Vascular Remodelling is Impaired in Parkinson Disease

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Abstract

Objective: We have previously reported vascular degeneration of human Parkinson disease (PD), in which we described degenerative pathology of endothelial cells and its association with increased string vessels in the grey matter of middle frontal gyrus (MFG). Growth factors, for example platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF), involve in vascular remodelling by promoting cell proliferations and angiogenesis through capillary pericytes. Thus current study examined the hypothesis whether vascular degeneration in human PD is associated with impairment of vascular remodelling.

Methods: Using tissue microarray method we conducted immunohistochemical staining in the grey matter of MFG of human PD (n=17) and age-matched control cases (n=17). The expression of PDGF receptor-beta, proliferating cell nuclear antigen and phosphorylation of IGF-1 receptor in capillaries, IGF binding protein-2 and VEGF were evaluated using automated image analysis software.

Results: PDGF receptor-beta was specifically expressed in the pericytes which formed capillary morphology. Compared to the age-matched control cases, there were significant decrease in PDGF receptor-beta positive capillaries (p<0.05; p<0.01), proliferating vascular cells (p<0.05) and VEGF (p<0.05) in the PD cases. There no difference in phosphorylation of IGF-1 receptors, expressed in the capillaries between the groups. We found a significant increase in IGF binding protein-2, expressed in the astrocytes of PD when compared to the control cases (p<0.05). Interestingly the levels of phosphorylated IGF receptors in the capillaries were significantly correlated with the numbers of pericytes and proliferating cells in capillaries (p=0.001).

Conclusion: Impaired PDGF function in the pericytes, reduced cell proliferation and VEGF suggested that the ability of vascular remodelling is impaired in PD. The maintained IGF-1 function appeared to be ineffective to retain vascular remodelling process in PD. The up-regulation of IGF binding protein-2 may suggest a role for autocrine/paracrine of IGF-1 in PD.

Keywords: Cerebrovascular diseases; Parkinson’s disease; Pericyte; Angiogenesis; Vascular biology; Immunohistochemistry

Introduction

We have recently reported vascular degeneration in the middle frontal gyrus (MFG) of cerebral cortex, substantia nigra (SN), caudate nuclei (CN) and brainstem nuclei as part of the pathology in human Parkinson disease (PD), in which endothelial cell degeneration has been characterized as the cause of the loss of capillary networks [1]. Subsequently, we have described the changes of basement membrane which is retained and leads to an increase in string vessel formation in the PD brains [2]. Given string vessels do not carry blood, the data may suggest a potential role for hypoperfusion in secondary neuronal degeneration of PD. Apart from the CN, the maintained basement membrane may prevent the leakage of the blood-brain barrier (BBB) and the activation of astrocytes in other brain regions examined in PD [2].

The mechanism underlying endothelial degeneration is so far not clear. Vascular remodelling through angiogenesis and vascular regeneration is the key to maintain functional vascular networks. As part of the capillary structure in the central nervous system (CNS), pericytes are embedded within the basement membranes and play important roles in vascular remodelling through promoting angiogenesis, endothelial proliferation and regulating cerebral blood flow [3,4].

Several growth factors play important roles in promoting vascular remodelling. While vascular endothelial growth factor (VEGF) is essential for angiogenesis [5], insulin-like growth factor-1 (IGF-1) plays a critical role in vascular remodelling in the CNS [6]. VEGF is broadly expressed in neurons [7], endothelial cells [8] and astrocytes [9]. Chronic hypoperfusion, for example, via increased string vessels in PD [2], can stimulate VEGF induced angiogenesis locally [8]. It is also known that VEGF stimulated angiogenesis is associated with the dysfunction of the BBB due to leakage of newly formed vessels [10]. IGF-1 is a neurotrophic factor which is also involved in vascular remodelling through regulating VEGF upstream by promoting the maturation of newly formed capillaries. IGF-1 dysfunction has been reported in neurological conditions [11]. The increase in circulating IGF-1 is used as a biomarker for IGF-1 resistance in PD [12]. The function of IGF-1 is mediated through activating its receptors which are widely distributed in different brain regions [13]. IGF-1 activity is also tightly regulated through the binding of IGF binding proteins (IGFBPs), in which IGFBP-2 is dominant in the CNS [14]. The binding to IGFBPs regulates IGF-1 function by preventing IGF-1 from being metabolised.

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Briefly, clinically diagnosed idiopathic PD cases were donated to the Neurological Foundation of New Zealand Human Brain Bank. After tissue processing a series of several key diagnostic regions all of the PD and control cases were examined by a neuropathologist. The anatomical areas sampled included the substantia nigra, pons at the level of locus coeruleus, middle frontal gyrus, middle temporal gyrus, hippocampus with the entorhinal cortex, caudate nucleus/putamen, cingulate gyrus, inferior parietal lobule, occipital cortex and cerebellum. Tissue blocks were processed for paraffin embedding, 4 µm thick tissue sections were cut, which were subsequently stained with haematoxylin and eosin, and immunohistochemistry was performed with antibodies to tau (DAKO A0024), beta-amyloid (DAKO 6F/3D) and alpha-synuclein (Leica clone KM51). Only cases consistent with idiopathic Parkinson's disease were chosen and age-matched control cases with absence of clinical neurological symptoms and only age related pathological changes in the brains were selected. All the selected cases have a post-mortem delay of less than 48 h. Information regarding the age, sex, post-mortem delay, clinical and pathological diagnosis, cause of death of PD and age-matched control cases are listed in Tables 1 and 2.

**Immunohistochemical staining**

The paraffin-embedded tissue microarray sections of the grey matter of the MFG were used. Immunohistochemistry was performed using antibodies labelling for pericytes, PCNA for proliferation of endothelial cells, VEGF, phosphorylated IGF-1R (IGF-1Rp) and IGFBP-2 (Table 3). The methodology has been described previously [1,16]. All washes, unless specially noted, were carried out three times for 5 minute each in PBS with gentle shaking. All antibodies were diluted with immunobuffer containing 1% donkey serum (v/v) and 0.4% merthiolate (w/v) in PBST.

The tissue microarray sections were incubated on a hotplate at 60°C for 1 hour before they were de-paraffinized in 2 changes of xylene, 10 min each. The sections were then rehydrated through a graded alcohol delay, clinical and pathological diagnosis, cause of death of PD and age-related pathological changes in the brains were selected. All the selected cases have a post-mortem delay of less than 48 h. Information regarding the age, sex, post-mortem delay, clinical and pathological diagnosis, cause of death of PD and age-matched control cases are listed in Tables 1 and 2.

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incubated in primary antibodies for 48 h at 4°C. The tissue microarrays were then washed and incubated with biotinylated secondary donkey anti-rabbit or donkey anti-mouse antibodies (1:500) for 24 h at 4°C, followed by washes and incubation with ExtrAvidin-HRP (1:1000) for 3 h at room temperature. The tissue microarrays were then washed and incubated in a DAB solution [2] for 10 to 20 min to generate a brown reaction product before the reaction was stopped by washing the sections in PBS. Sections were then dehydrated through a graded alcohol series and xylene before cover-slipping.

**Image processing and analysis**

The methods for image capture had been recently published [2]. Briefly, 20 images were obtained using a 20x objective lens on the Nikon E800 microscope from each “core” of the tissue microarray by automated random imaging. An image of the whole tissue microarray section with VEGF staining was also obtained at 0.8x magnification using a Leica MZ6 modular stereomicroscope.

**Image**

PDGFRβ positive pericytes exhibited capillary-like morphology (Figures 1A and 1B). Phosphorylated IGF-1 receptors were expressed in morphologically recognised capillaries and other brain cell types. The staining of phosphorylated IGF-1 receptors in capillaries also formed a structure equivalent to capillaries seen with other markers (Figure 3A). ImageJ software (V1.46) was used to analyse the number and length of PDGFRβ positive capillaries, number of vascular PCNA staining, number and length of IGF-1Rp positive capillaries, number of IGFBP-2 positive astrocytes, and density of VEGF staining in the acquired images. For PDGFRβ, PCNA and IGF-1Rp staining, macros were developed in ImageJ based on previously described analysis

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**Table 2:** Clinical and pathological details of PD cases.

| Case | Age | Sex | PM delay | Clinical diagnosis | Pathology | Cause of death |
|------|-----|-----|----------|-------------------|-----------|----------------|
| PD48 | 84  | M   | 18-20 h  | Idiopathic PD     | SN: patchy neuronal loss with gliosis; a few LBs | Not known |
| PD46 | 77  | M   | 34 h     | Idiopathic PD     | SN: Marked loss of pigmented neurons; low numbers of laminated LBs in the neuropil; LC: no LB | Pneumonia |
| PD42 | 84  | M   | 21 h     | Idiopathic PD     | Moderate loss of pigmented neurons with mild gliosis. Moderate loss of pigmented neurons; mild gliosis. Pigment incontinence; occasional LBs; LC: loss of pigmented neurons and pigment incontinence; classic LBs | Myocardial infarction |
| PD37 | 81  | M   | 4 h      | Idiopathic PD     | SN: Moderate to marked loss of pigmented neurons, mild gliosis, pigment incontinence; occasional LBs; LC: loss of pigmented neurons and pigment incontinence; classic LBs | PD |
| PD33 | 91  | M   | 4 h      | Idiopathic PD     | SN and LC: moderate neuronal loss, gliosis and poor pigmentation of some remaining neurons; classic LBs in a few neurons. | Pneumonia |
| PD31 | 67  | M   | 25 h     | Idiopathic PD     | SN: Patchy loss of pigmented neurons and fibrillary gliosis. Small LBs in some residual neurons and some larger LBs in neuropil. LC: neuronal loss not obvious; a few LBs in cytoplasm | Respiratory failure |
| PD28 | 76  | F   | 27 h     | Idiopathic PD     | SN not available. Some loss of pigmented neurons and LBs in LC | Pneumonia |
| PD27 | 77  | M   | 4 h      | Idiopathic PD     | SN: Marked loss of pigmented neurons and gliosis; Laminated LBs in some residual neurons | End stage PD |
| PD23 | 78  | F   | 18.5 h   | Idiopathic PD     | SN: Severe loss of pigmented neurons and gliosis; LBs rarely seen. Little neuronal loss and numerous LBs in LC | Pneumonia |
| PD21 | 79  | F   | 9.5 h    | Idiopathic PD     | SN: Marked loss of pigmented neurons; 2 LBs. LC: less loss of pigmented neurons; many LBs. | PD |
| PD20 | 73  | M   | 14 h     | Idiopathic PD     | SN: sparsely cellular and fibrillary gliosis | Congestive heart failure |
| PD16 | 79  | M   | 8.5 h    | Idiopathic PD     | SN: severe loss of pigmented neurons and a commensurate degree of fibrillary astrogliosis; rare small LBs in stroma. | Pneumonia |
| PD14 | 81  | M   | 11 h     | Idiopathic PD     | SN: marked loss of pigmented neurons and fibrillary gliosis; scanty eosinophilic cytoplasmic LBs in residual neurons. | Pneumonia |
| PD13 | 70  | M   | 12 h     | Idiopathic PD     | SN: marked loss of pigmented neurons; laminated eosinophilic LBs in neuropil and to a lesser extent in the cytoplasm of residual pigmented neurons. | Septicaemia, myelodysplasia |
| PD12 | 76  | F   | 3 h      | Idiopathic PD     | SN: Marked loss of pigmented neurons and severe fibrillary gliosis; some small intra- and extra-laminated LBs | Perforated gastric ulcer and peritonitis |
| PD11 | 69  | F   | 36 h     | Idiopathic PD     | SN: marked loss of pigmented neurons; a few laminated eosinophilic LBs in residual neurons. | Pulmonary embolism |
| PD10 | 70  | M   | NA       | Idiopathic PD     | SN: mild loss of pigmented neurons; small laminated LB in a number of neurons | NA: Not available. |

**Table 3:** List of antibodies used for DAB immunohistochemistry.

| Antibody          | Source                                | Host | Dilution |
|-------------------|---------------------------------------|------|----------|
| PDGFBR            | Cell Signalling Technology, Danvers, MA, USA | Rabbit | 1:200    |
| VEGF              | Santa Cruz Biotechnology, Dallas, Texas, USA | Rabbit | 1:100    |
| IGF1Rp            | Abcam, Melbourne, Australia           | Rabbit | 1:100    |
| PCNA              | Santa Cruz Biotechnology, Dallas, Texas, USA | Rabbit | 1:100    |
| IGFBP2            | Abcam, Melbourne, Australia           | Rabbit | 1:100    |
| Biotinylated anti-mouse IgG | Jackson ImmunoResearch Laboratories, West Grove, PA, USA | Donkey | 1:500    |
| Biotinylated anti-rabbit IgG | Jackson ImmunoResearch Laboratories, West Grove, PA, USA | Donkey | 1:500    |
| Tertiary Antibody | Sigma-Aldrich Corporation, Saint Louis, MO, USA | N/A  | 1:1000   |

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Figure 1: Shows the PDGFRβ positive capillaries in the MFG of the age-matched control (A) and the PD (B) cases. PDGFRβ positive pericytes show the morphology of longitudinal and cross sectional capillaries, evenly distributed in the grey matter of the MFG of both groups. PDGFRβ staining was clearly located in capillary walls, which was less distinctive in the PD cases (insert C) compared with the age-matched control cases (insert D). There was no statistically significant difference between the densities (number/mm², E) of PDGFRβ positive capillaries of the two groups. The density (F; *p<0.05), the total length (G; *p<0.05) and average length (H; **p<0.01) of the PDGFRβ positive capillaries longer than 15 µm were significantly reduced in the PD cases compared with the aged-matched control cases. There was no significant difference in average width (I) of PDGFRβ positive capillaries between the two groups. MFG: middle frontal gyrus; Scale bars: 100 µm and 20 µm (Inserts)
methods regarding vascular staining [1] to enable automated batch processing of images. The images were first converted into 8-bit format and background was subtracted. Automatic thresholding method called “MaxEntropy” was used in the “Threshold” tool to select the targets of interest and create binary images. The “noise” of the binary images was reduced using the “Median” tool where the radius setting was 5 pixels for PDGFRβ, 2 pixels for PCNA and 4 pixels for IGF-1R. For the analyses of PDGFRβ and IGF-1R positive blood vessels, the “Fill Holes” tool was used to fill the enclosed hollow spaces of the vessels so that the length of the blood vessels would be measured accurately. The “Analyse Particles” tool was used to obtain the number and total length of the blood vessels. In the “Analyse Particles” tool, the shapes of the targets were filtered by setting the “circularity” range of the targets, where a circularity value of 1.0 indicates a perfect circle and a value closer to 0.0 indicates an increasingly elongated polygon. Therefore, to analyse only the blood vessels oriented longitudinally to the tissue section, non-vascular staining and cross sections of vessels were excluded from the images by setting the “circularity” option in the “Analyse Particles” tool to 0.00-0.50 for image processing. The density of blood vessels was calculated by dividing the number of vessels per image by the actual sample area of the image (0.26 mm²). The average length of the vessels in an image was acquired by dividing the total length of blood vessels by the number of vessels in the image. For vascular PCNA analysis, after the “noise” of the binary images was reduced using the “Median” tool, the “Analyse Particles” tool was used to obtain the number of vascular PCNA staining by setting “circularity” to 0.00-0.70 to include only the linear and irregular shaped staining generally found in the vessels. The density of the vascular PCNA staining was calculated by dividing the average number of vascular PCNA staining in an image by the sample area of the image (0.26 mm²). For IGFBP-2 positive astrocyte analysis, the IGFBP-2 positive astrocytes in the original images were counted using the “cell counter” tool of ImageJ, where the images were coded for blinding.

The method used for analysing the density of VEGF staining was based on the previously described intensity analysis method [2]. Briefly, the image was converted to 8-bit format before the grayscale pixel values were inverted so that stronger staining has higher pixel intensity value. Both the “cores” of the tissue microarray and a blank region of the slide were marked by using the “Freehand Selections” tool. The intensity of the selected regions was obtained using the “Measure” tool. The pixel intensity values of the “cores” were subtracted by that of the blank region to obtain the values that represent the density of the staining on the “cores”.

**Statistical analysis**

The difference between the PD and age-matched control cases was analysed using two-tailed unpaired t-test. Correlation was analysed using two-tailed Pearson correlation test. The r value in the correlation test represents the Pearson correlation coefficient. The data were presented as Mean ± SEM. P value less than 0.05 was considered to be significant.

**Results**

**Changes in pericyte associated capillaries**

The photographic images in figure 1 show the expression of PDGFRβ in pericytes in the MFG of an age-matched control (Figure 1A) and a PD cases (Figure 1B). The positively stained pericytes showed typical capillary structures in either longitudinal or cross section morphology. The staining was located to capillary walls where the structural images were more complete and clearer in the age-matched controls case (Figure 1, insert C) than that in the PD cases (Figure 1, insert D). Unpaired t-test analysis showed that the density (number/mm²) of pericyte associated capillaries was similar between the PD and age-matched control cases (Figure 1E). When the analysis excluded the vessels shorter than 15 µm the density (p<0.05, Figure 1F), the total length (p<0.05, Figure 1G) and the average length of PDGFRβ positive capillaries were reduced in the PD cases compared with the age-matched control cases. The average width of PDGFRβ positive capillaries on the other hand, did not show a statistically significant difference between the two groups (Figure 1H).

**Vascular proliferation**

The photographic images in Figure 2A show PCNA positive staining, in which the darkly stained nuclei were observed in both vascular (long arrows) and non-vascular cells (arrowheads) in the MFG of both age-matched control and the PD cases. The PCNA staining in the non-vascular cells was round in shape and the staining in the vascular cells had elongated shapes. We only included the vascular
PCNA positive cells for quantitative analysis. Unpaired t test analysis showed that the density of vascular related PCNA positive staining evaluated as cell number/mm² in the PD cases was reduced compared with the age-matched control cases (p<0.05, Figure 2B).

**Phosphorylation of IGF-1 receptors in capillaries**

Figure 3A shows phosphorylated IGF-1R staining in the capillary walls with both longitudinal (long arrow) and cross sectional (arrowheads) views and morphologically recognised neurons (short arrows) in the MFG of both groups. The IGF-1R staining formed a clear capillary structure where the capillaries branching off showed absence of staining. Unpaired t-test analysis showed that the density (number/mm², Figure 3B), total length (total length/mm², Figure 3C) and average length (Figure 3D) of the IGF-1Rp positive capillaries were similar between the PD and the age-matched control cases.

Pearson correlation analysis showed that the density of vascular PCNA positive cells was significantly correlated with the densities of PDGFRβ positive capillaries (r=0.5419, p=0.0011, Figure 4A) and vascular IGF-1Rp positive cells (r=0.4228, p=0.0142, Figure 4B).

**IGFBP-2**

Figure 5 shows the distribution and morphology of IGFBP-2 positive astrocytes in the MFG of a PD case (Figures 5A and 5B). The distribution of IGFBP-2 positive astrocytes varied largely in both PD and age matched controls where IGFBP-2 positive astrocytes were broadly spread out in some cases but clustered together in other cases. There were cases from both groups showing absence of IGFBP-2 positive astrocyte. Morphologically, IGFBP-2 staining was found in both the cytoplasm and processes of the astrocytes with some of them showing clear association with blood vessels (Figure 5B). Unpaired t-test analysis showed that the density (number/mm²) of IGFBP-2 positive astrocytes was increased in the PD cases compared with the age-matched control cases (p<0.05, Figure 5C).

**VEGF expression**

Figure 6 shows VEGF staining in the MFG (Figure 6A). VEGF expression was observed mostly in capillaries (arrowheads) and cells morphologically recognised as neurons (long arrows) and astrocytes (short arrows). Morphologically the dotty staining was clearly located around the capillaries and in the extracellular spaces (inserts). In neurons, VEGF staining was observed in cytoplasm and dendrites/
axon (insert). The staining in astrocytes was mainly located on the processes (insert). The overall staining of the PD cases was generally lighter than that of the age-matched control cases. Unpaired t-test analysis showed that the density of the VEGF staining in the PD cases was reduced compared with the age-matched control cases ($p<0.05$, Figure 6E).

**Discussion**

In general we found the loss of pericytes, VEGF expression and reduced vascular proliferation in the MFG, suggesting the impaired ability in vascular remodelling of human PD brains. IGF-1 function in PD was maintained and was closely related to the loss of pericytes and vascular proliferation. The elevated IGFBP-2 in astrocytes might suggest a role for autocrine/paracrine regulation of IGF-1 in PD pathology.

The role of pericytes in vascular remodelling has been well documented [3]. As specialized mural cells, pericytes are involved in vascular remodelling by modulating endothelial cell proliferation and angiogenesis [3]. Using post mortem brain tissue from PD cases where endothelial degeneration was reported [1], we found a significant loss of pericyte-associated capillaries. Using a marker for pericytes the staining was clearly associated with capillaries with typical tubular structure (Figures 1A-1D). By labelling the endothelial cells we have previously characterised the increase of fragmentation of the capillaries in PD [1]. Similarly while there was no statistically significant difference between the PD and control cases when the number and average width of the pericyte-associated capillaries were measured (Figures 1E and 1I), we found a significant reduction in the number and length of pericyte-associated capillaries when the evaluation only included the longer vessels (Figures 1F-1H). The data suggest that the loss of pericytes also involves in shorter and more fragmented capillaries, possibly due to more severe impairment in vascular remodelling. Due to tissue availability we only evaluated the changes in the MFG, in which the pericyte changes were similar to that of endothelial cells [1]. The interactions between endothelial cell and pericytes on vascular remodelling are well documented [18-20]. For example, pericytes can regulate the proliferation and survival of endothelial cells and endothelial cells mediate the recruitment of pericytes [21]. The reduced pericytes may contribute to the endothelial degeneration seen in the PD cases [1,2]. More importantly, the expression of PDGFRβ was used for marking the pericytes, which is much more than just a marker of pericytes as PDGF signalling is essential for pericyte-endothelial interactions which lead to

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**Figure 4:** Correlations of vascular IGF-1Rp positive capillaries and PDGFRβ positive capillaries with vascular PCNA staining. The density of vascular PCNA staining showed significant positive correlation with the densities of PDGFRβ positive capillaries (A; $r=0.5419$, $p=0.0011$) and IGF-1Rp positive capillaries (B; $r=0.4228$, $p=0.0142$).

**Figure 5:** Shows IGFBP-2 expressing glial cells in the MFG of a PD case. IGFBP-2 staining was observed in the cytoplasm and processes of glial cells morphologically recognised as astrocytes (B; long arrows) and walls of blood vessels (B; arrowheads). In some cases, the IGFBP-2 positive astrocytes were distributed in groups (A). Some IGFBP-2 positive astrocytes were closely associated with blood vessels showing IGFBP-2 staining (B). The density of IGFBP-2 positive astrocytes in the PD cases was higher compared with the age-matched control cases (*$p<0.05$). MFG: middle frontal gyrus; Scale bars: 50 µm and 20 µm
vascular remodelling through promoting vasogenesis and angiogenesis [3]. Other than PDGF receptors pericytes also express receptors for other growth factors such as VEGF, transforming growth factor-β (TGF-β) and IGF-1 receptors [22,23]. Apart from the quantitative analysis the images of PD cases (Figures 1B and 1D) also suggested the vascular degeneration by showing the morphology of shortened capillaries with indistinct and palely stained pericytes.

Severe loss of pericytes can lead to increased permeability of the BBB through dysfunction of endothelial cells and basement membrane deposition by astrocytes [24,25]. The reduced pericytes in PD in our study might not be severe enough to compromise the permeability of the BBB in the MFG as we have previously reported there was no increased BBB leakage and no change in basement membrane in the PD cases compared to the age matched control cases [2]. It is clear that the preserved basement membrane prevents the leakage of large molecules; however the increase of string vessel formation due to endothelial degeneration and the remaining basement membrane may impair other functions of capillaries, such as blood supply, regulation of blood flow [26] and transport of macromolecules such as insulin, IGF-1 and low density lipoproteins from the circulation to the brain parenchyma [27].

Cell proliferation is another key mechanism of vascular remodelling. PCNA is a commonly used marker for cell proliferation, which includes endothelial proliferation. By counting the number of PCNA positive nuclei that were expressed in capillaries we also identified a decrease of cell proliferation in the capillaries. Even though we did not successfully identify the cell types that proliferate, the proliferation is likely to be associated with endothelial cells and/or pericytes due to their close associations with the capillaries (Figures 1A) [1,18,21-23].

Figure 6: VEGF staining in the MFG. Positive staining was observed in neurons (A; long arrows), capillaries (A; arrowheads), astrocytes (A; short arrow) and the extracellular spaces. Punctate morphology of VEGF staining could be observed in the high resolution images (inserts B-D). In neurons, VEGF was located in the cytoplasm and the processes (insert B). In capillaries, VEGF staining was visualized along the tubular walls of the vessels (insert C). In astrocytes, VEGF staining was observed mainly on the processes (insert D). In the PD cases, the average density of VEGF staining were significantly reduced compared with the age-matched control cases in the MFG. (E; *p<0.05). MFG: middle frontal gyrus; Scale bars: 50 µm and 20 µm (inserts)
IGF-1 deficiency has been reported in PD patients by showing increased plasma IGF-1 and IGF binding protein (IGFBP)-3 [28]. While over 75% of circulating IGFBPs are IGFBP-3 [29], the dominant binding protein in the CNS is IGFBP-2 [30]. IGFBPs regulate IGF-1 function by both inhibitory and stimulatory mechanisms [29,31]. IGFBP-1 has higher affinity to IGFBPs than that of IGF-1 receptors, thus the binding to IGFBPs can prevent IGF-1 activating IGF receptors, the key for initiating the downstream pathways of IGF-1 signalling [31]. Therefore, the reduction of IGFBPs could be a compensatory response to IGF-1 deficiency through increasing IGF-1 bioavailability.

By evaluating the expression of IGFBP-2 in cells morphologically resembling astrocytes, our study found increased IGFBP-2 in PD patients compared to the age-matched controls (Figure 5B) [32]. We do not have a clear explanation for the cause of the increase, however given the potential role for IGFBP-2 in regulating IGF-1 bioavailability in the CNS, the increased IGFBP-2 might associate with PD pathology possibly through IGF-1 resistant.

Using phosphorylated IGF-1R as an indicator for the function of IGF-1 in capillaries, we found no change in IGF-1 function between PD and control cases. A similar finding has been recently reported by another group [12], where the reduced IGF-1 receptor is only associated with Lewy bodies disease, but not PD. Given the PD cases used for current study were not Lewy body disease the changes of IGF-1 function may be the hallmark for PD with dementia [28]. Interestingly, the activation of IGF-1R was in close correlation with cell proliferation in capillaries, in which these proliferating cells were highly associated with the pericytes (Figure 4). It is known that IGF-1Rs are present in both endothelial cells [33] and pericytes [22,23]. Activation of IGF-1R in the capillaries protects capillaries from ischemic brain injury [22]. Thus the maintained IGF-1 function in PD could be a trophic response to the impaired vascular remodelling prior to development of dementia in PD. However, activation of IGF-1R may not be a specific measure for determining the presence of bioavailable IGF-1 in situations with IGF-1 function deficiency as both insulin and IGF-2 can activate IGF-1R [34], which provides compensatory pathways for insufficient IGF-1 function.

The plasma IGF-1 increases in PD patients, but reduces in PD with cognitive decline [28,35]. Although the transport of IGF-1 across the BBB is very restricted, circulating IGF-1 can directly act on IGF-1 receptors located in the luminal side of vessels to promote vascular remodelling [36]. The PD cases used for the current study were PD with no obvious cognitive impairment which may also explain the unchanged activation of IGF-1 receptors, and perhaps, maintains the BBB function through the protected basement membrane. Thus our hypothesis is that the BBB dysfunction may be an indicator for development of cognitive impairment in PD.

The effect of VEGF on neuronal survival and vascular remodelling in neurodegeneration are well documented [37-39]. Expressions of VEGF in brain tissue, particularly which in astrocytes and blood vessels are commonly used for indicating the ability in vascular remodelling. This is supported by similar reports that the VEGF was diffusely expressed on vascular walls, extracellular spaces, neurons and astrocytes [7,40-42]. By analysing the overall density of the VEGF expression, we found a general reduction of VEGF in the MFG of the PD cases. The neuronal role in vascular remodelling and the vascular role in neuronal survival through VEGF are examples of neuronal-vascular interactions [7,37]. Thus the overall expression of VEGF may provide a reliable indication of vascular remodelling [5] and therefore the decline of cerebral VEGF level may also contribute to impaired vascular remodelling and thus vascular degeneration in the PD cases. Given that IGF-1 regulates the function of VEGF [36] the maintained IGF-1 function may be an ineffective regulatory response to the decline of VEGF.

In summary, we propose that vascular degeneration in human PD was associated with impaired vascular remodelling, possibly mediated through impaired PDGF function and the loss of pericytes. The maintained IGF-1 function was associated with vascular cell proliferation and may be an ineffective response to the loss of VEGF function. Up-regulation of IGFBP-2 may suggest a role for autocrine/paracrine regulation of IGF-1 in PD pathology.

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