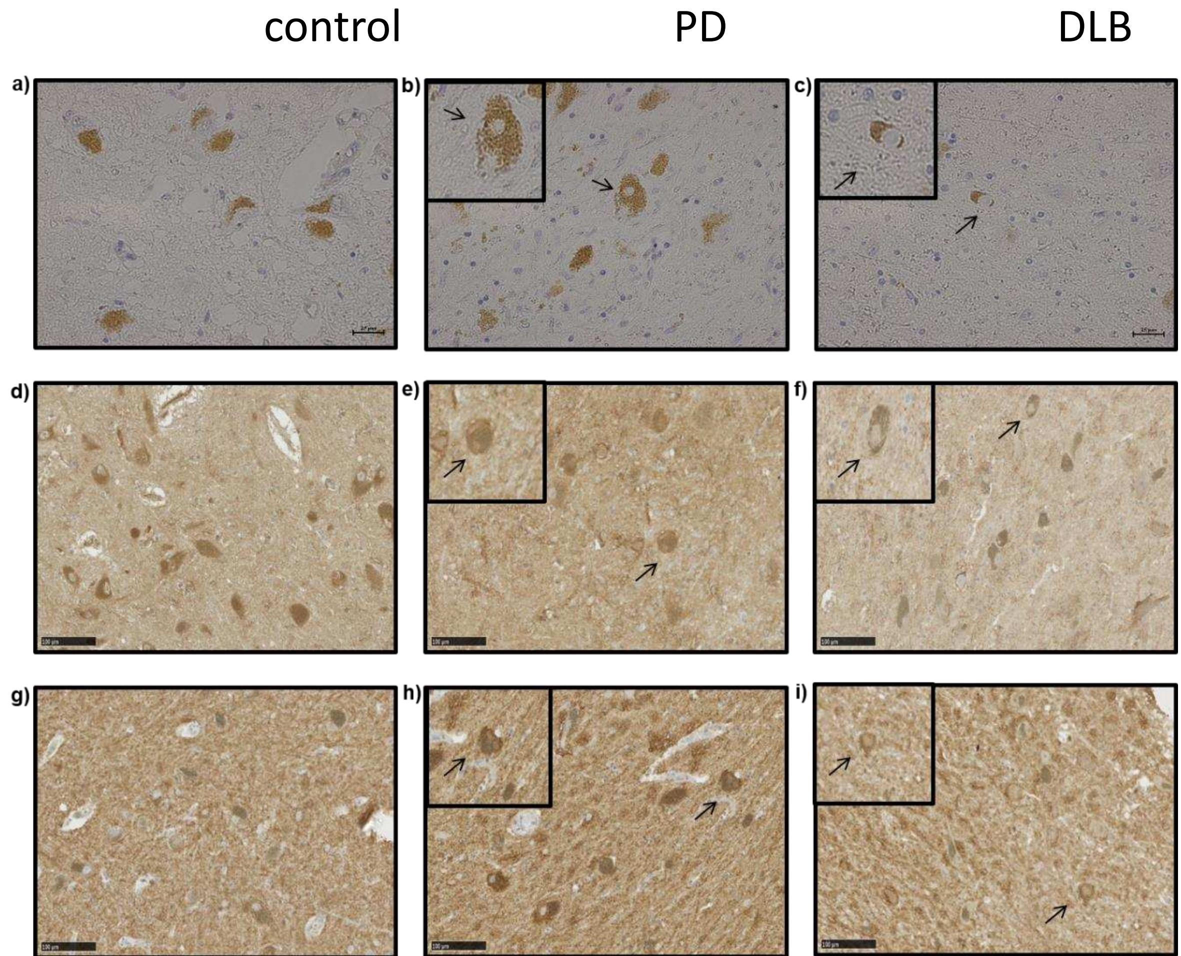




Supplementary figure 1. Immunohistochemistry for TIGAR using antibodies to the central portion and the region of the C terminus of the protein showing labelling of Lewy bodies in the C terminus antibodies (arrows).



Supplementary figure 2. Double labelling immunohistochemistry for-alpha synuclein. When TIGAR is labelled with DAB (figures a and c; ThermoScientific PA5-29152 antibody), and alpha-synuclein is labelled in fluorescence (figures b and d) both Lewy bodies (white arrow head) and Lewy neurites (blue arrow head) can be seen to co label for both antisera. When both TIGAR and alpha-synuclein are labelled in fluorescence (figures e-h), there is again, co-localisation (bar =50μm).



Supplementary figure 3. p53 (a, b, c), HK-I (d, e, f) and HK-II (g, h, i) immunohistochemistry in the substantia nigra in control (a, d, g), PD (b, e, h) and DLB (c, f, i) cases. Negative Lewy bodies demonstrated by arrow. HK-I had slightly greater immunopositivity than controls on image analysis. There was no staining for p53 and only low-level non specific labelling was seen for HK-II. Bar=100μm.

ID	Sex	Diagnosis	Age at death (years)
LP0079/06	M	DLB	78
LP112/06	M	DLB	86
LP003/04	F	DLB	66
LP016/05	M	DLB	72
LP097/09	F	sPD	84
LP052/09	M	sPD	76
LP015/03	F	sPD	74
LP087/03	M	sPD	71
LP073/09	M	MSA	69
LP120/04	F	MSA	63
LP129/01	M	MSA	62
LP014/90	M	ALS sporadic	51
LP059/09	F	ALS sporadic	80
LP072/05	M	ALS sporadic	66
LP094/06	M	ALS sporadic	71
LP141/90	M	ALS sporadic	55
LP085/07	F	Control	59
LP005/07	M	Control	63
LP098/07	M	Control	67
NA188/96	F	Control	82
LP056/90	M	Control	51
LP309/90	M	Control	82
LP335/90	F	Control	29

Supplementary Table S1. Sheffield Brain Bank Tissue used in this study. F, female; M, male; sPD, sporadic Parkinson´s Disease; DLB, Dementia of Lewy Bodies; MSA, Multiple System Atrophy; ALS Amytrophic lateral sclerosis.

ID	Sex	Diagnosis	Age (years)
P2/10	F	sPD	82
P54/11	M	sPD	80
P89/10	M	sPD	77
P21/12	M	sPD	83
P33/12	M	sPD	74
P22/12	M	sPD	85
P11/11	M	DLB	60
P34/07	M	DLB	80
P28/11	F	DLB	86
P68/10	M	DLB	73
P48/03	F	DLB	74
P80/10	M	DLB	67
P72/07	M	Control	85
P47/11	F	Control	79
P75/10	F	Control	83
P64/11	F	Control	80
P82/10	F	Control	87
P78/06	F	Control	68

Supplementary Table S2. Queen Square Brain Bank Tissue used in this study. F, female; M, male; sPD, sporadic Parkinson´s Disease; DLB, Dementia of Lewy Bodies

Antibody	Region	Antigen retrieval	Dilution/time
Anti-TIGAR antibody. Millipore (AB10545) Polyclonal / rabbit	KLH-conjugated linear peptide that lies in the central region of the protein	PC, pH9	1:400 / 30 min
Anti-TIGAR antibody. ABCAM (ab62533) Polyclonal / rabbit	Synthetic peptide (Human) of 19 amino acids from a region near the centre of TIGAR	PC, pH9	1:800 / 30 min
Anti-TIGAR antibody. ABCAM (ab129333) Polyclonal / rabbit	Synthetic peptide corresponding to a region within C terminal amino acids 220-270 of Human TIGAR	PC, pH9	1:750/ 30 min
***TIGAR Polyclonal antibody Thermo Scientific (PA5-29151) Polyclonal/rabbit**		PC, pH9	1:400 / 1 hr
Anti-TIGAR Antibody LS-C286858 (LifeSpan Biosciences) Polyclonal / rabbit		PC, pH	1:50 / 1 hr
Anti alpha-synuclein antibody (NCL-L-ASYN) Molyclonal / mouse	Prokaryotic recombinant protein corresponding to the majority of the full length alpha-synuclein molecule	PC, pH9*	1:1000 / 1hr
Anti-HK-I [4D7]. ABCAM (ab105213) Molyclonal / mouse	Recombinant full length protein, corresponding to amino acids 1-917.	PC, pH	1:2000 / 1 hr
Anti-HK-II. Thermo Fisher (MA5-14849) Molyclonal / rabbit	Synthetic peptide corresponding to the sequence of human HK-II	PC, pH	1:50 / 1 hr
Anti-p53 Dako GA616 Molyclonal / mouse	Recombinant human wild-type p53 protein.	High pH Target Retrieval Solution	NA^{+} / 20 min

Supplementary table S3: Antibodies tested and optimised for immunohistochemistry in Human brain tissue. Hexokinase I (HK-I); Hexokinase II (HK-II); Mouse (M), Rabbit (R); Monoclonal (Mono); Polyclonal (Poly); Non applicable (NA) Pressure cooker (PC). * Pre-treatment for 1 hr with formic acid followed by antigen retrieval. $^{+}$Anti-p53 antibody Ready to Use (prediluted).