Acta Histochemica 119 (2017) 142–149

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Adult human retinal Müller glia display distinct peripheral and macular expression of CD117 and CD44 stem cell-associated proteins

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ARTICLE INFO

Article history:
Received 23 September 2016
Received in revised form
30 November 2016
Accepted 20 December 2016

Keywords:
CD117
CD44
Hyaluronan
Müller glia
Human retina
Stem cell-associated proteins

ABSTRACT

Experimental evidence suggests human Müller glia exhibit neural progenitor properties in vitro. CD117 and CD44 are known to be expressed by stem cells, the survival of which appears to depend critically on interactions with hyaluronan-rich extracellular matrix (ECM). Here, we characterise Müller glia expression of CD117 and CD44 in normal adult human retina and describe how it correlates with hyaluronan distribution in ocular ECM. By using chromogen-based immunohistochemistry, CD117 expression was found in entire Müller glia cytoplasm spanning from inner to outer limiting membrane in both peripheral retina (PR) and macular retina (MR), mirroring expression of the established Müller glia marker vimentin. Unlike vimentin, CD117 was also strongly expressed by Müller glia nuclei. Relative to total inner nuclear layer (INL) nuclei, more CD117+ Müller glia nuclei were seen in PR than MR. By contrast, CD44 expression was found predominantly in Müller glia apical processes of PR; no expression was found in MR. Astral blue staining demonstrated the presence of hyaluronan in cortical vitreous and the interphotoreceptor matrix (IPM) in both MR and PR. Our findings demonstrate that: (i) both CD117 and CD44 are expressed by human adult Müller glia; (ii) CD117 is a robust nuclear and cytoplasmic immunohistochemical marker of Müller glia; and (iii) that while CD117 is expressed by the entire Müller glia in both PR and MR, CD44 is only expressed by Müller glia apices in PR. Since the apices of Müller glia are in direct contact with the hyaluronan-rich IPM, the Müller glia-IPM interface in PR is likely a favourable region for supporting progenitor or stem cell-like signalling. These observations provide novel insights into potential stem-cell favouring microenvironments in mature human retina.

1. Introduction

A number of adult tissue types are increasingly thought to harbour stem cell niches. In retina, Müller glia have been proposed as a potential endogenous source of retinal stem cells (Jeon and Oh, 2015).

Müller glia perform multiple functions to support retinal neuronal metabolism and homeostasis. Numerous experimental data in animal models have also shown that Müller glia can behave like neural progenitor cells (Reichenbach and Bringmann, 2013). The potential of Müller glia to express stem cell markers, and to divide and differentiate into retinal neurons varies among animal species (Bernardos et al., 2007; Blackshaw et al., 2004; Roesch et al., 2008; Rowan and Cepko, 2004). In humans, *in vitro* evidence suggests Müller glia have the potential to serve progenitor cell functions. For example, human Müller glia can grow infinitely under standard tissue culture conditions and express progenitor markers, including Chx10, Notch 1, Pax6, Sox2 and βIII tubulin, upon stimulation by growth factors in the presence of extracellular matrix (ECM) (Lawrence et al., 2007). The development of stem cell-like properties in both animal and human cell lines appears to depend critically on ECM that is rich in hyaluronan (Solis et al., 2012).

Abbreviations: AB/PAS, astral blue/periodic acid Schiff; ECM, extracellular matrix; FPR, far peripheral retina; GCL, ganglion cell layer; H&E, haematoxylin and eosin; ILM, inner limiting membrane; INL, inner nuclear layer; IPM, interphotoreceptor matrix; MFPR, microcystoid degeneration in far peripheral retina; MR, macular retina; NFL, nerve fibre layer; NPR, near peripheral retina; OLM, outer limiting membrane; ONL, outer nuclear layer; *Pax6*, paired box gene 6; POS, photoreceptor outer segment; PR, peripheral retina.

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http://dx.doi.org/10.1016/j.acthis.2016.12.003

CD117 (c-kit) – a type III receptor tyrosine kinase – binds to stem cell factor to regulate cell differentiation and proliferation, chemotaxis, and apoptosis (Ronnstrand, 2004). Its expression has been shown in neoplasms of some hematopoietic stem cells, germ cells and melanocytes (Ronnstrand, 2004). Moreover, CD117+ human retinal progenitor cells are able to differentiate into retinal cells that express markers of glia, photoreceptors and ganglion cells *in vitro* (Zhou et al., 2015). On the other hand, CD44 is a cell surface glycoprotein with multiple cell–cell and cell-matrix interactions (Goodison et al., 1999). Amongst these is the ability to function as a receptor for hyaluronan (Solis et al., 2012). The interaction between hyaluronan and CD44 is believed to regulate cellular stem cell niches in response to microenvironmental changes (Misra et al., 2011; Solis et al., 2012). CD44 is expressed in parallel to hyaluronan distribution in pathological conditions, such as in cancer to drive tumour progression (Toole, 2009), and in experimental autoimmune encephalomyelitis to modulate differentiation of *oligodendrocyte* precursor cells (Back et al., 2005).

In humans, Müller glia are in direct contact with hyaluronan-rich ECM both basally and apically. The ILM of the retina is the basement membrane elaborated by Müller glia. It is in direct contact with the hyaluronan-rich vitreous body (hyaloid body). Apically, Müller glia contact another hyaluronan-rich ECM compartment – the interphotoreceptor matrix (IPM) – via their apical processes (also known as Müller glia fibre baskets) (Clark et al., 2011; Hogan et al., 1971b; Meyer, 1934). These two points of contact between Müller glia and hyaluronan-rich ECM therefore represent potential stem-cell favouring environmental niches.

Evidence of Müller glia expression of stem cell-associated markers and their association with hyaluronan-rich ECM in human retinal tissue however, remains unexplored. In this study, we describe Müller glia expression of CD117 and CD44 and discuss the topological interrelationship between hyaluronan-rich ECM and Müller glia expression of these proteins.

2. Methods

2.1. Human eye tissue

Post-mortem human eyes were allocated by the Lions NSW Eye Bank. The inclusion criteria for study eyes were: (i) no history of eye disease and (ii) no macroscopic or microscopic pathology. A total of 6 eyes from 4 males aged between 52 and 59 years were included. The eyes were fixed for at least 24 h in 10% neutral buffered formalin (mean *post-mortem* delay 15 h). Superior and inferior calottes were removed from each eye to generate a central pupil-optic nerve (PO) block, subsequently processed for paraffin embedding and for histology and immunohistochemistry.

The study protocol was approved by the University of New South Wales (HC10323) and the University of Sydney (USYD14-792) Human Research Ethics Committees in accordance with the tenets of the Declaration of Helsinki (1964).

2.2. Histochemical staining

2.2.1. Haematoxylin and eosin (H&E)

H&E staining was performed on 3 μm paraffin sections according to the standard protocols of the South Eastern Area Laboratory Services (SEALS) of Prince of Wales Hospital (Sydney, Australia). Briefly, tissue sections were heated at 80 °C for 12 min, then deparaffinised in two changes of xylene (2 min each). The sections were re-hydrated through a series of graded alcohols, followed by staining with Harris' haematoxylin for 3 min, and differentiating briefly in 0.1% acid alcohol for 1 s. Subsequently, blueing was performed in Scott's Tap Water Substitute for 5 s. The sections were stained in 0.25% (w/v) eosin after being rinsed briefly in the running water, 70% and 95% ethanol (5 s each). Finally, sections were dehydrated, cleared in xylene and coverslipped. At least two H&E stained slides from each donor eye were examined for microscopic pathology. A representative H&E stained retinal section is shown in Fig. 1.

2.2.2. Astral blue/Periodic acid Schiff (AB/PAS)

Sections were stained with AB/PAS to visualise hyaluronan in tissue. With this staining, hyaluronan appears pale blue and is PAS negative, while the other acid or neutral mucins appear pink to purple (Bishop et al., 2011). In contrast, PAS stains basement membranes, highlighting the ILM. The staining was performed according to the standard protocol of SydPath (St. Vincent's Hospital, Sydney, Australia). The tissue sections were initially placed in 3% acetic acid for 3 min prior to staining in 1% Astral blue in 3% acetic acid (pH 2.5) for 30 min. Slides were subsequently washed in water for 5 min followed by oxidation of tissue section in 1% periodic acid for 10 min. Following brief tissue rinsing in the water for 2 min, PAS colour staining was developed by treatment with Schiff's reagent for 10 min. Subsequently, the tissue sections were counterstained in haematoxylin for 3 min, followed by differentiation in acid alcohol and treatment in Scott's blueing solution. Finally, the tissue sections were dehydrated, cleared and coverslipped.

2.3. Immunohistochemistry (IHC)

2.3.1. CD117 and vimentin

CD117 immunostaining is rarely reported in human retinal tissue. We observed its expression coincidentally in Müller glia when characterising uveal melanocytes and melanomas. To confirm the specificity of CD117 Müller glia immunostaining, we compared it to vimentin, a well-established Müller glia marker. IHC was performed on an automated Leica Bond II IHC stainer (Leica Biosystems Melbourne Pty Ltd, Mt Waverley VIC Australia) with Leica Bond Polymer Refine Red Detection kit. Briefly, tissue section was dewaxed using Leica Dewax solution for 30 s at 72 °C, washed in Leica Bond wash buffer, and subsequently heat-treated for epitope retrieval (ER) in ER2 solution (for CD117 IHC) or ER1 solution (for vimentin IHC) for 30 min at 100°. Following this, tissue section was incubated with 1:50 diluted mouse monoclonal CD117 antibody (clone T595, Catalogue No.: NCL-CD117, Leica Microsystems, New South Wales, AUS) for 15 min at room temperature or 1:200 diluted mouse monoclonal vimentin antibody (clone V9, Catalogue No.: PA0640, Leica Microsystems, New South Wales, AUS) for 30 min at room temperature, and then with Leica Bond Polymer Refine Red Detection kit to stain CD117 antigen with Fast Red chromogen and counterstain with haematoxylin. Human gastrointestinal stromal tumour tissue was used as positive control for CD117 IHC, while colon tissue (mesenchymal compartment) was used for vimentin IHC. Two negative controls were used for both CD117 and vimentin: (i) omission of the primary antibody in the automated staining protocol; (ii) colonic and tonsillar epithelium.CD117 or vimentin IHC was accepted for evaluation in the present study only with appropriate staining in the controls.

2.3.2. CD44

Three-micron paraffin-embedded formalin fixed tissue sections were collected on SuperFrost Plus charged glass slides and IHC performed on a BenchMark ULTRA 2 Automated IHC/ISH slide staining system (Ventana Medical Systems, Azusa, USA) with Ventana ultraView Universal Alkaline Phosphatase Red Detection Kit. Briefly, pre-melted sections (80 °C for 10 min in oven) were (1) incubated at 75 °C for 4 min, (2) deparaffinised at 72 °C with Ventana EZ Prep solution, (3) heat-pretreated with Ventana Cell Conditioning 1 (CC1) using "mild CC" for antigen retrieval at 95 °C