International Immunology, Vol. 35, No. 4, pp. 171–179
https://doi.org/10.1093/intimm/dxac060
Advance Access publication 16 December 2022

Osteopontin (OPN)/SPP1: from its biochemistry to biological functions in the innate immune system and the central nervous system (CNS)

Elliot Yi-Hsin Lin[1], Wen Xi[1], Nupur Aggarwal[1] and **Mari L. Shinohara[1,2,]**

[1]Department of Immunology, Duke University School of Medicine, Durham, NC 27710, USA
[2]Department of Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, NC 27710, USA

Correspondence to: M. L. Shinohara; E-mail: mari.shinohara@duke.edu

Received 17 September 2022, *editorial decision* 12 December 2022, *accepted* 14 December 2022

REVIEW

Abstract

Osteopontin (OPN) is a multifunctional protein, initially identified in osteosarcoma cells with its role of mediating osteoblast adhesion. Later studies revealed that OPN is associated with many inflammatory conditions caused by infections, allergic responses, autoimmunity and tissue damage. Many cell types in the peripheral immune system express OPN with various functions, which could be beneficial or detrimental. Also, more recent studies demonstrated that OPN is highly expressed in the central nervous system (CNS), particularly in microglia during CNS diseases and development. However, understanding of mechanisms underlying OPN's functions in the CNS is still limited. In this review, we focus on peripheral myeloid cells and CNS-resident cells to discuss the expression and functions of OPN.

Keywords: innate immunity, intracellular osteopontin (iOPN), myeloid cells, secreted osteopontin (sOPN), *SPP1*

Introduction

Osteopontin (OPN) is a pleiotropic protein encoded by the *SPP1* (or *Spp1* in rodents) gene and found in various cells, tissues, and fluids, identified as a versatile protein with multiple functions (1–3). Upregulation of *Spp1* gene expression is frequently associated with inflammation elicited by events, including but not limited to infections (4–9), allergic responses (10–12), autoimmunity (13–16) and tissue damage (16, 17). OPN plays an important role in immune responses of different immune cell types, including both lymphocytes and myeloid cells.

Initially identified in osteosarcoma cells (18), OPN was reported to play a critical role in bone synthesis and resorption by mediating osteoblasts adhesion (19). Later, OPN was found as early T lymphocyte activation-1 (Eta-1) because activated T cells express copious OPN (20). Of note, the *Spp1* gene is not transcribed in naive T cells unless hosts are aged (21, 22). However, macrophages constitutively express OPN (21). More recent studies identified OPN transcript in the central nervous system (CNS), particularly in microglia under pathogenic conditions and during brain development (23–25). A majority of functional studies on OPN has been performed with cells in the peripheral immune system, but the functions of OPN in the CNS have yet to be largely explored. Here, our review starts with the biochemistry of OPN and discusses the biological roles of OPN in peripheral innate immune cells and CNS-resident cells.

Gene and protein structures of OPN

The Human *SPP1* gene locus locates in 4q22.1 and spans 7.7-kb with 7 exons. The full length of human OPN (hOPN) contains 314 aa residues. In contrast, the mouse *Spp1* gene locates in chromosome 5 with the approximately 6-kb coding region including 8 exons. The full length of mouse OPN (mOPN) protein has 294 aa. Although human and mouse OPN peptide sequences only share 63% of homology, several structures and motifs in OPN are highly conserved across species (26). The molecular weight of hOPN is around 35 kDa, but hOPN protein migrates on SDS-PAGE gels from 40 to 80 kDa due to extensive posttranslational modifications (27, 28). The calculated size of mOPN is 32 kDa, but it also appears between 40 and 80 kDa on SDS-PAGE gels.

OPN has isoforms: alternative splicing isoforms and alternative translation isoforms. Alternative splicing isoforms were studied in hOPN because mOPN does not show splicing isoforms, at least in leukocytes (29). Splicing isoforms are termed OPNa, OPNb, and OPNc. OPNa contains Exons 2–7, including the full-length protein-coding regions. OPNb and OPNc lack Exon 5 and 4, respectively. All the human alternative splicing isoforms retain the signal sequence for protein secretion and other critical OPN domains and sites, such as the calcium-binding domain, heparin-binding domain, cleavage sites by thrombin and matrix metalloproteases (MMPs), and domains with amino acid sequences with

SVVYGLR and RGD. These three isoforms have been intensively studied, mainly in cancer settings.

OPN also has alternative translation isoforms. *Spp1* alternative translation initiation excludes the signal sequence and prevents OPN secretion, resulting in the generation of intracellular OPN (iOPN) (29). In contrast, the full *Spp1* transcript, including the signal sequence, generates secreted OPN (sOPN). Indeed, both mouse and human OPN were identified in the cytoplasm and the nucleus of cells (29–35), although only a limited number of human studies evaluated the impact of the balance between iOPN and sOPN on human diseases (35–37). sOPN and iOPN are considered to have distinct phosphorylation and glycosylation status because of their differential subcellular localization.

Conserved domains and motifs

Both human and mouse OPN contains the classical RGD motif that binds αvβ1, αvβ3, αvβ5, αvβ6, α5β1, and α8β1 integrins (38–40). An additional integrin-binding motif is SVVYGLR^{168} for hOPN or SLAYGLR^{153} for mOPN. The 7-aa motifs are located immediately downstream of RGD and are called OPN 'cryptic epitopes' because of their exposure upon thrombin cleavage between R^{168} and S^{169} residues in hOPN and between R^{153} and S^{154} in mOPN (39, 41). In addition to thrombin, OPN is also cleaved by MMP-3 and MMP-7 between the G^{166} and L^{167} residues in hOPN and between G^{151} and L^{152} in mOPN (42–47), which are within the SVVYGLR and SLAYGLR cryptic epitopes. MMP cleavage disrupts the cryptic epitopes and prevents α5β1 binding to RGD, although RGD-binding αv integrin recognition is not affected (41). Heparin-binding domain (HBD) YGLRSKSKKF in hOPN or YGLRSKSRSF in mOPN overlaps the SVVYGLR or SLAYGLR cryptic epitopes, respectively. There are other biologically functional domains in OPN, such as the calcium-binding domain. These are summarized in excellent reviews (48, 49).

Enzymes that act on OPN

We mentioned above that OPN is a substrate of thrombin and MMP-3/7. Studies also showed that OPN is a substrate of other enzymes. One of those is transglutaminase 2, which covalently crosslinks OPN residues to form an OPN polymer (polyOPN). The OPN conformation change by polymerization makes polyOPN bind integrins, especially $\alpha_9\beta_1$, through the exposure of the cryptic SVVYGLR epitope (50–52). Another enzyme, phosphate-regulating endopeptidase homolog X-linked (PHEX), digests OPN (53). Accumulation of OPN and other SIBLING proteins is caused by loss-of-function mutation of PHEX, resulting in the genetic disease X-linked hypophosphatemia (54, 55).

Posttranslational modifications of OPN

A comprehensive study of posttranslational modifications identified 34 phospho-serines, 2 phospho-threonines, 5 *O*-glycosylated threonines and no apparent *N*-glycosylation in milk hOPN (28), although different cell types generate various degrees of OPN phosphorylation (56). Another study using milk hOPN showed that *O*-glycosylation at the 5 threonine residues protects the integrin binding motifs from pepsin digestion, thus retaining the ability to mediate cell adhesion after pepsin digestion (57). For OPN phosphorylation, Golgi-casein kinases, such as FAM20C and VLK, are involved (58). Phosphorylation and glycosylation can regulate the OPN-integrin interactions if the modification sites are within or close to integrin binding motifs. For example, serine phosphorylation in SVVYGLR significantly reduces the $\alpha_v\beta_3$ integrin binding affinity to RGD (58, 59). These studies suggested that posttranslational modifications of OPN can alter the functions of OPN.

Receptors detecting sOPN

As we mentioned, various integrins bind the RGD motif of sOPN. The OPN cryptic epitope is recognized by α4β1 and α4β7, while α9β1 recognizes the cryptic epitope only after thrombin cleavage (40). The second type of sOPN receptor is the splicing variants of CD44 containing exon v6 or v7 (CD44v6 or CD44v7) (60–62). The binding of CD44v6 or CD44v7 to OPN requires the presence of β1 integrin but is RGD-independent. Thrombin cleaved OPN N-terminal and C-terminal fragments have similar affinity to the CD44v6 or CD44v7, suggesting multiple epitopes are involved in the sOPN-CD44 interaction (61). Recently, a cell surface receptor, inducible T-cell costimulator ligand (ICOSL), was also discovered to bind sOPN in several types of tumor cells (63). The OPN-ICOSL interaction promotes tumor metastasis and angiogenesis (64).

Involvement of iOPN in signal transduction in the cytoplasm

As an intracellular molecule, iOPN is physically segregated from all the cell surface receptors, exerting distinctive functions from those of sOPN. Previous studies demonstrated that iOPN is an adaptor protein, without enzymatic functions, in cytoplasmic signal transduction (9, 32, 34, 65–67). The first report of such a function of iOPN indicated that iOPN promotes IRF7 activation to induce type-1 interferon (IFN-I) gene expression downstream of TLR7 and TLR9 in plasmacytoid dendritic cells (pDCs) (9). iOPN also associates with MyD88, TRAF2, IRAK1 and Syk, in mononuclear phagocytes (MNPs) and controls TLR4 and CD40 dual signaling, as well as 'tethering' multiple cell surface receptors (TLR2, dectin-1, mannose receptor) to cluster receptors to synergize signal transduction triggered by the receptors (32, 33). Another study also suggested that tumor necrosis factor (TNF) receptor-associated factor 3 (TRAF3) associates with iOPN through an OPN C-terminal region in macrophages to stabilize TRAF3, promoting anti-viral responses through upregulating IFNβ production (68). iOPN also interacts with vimentin, a structural protein of intermediate filaments and stabilizes vimentin by extending the vimentin half-life. Increased vimentin is considered a hallmark of cancer metastasis (69).

iOPN in the nucleus

iOPN also localizes in the nucleus. An earlier study identified nuclear iOPN in a human cell line, HEK294, and demonstrated nuclear translocation of iOPN correlating to chromatin condensation during cell cycles (35). Another study on hypoxia/reoxygenation showed that iOPN is cleaved by caspase-9,

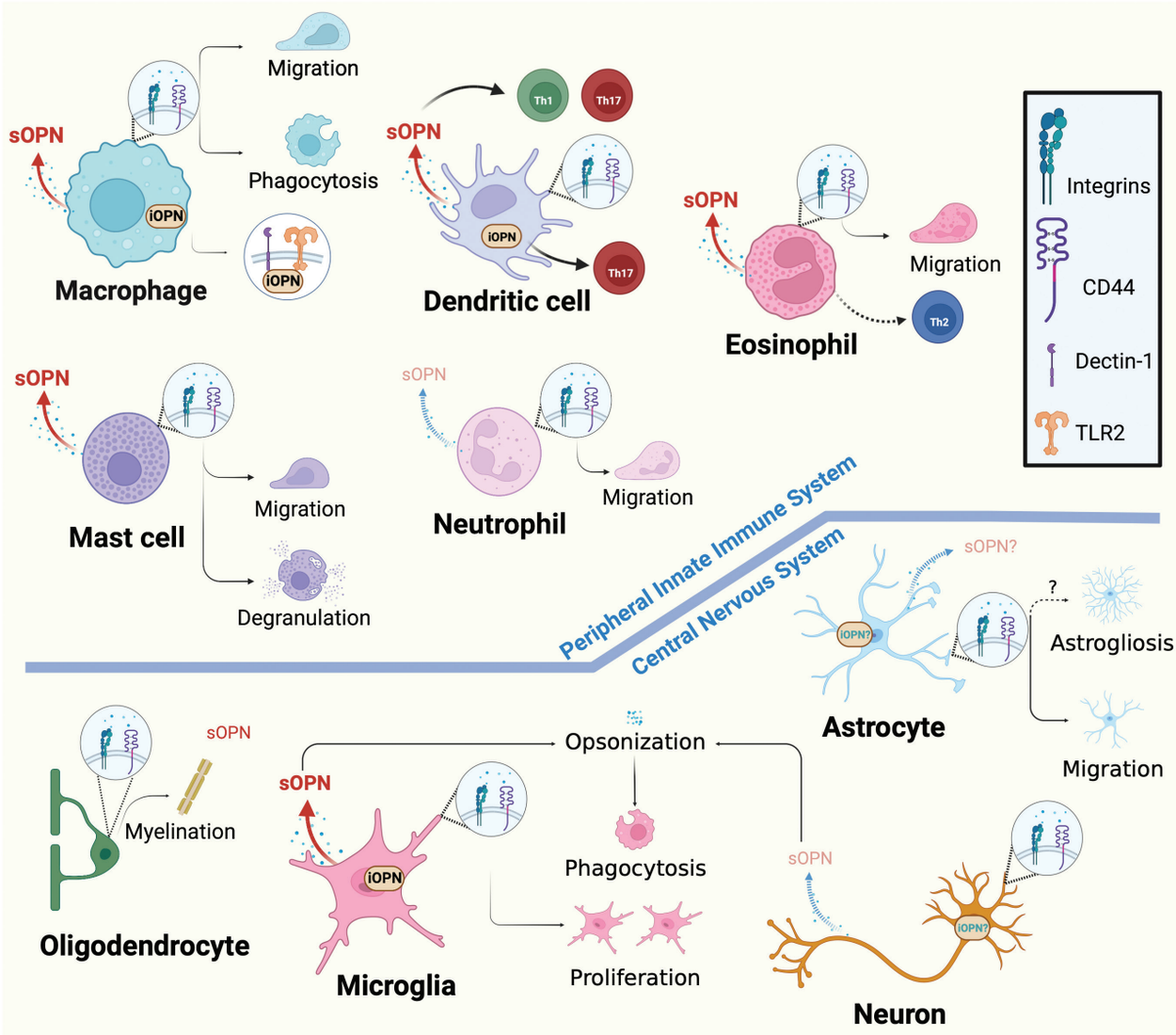

Fig. 1. OPN expression and its outcomes in various cell types. OPN receptors, integrins and CD44, are commonly expressed on the surface of cell types described here. sOPN has been extensively studied, particularly in peripheral immunity, and generally promotes cell migration and effector functions, such as opsonization and cell proliferation. iOPN has been studied in the immune system but not in the CNS-resident cells. For some cells, mainly CNS-resident cells other than microglia, OPN expression was reported but not to the extent of sOPN and/or iOPN. For astrocytes and neurons, we interpreted that sOPN and/or iOPN may be produced based on data from published articles (indicated as 'sOPN?' or 'iOPN?'). Oligodendrocytes and OPC detect sOPN, but the source of sOPN is unclear *in vivo*. Degenerated neurons are considered to be opsonized by sOPN for phagocytosis by microglia.

and its C-terminal fragment translocates into the nucleus, leading to upregulating the transcriptional activity of a pro-apoptotic p53 molecule and apoptosis of HeLa cells (70). In mouse T follicular helper (Tfh) cells, a molecule p85α chaperones iOPN for its nuclear entry (67). Nuclear iOPN promotes functional Tfh differentiation by interacting and stabilizing Bcl-6 (67). Another study showed that nuclear iOPN in human cell lines interacts with HIF2α to eventually impact lung metastasis in patients (36).

OPN in peripheral innate immunity

Currently, many articles describing OPN expression by all kinds of leukocytes are available, suggesting the critical involvement of OPN in various immune responses (Fig. 1 focuses on myeloid cells and CNS-resident cells only). OPN was studied in autoimmune diseases [such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and multiple sclerosis (MS)], various types of infections, cancer and other pathological conditions. OPN in innate immunity is critically involved in pathogenesis and health.

Macrophages/monocytes

Among cell types in the innate immune system, OPN may have been most intensively studied in macrophages. Many early studies already showed that sOPN plays critical and pleiotropic roles in mediating macrophage functions, including cell

migration, proliferation, phagocytosis and MMP production (71–75). Furthermore, exposure of monocytes to sOPN promotes their differentiation to macrophages through a sOPN-integrin interaction (76). There are many studies of sOPN and macrophages, and we would like to leave further discussions about sOPN and macrophages to outstanding review articles (77–79).

iOPN has also been reported to be involved in immune regulation. iOPN supports receptor clustering of dectin-1, TLR2 and mannose receptor on the surface of macrophages to detect a fungus, *Pneumocystis murina*, and enhance collaboration of dectin-1/TLR2 signaling pathways to elicit strong host cell responses (33). In peritoneal macrophages, iOPN also participates in the innate anti-viral response by inhibiting ubiquitination and degradation of TRAF3 (68). iOPN in macrophages also negatively controls TLR4 signaling through CD40 ligated by CD40L on T cells (32). Here, iOPN plays a role as an adaptor molecule to couple IRAK1 and TRAF2 to promote IRAK1 sumoylation (32). The outcome of the iOPN's function during CD40L/TLR4 co-stimulation is promoting IL-10 expression and downregulation of TNFα to protect hosts from LPS endotoxemia (32).

Dendritic cells

One of the initial studies on OPN and dendritic cells (DCs) demonstrated that sOPN enhances the production of TNFα and IL-12 by human DCs (80), resulting in enhanced stimulation of allogeneic T cells and Th1 polarization (80). Another study showed that sOPN secreted by $CD103^-$ DCs mediated exacerbation of TNBS-induced experimental colitis by promoting Th17 and Th1 polarization in mesenteric lymph nodes (81). The study also demonstrated that the SLAYGLR motif of sOPN ligates α9 integrins to elicit the pathogenic role of sOPN in colitis (81). iOPN expressed in conventional DCs (cDCs) promotes Th17 responses in a model of MS by suppressing IL-27 expression, suggesting the pro-inflammatory role of sOPN (14). Yet, iOPN expression in cDCs is negatively controlled by type-1 interferon receptor (IFNAR) (14). Here, another subset of DCs, pDCs, potently produce IFN-I; and iOPN promotes the IFN-I production downstream of TLR9 by interacting with MyD88 to promote IRF7 activation (9). Thus, iOPN and IFN-I are mutually controlling their expression in cDCs and pDCs. A different study on cancer showed that a hypoxic environment within tumors promotes OPN expression by DCs, and DC-derived sOPN promotes tumor cell migration in a tissue culture setting (82). OPN also modulates a cross-talk between DCs and mesenchymal stem cells (MSCs) (83). Resting MSCs promote OPN expression by DCs in their co-culture, but activated MSCs suppress OPN production by DCs in the presence of pro-inflammatory cytokines (IL-1β, IL-6 and TNFα) (83). This suggests the DC-derived sOPN production is modulated by cytokine milieu. Many other studies demonstrated DCs as an OPN producer and an OPN target, and we leave further discussion on this another excellent review article (84) focusing on that aspect.

Neutrophils

Neutrophils are the most abundant blood-circulating leukocytes, and their destructive function against pathogenic microbes is occasionally detrimental to host cells. Mouse neutrophils express OPN, but the expression was 75-fold less than the RAW264.7 macrophage cell line and 25-fold less than peritoneal macrophages (85), suggesting that neutrophils may not be as potent OPN producers as macrophages. Instead, sOPN is detected by neutrophils and enhances neutrophil migration in a CD44-independent fashion (86). Yet, OPN-deficient neutrophils are not impaired with phagocytosis, generation of reactive oxygen species, MMP-9 production and cytokine production, such as IFN-γ, TNF-α, IL-1β and IL-4 (85). Interestingly, OPN-mediated enhancement of neutrophil migration could favor pathogen dissemination. For example, OPN promotes the migration of neutrophils, infected by West Nile virus (WNV), into the CNS and causes encephalitis and meningitis (87). Indeed, *Spp1*$^{-/-}$ mice showed an improved survival rate after WNV infection, and the WNV viral burden in the brain was significantly decreased (87). Thus, sOPN could also promote host-detrimental roles of neutrophils in microbial infections.

Eosinophils

Eosinophils express OPN. Studies indicated that OPN and eosinophils are a detrimental combination in type-2 diseases. Resting eosinophils secrete OPN, which promotes airway angiogenesis in asthma (88). Another study also suggested the association of OPN with severe chronic rhinosinusitis (89). A mouse lung fibrosis model using house dust mite indicated OPN expression triggered airway fibrosis through Th2 responses (90). In patients with asthma, a positive correlation between the sputum eosinophil frequency and the sputum sOPN levels was found (91), suggesting that sOPN in the sputum might be a diagnostic marker. Antibody neutralization of sOPN limited *ex vivo* migration of human eosinophils by blocking OPN's interactions with integrin α4 and attenuated eosinophilic inflammation in a mouse asthma model (91). Thus, eosinophils can be an sOPN source and responders to OPN with detrimental impacts on pulmonary diseases. The impact of iOPN in eosinophils is not known yet.

Mast cells

Mast cells are found in mucosal sites, including the gastrointestinal tract, the lung epithelium, and other vascularized mucosal tissues. Mast cells carry large granules containing inflammatory mediators, such as histamine, heparin, proteases and pro-inflammatory cytokines (92). A study demonstrated that a basal level of OPN is produced by murine fetal skin-derived cultured mast cells (FSMCs) and enhances IgE-mediated degranulation in FSMCs (93). In addition, migration of FSMCs is also enhanced by sOPN by its interaction with αv integrins (93). Another study, using IgE-activated human cultured mast cells (HCMCs) derived from peripheral blood $CD34^+$ progenitors, showed that sOPN enhanced HCMC adhesion and suppressed cytokine productions such as IL-5, IL-8 and TNFα (94). Therefore, CD44 and integrins on mast cells are expected to mediate the impacts of sOPN on mast cell function and migration, but the roles of iOPN are still unclear.

OPN in the CNS

Recent single-cell RNA sequence studies indicated upregulation of *Spp1* mRNA, as well as OPN expression,

during CNS development and various CNS disease models (24, 25, 95), which indicates the roles of OPN in CNS development, pathology, and health. However, OPN functions in the CNS are still largely unexplored. In this section, we will focus on the expression and known functions of OPN in CNS-resident cells (Fig. 1).

Microglia

Microglia may be the most intensively studied cell type of CNS-resident cells regarding OPN. Microglia are macrophage-like cells derived from primitive macrophage progenitors in the yolk sac at the early stage of embryonic development (96). Upregulation of *Spp1* and its product, OPN, was observed in microglia in both humans and mice with neurodegenerative diseases, such as AD and MS, and in a cuprizone-induced demyelination animal model (24, 95, 97). Elevated *Spp1* expression in microglia was also identified in the early postnatal mouse brain, in which microglia are crucial for regulating the growth and pruning of synapses under a homeostatic condition (25, 98, 99). *Spp1* upregulation in microglia also appears to be age-related, on the basis of the increase of *Spp1* transcripts in microglia from human individuals over 50 years old (100). Of note, age-related *Spp1* expression was previously reported in T cells too (22). Studies showed that microglial *Spp1* expression is upregulated in microglia subsets (98, 100), which are described as disease-associated microglia (DAM) in AD (24), microglial neurodegenerative (MGnD)-neurodegenerative microglia highly expressing *Apoe* (95), CD11c$^+$ microglia providing survival factors to support myelin development (101) and the axonal tract-associated and proliferative region-associate microglia (ATM and PAM, respectively) that appear in the early postnatal development (25, 99). These microglia subsets described in different studies appear to have some overlap in gene expression patterns and possibly in their functions.

Microglia express OPN receptors, such as CD44 and integrins; thus, an autocrine impact of sOPN is likely. A study on ischemic brain injury demonstrated detailed imaging of *Spp1* mRNA and OPN protein in the brain, showing clear upregulation of *Spp1* mRNA expression in Iba-1$^+$ cells (i.e. macrophages and/or microglia) 3 days after ischemia induction at latest and lasting until the last day of evaluation on 28 days (102). OPN protein was detected in the extracellular matrix (ECM) of ischemic cores, but cytoplasmic OPN was apparent on day 28 (102), suggesting that sOPN is released at the early stage and iOPN starts to accumulate in the cytoplasm later. The study also suggested by using electron microscopy that sOPN accumulates at the surface of degenerated neurons, and microglia and/or macrophages engulf fragments of degenerated neurons (102). Indeed, OPN's role as an opsonin for microglia was suggested (103). Recombinant OPN (rOPN; i.e. sOPN) promotes the proliferation of porcine microglia *ex vivo*, and sOPN neutralization using antibody abolishes the proliferation (104), suggesting sOPN's role in microglia proliferation. However, rOPN treatment of LPS-stimulated mouse primary microglia does not enhance microglia proliferation; instead, cells treated with rOPN show an anti-inflammatory phenotype with reduced iNOS expression and decreased pro-inflammatory cytokine expression, such as IL-6 and TNFα (105). These results suggest that the role of OPN on microglia is complex, and the outcomes appear to be context-dependent. In fact, the complexity of OPN-mediated outcomes is not specific to microglia, but other immune cells exhibit the multi-faceted roles of OPN in immunological responses. To clarify the complexity, dissecting the differential role of OPN isoforms may be an important next step to be elucidated.

Astrocytes

Primary astrocyte culture showed expression of OPN (106). OPN expression in astrocytes was also detected in demyelinated brain areas in a cuprizone demyelination model (97). Elevated OPN expression was also demonstrated in intratumoral astrocytes (107). Fluorescent microscope images from these studies suggested that OPN is localized in the cytoplasm because of its colocalization with glial fibrillary acidic protein (GFAP) (97, 107), but this point is not discussed in the articles. rOPN treatment of astrocytes in tissue culture showed enhanced proliferation and survival particularly with pre-treatment with pro-inflammatory cytokines (TNFα, IL-1β and IFN-γ), although the impact of sOPN on proliferation and survival was modest without the cytokine pre-treatment, despite a significant increase in the astrocyte migration (108). OPN-deficient astrocytes reduced expression of GFAP, when stimulated with LPS *ex vivo* and OPN-deficient mice had reduced reactive astrocytes (106), suggesting the involvement of OPN in promoting astrogliosis. OPN-deficient mice with ischemic stroke demonstrated that astrocytes failed to extend their processes (109). The same study also showed that sOPN promoted the re-establishment of the blood-brain barrier (BBB) after ischemic stroke. The study concluded that the source of OPN was macrophages using a clodronate liposome approach (109). (Here, cell depletion with clodronate liposome is not specific; thus, identification of OPN producers requires more precise approaches, such as mouse genetic models.) Yet, these studies strongly suggested the impacts of sOPN on astrocytes. It is still unknown which mechanism enhances OPN expression in astrocytes, which sOPN receptors on astrocytes exert the impacts on astrocytes, and if iOPN also plays some roles in astrocytes.

Neurons

Although microglia and astrocytes can be sources of OPN in the CNS, other CNS resident cells, such as neurons, particularly motor neurons (MNs), are also capable of expressing OPN (110). OPN is detected in the cytoplasm (i.e. iOPN) in a majority of alpha MNs in the spinal cord of SOD^{G93A} amyotrophic lateral sclerosis (ALS) model mice and also in extracellular spaces as granular deposits (i.e. sOPN) (103). sOPN accumulated in ECM is detected by the αvβ3 integrin on MNs and upregulates MMP-9 expression by the cells, resulting in second-wave neurodegeneration in the ALS mouse model (103). Another study using a rat acute striatal injury model suggested that OPN granular deposits are from degenerated neurites and are engulfed by astrocytes (and microglia) in astroglial scar (111). In contrast, a study using macaques showed OPN localization in the neuron cell bodies but neurons may not secrete detectable levels of OPN (112).

Oligodendrocyte and oligodendrocyte precursor cells

In a cuprizone demyelination model, OPN was not identified in mature oligodendrocytes and oligodendrocyte precursor cells (OPCs), although OPN is produced by microglia and astrocytes in the corpus callosum in the model (97). Yet, rOPN induced cell proliferation of OPC-like cell lines *ex vivo*, and stimulated myelin basic protein (MBP) synthesis and myelin sheath formation (97). Thus, OPN may protect myelin, and the OPN-mediated outcomes may involve oligodendrocytes.

Conclusions

Because OPN and its transcript (*Spp1*) are often highly expressed and detected in many pathogenic conditions, OPN has been generally considered to be a pro-inflammatory and pathogenic molecule. However, recent studies indicate that OPN is multi-faceted. Indeed, multiple studies using OPN knockout mice showed that OPN could be pro- and anti-inflammatory, as well as detrimental and beneficial even in similar disease settings (74). As discussed in this review, OPN has diverse functions not only because of its involvement in numerous biological settings but also because of its various forms as mature proteins. For example, alternative intron splicing and translation initiation generate multiple isoforms. OPN also gains functions when cleaved by various proteases. In addition, various levels of posttranslational modifications of OPN in different cell types contribute to altering OPN's function. Therefore, it is essential to understand how and when the impact of OPN becomes host beneficial or harmful by considering various forms of OPN. This aspect is critically important, especially because OPN is a potential pharmaceutical target in many diseases.

Functions of OPN in the CNS have not been largely explored. In addition to considering various forms of OPN to understand its function, it is critical to identify specific roles of OPN to various CNS cell types.

Funding

This work was supported by National Institutes of Health (R01-AI088100, R01-NS120417, and R01-AI160737) to M.L.S.

Author contributions

E.Y.-H.L., W.X. and M.L.S. wrote the manuscript, and N.A. edited the manuscript.

Conflicts of interest statement: the authors declare no conflicts of interest.

References

1 Fisher, L. W. and Fedarko, N. S. 2003. Six genes expressed in bones and teeth encode the current members of the SIBLING family of proteins. *Connect. Tissue Res.* 44(Suppl 1):33. doi:10.1080/03008200390152061.
2 Bellahcene, A., Castronovo, V., Ogbureke, K. U. *et al.* 2008. Small integrin-binding ligand N-linked glycoproteins (SIBLINGs): multifunctional proteins in cancer. *Nat. Rev. Cancer* 8:212.
3 Platzer, G., Schedlbauer, A., Chemelli, A. *et al.* 2011. The metastasis-associated extracellular matrix protein osteopontin forms transient structure in ligand interaction sites. *Biochemistry* 50:6113. doi:10.1021/bi200291e.
4 Wang, J., Li, X., Wang, Y. *et al.* 2022. Osteopontin aggravates acute lung injury in influenza virus infection by promoting macrophages necroptosis. *Cell Death Discov.* 8:97.
5 Sampayo-Escobar, V., Green, R., Cheung, M. B. *et al.* 2018. Osteopontin plays a pivotal role in increasing severity of respiratory syncytial virus infection. *PLoS One* 13:e0192709. doi:10.1371/journal.pone.0192709.
6 van der Windt, G. J. W., Hoogendijk, A. J., Schouten, M. *et al.* 2011. Osteopontin impairs host defense during pneumococcal pneumonia. *J. Infect. Dis.* 203:1850. doi:10.1093/infdis/jir185.
7 Koguchi, Y., Kawakami, K., Uezu, K. *et al.* 2003. High plasma osteopontin level and its relationship with interleukin-12-mediated type 1 T helper cell response in tuberculosis. *Am. J. Respir. Crit. Care Med.* 167:1355. doi:10.1164/rccm.200209-1113oc.
8 Rittling, S. R., Zetterberg, C., Yagiz, K. *et al.* 2010. Protective role of osteopontin in endodontic infection. *Immunology.* 129:105.
9 Shinohara, M. L., Lu, L., Bu, J. *et al.* 2006. Osteopontin expression is essential for interferon-alpha production by plasmacytoid dendritic cells. *Nat. Immunol.* 7:498.
10 Kasetty, G., Bhongir, R. K. V., Papareddy, P. *et al.* 2019. Osteopontin protects against pneumococcal infection in a murine model of allergic airway inflammation. *Allerg.* 74:663.
11 Trinh, H. K. T., Nguyen, T. V. T., Kim, S. H. *et al.* 2020. Osteopontin contributes to late-onset asthma phenotypes in adult asthma patients. *Exp. Mol. Med.* 52:253. doi:10.1038/s12276-020-0376-2.
12 Samitas, K., Zervas, E., Vittorakis, S. *et al.* 2011. Osteopontin expression and relation to disease severity in human asthma. *Eur. Respir. J.* 37:331.
13 Ohshima, S., Yamaguchi, N., Nishioka, K. *et al.* 2002. Enhanced local production of osteopontin in rheumatoid joints. *J. Rheumatol.* 29:2061.
14 Shinohara, M. L., Kim, J. H., Garcia, V. A. *et al.* 2008. Engagement of the type I interferon receptor on dendritic cells inhibits T helper 17 cell development: role of intracellular osteopontin. *Immunity* 29:68. doi:10.1016/j.immuni.2008.05.008.
15 Miyazaki, T., Ono, M., Qu, W. M. *et al.* 2005. Implication of allelic polymorphism of osteopontin in the development of lupus nephritis in MRL/lpr mice. *Eur. J. Immunol.* 35:1510. doi:10.1002/eji.200425672.
16 Weber, G. F. and Cantor, H. 2001. Differential roles of osteopontin/Eta-1 in early and late lpr disease. *Clin. Exp. Immunol.* 126:578. doi:10.1046/j.1365-2249.2001.01702.x.
17 Cui, G., Chen, J., Wu, Z. *et al.* 2019. Thrombin cleavage of osteopontin controls activation of hepatic stellate cells and is essential for liver fibrogenesis. *J. Cell. Physiol.* 234:8988.
18 Oldberg, A., Franzen, A. and Heinegard, D. 1986. Cloning and sequence analysis of rat bone sialoprotein (osteopontin) cDNA reveals an Arg-Gly-Asp cell-binding sequence. *Proc. Natl Acad. Sci. U.S.A.* 83:8819.
19 Sodek, J., Chen, J., Nagata, T. *et al.* 1995. Regulation of osteopontin expression in osteoblasts. *Ann. N. Y. Acad. Sci.* 760:223.
20 Patarca, R., Freeman, G. J., Singh, R. P. *et al.* 1989. Structural and functional studies of the early T lymphocyte activation 1 (Eta-1) gene. Definition of a novel T cell-dependent response associated with genetic resistance to bacterial infection. *J. Exp. Med.* 170:145.
21 Shinohara, M. L., Jansson, M., Hwang, E. S. *et al.* 2005. T-bet-dependent expression of osteopontin contributes to T cell polarization. *Proc. Natl Acad. Sci. U.S.A.* 102:17101.
22 Shimatani, K., Nakashima, Y., Hattori, M. *et al.* 2009. PD-1+ memory phenotype CD4+ T cells expressing C/EBPalpha underlie T cell immunodepression in senescence and leukemia. *Proc. Natl Acad. Sci. U.S.A.* 106:15807.
23 Deczkowska, A., Keren-Shaul, H., Weiner, A. *et al.* 2018. Disease-associated microglia: a universal immune sensor of neurodegeneration. *Cell* 173:1073. doi:10.1016/j.cell.2018.05.003.
24 Keren-Shaul, H., Spinrad, A., Weiner, A. *et al.* 2017. A unique microglia type associated with restricting development of Alzheimer's disease. *Cell* 169:1276. doi:10.1016/j.cell.2017.05.018.
25 Hammond, T. R., Dufort, C., Dissing-Olesen, L. *et al.* 2019. Single-cell RNA sequencing of microglia throughout the mouse lifespan

and in the injured brain reveals complex cell-state changes. *Immunity* 50:253.
26 Weber, G. F. 2018. The phylogeny of osteopontin-analysis of the protein sequence. *Int. J. Mol. Sci.* 19:2557.
27 Sorensen, E. S., Hojrup, P. and Petersen, T. E. 1995. Posttranslational modifications of bovine osteopontin: identification of twenty-eight phosphorylation and three O-glycosylation sites. *Protein Sci.* 4:2040.
28 Christensen, B., Nielsen, M. S., Haselmann, K. F. *et al.* 2005. Post-translationally modified residues of native human osteopontin are located in clusters: identification of 36 phosphorylation and five O-glycosylation sites and their biological implications. *Biochem. J.* 390:285.
29 Shinohara, M. L., Kim, H. J., Kim, J. H. *et al.* 2008. Alternative translation of osteopontin generates intracellular and secreted isoforms that mediate distinct biological activities in dendritic cells. *Proc. Natl Acad. Sci. U.S.A.* 105:7235.
30 Zohar, R., Lee, W., Arora, P. *et al.* 1997. Single cell analysis of intracellular osteopontin in osteogenic cultures of fetal rat calvarial cells. *J. Cell. Physiol.* 170:88.
31 Zohar, R., Suzuki, N., Suzuki, K. *et al.* 2000. Intracellular osteopontin is an integral component of the CD44-ERM complex involved in cell migration. *J. Cell. Physiol.* 184:118.
32 Inoue, M., Arikawa, T., Chen, Y. H. *et al.* 2014. T cells down-regulate macrophage TNF production by IRAK1-mediated IL-10 expression and control innate hyperinflammation. *Proc. Natl Acad. Sci. U.S.A.* 111:5295.
33 Inoue, M., Moriwaki, Y., Arikawa, T. *et al.* 2011. Cutting edge: critical role of intracellular osteopontin in antifungal innate immune responses. *J. Immunol.* 186:19.
34 Kanayama, M., Xu, S., Danzaki, K. *et al.* 2017. Skewing of the population balance of lymphoid and myeloid cells by secreted and intracellular osteopontin. *Nat. Immunol.* 18:973. doi:10.1038/ni.3791.
35 Junaid, A., Moon, M. C., Harding, G. E. *et al.* 2007. Osteopontin localizes to the nucleus of 293 cells and associates with polo-like kinase-1. *Am. J. Physiol. Cell Physiol.* 292:C919.
36 Jia, R., Liang, Y., Chen, R. *et al.* 2016. Osteopontin facilitates tumor metastasis by regulating epithelial-mesenchymal plasticity. *Cell Death Dis.* 7:e2564. doi:10.1038/cddis.2016.422.
37 Assidi, M., Gomaa, W., Jafri, M. *et al.* 2019. Prognostic value of Osteopontin (SPP1) in colorectal carcinoma requires a personalized molecular approach. *Tumour Biol.* 41:1010428319863621010428319863627. doi:10.1177/1010428319863627.
38 Sodek, J., Ganss, B., and McKee, M. D. 2000. Osteopontin. *Crit. Rev. Oral Biol. Med.* 11:279.
39 Yokosaki, Y., Tanaka, K., Higashikawa, F. *et al.* 2005. Distinct structural requirements for binding of the integrins alphavbeta6, alphavbeta3, alphavbeta5, alpha5beta1 and alpha9beta1 to osteopontin. *Matrix Biol.* 24:418.
40 Kon, S. and Uede, T. 2018. The role of alpha9beta1 integrin and its ligands in the development of autoimmune diseases. *J. Cell Commun. Signal.* 12:333.
41 Ito, K., Kon, S., Nakayama, Y. *et al.* 2009. The differential amino acid requirement within osteopontin in alpha4 and alpha9 integrin-mediated cell binding and migration. *Matrix Biol.* 28:11.
42 Barry, S. T., Ludbrook, S. B., Murrison, E. *et al.* 2000. Analysis of the alpha4beta1 integrin-osteopontin interaction. *Exp. Cell Res.* 258:342. doi:10.1006/excr.2000.4941.
43 Green, P. M., Ludbrook, S. B., Miller, D. D. *et al.* 2001. Structural elements of the osteopontin SVVYGLR motif important for the interaction with alpha(4) integrins. *FEBS Lett.* 503:75. doi:10.1016/s0014-5793(01)02690-4.
44 Bayless, K. J. and Davis, G. E. 2001. Identification of dual alpha 4beta1 integrin binding sites within a 38 amino acid domain in the N-terminal thrombin fragment of human osteopontin. *J. Biol. Chem.* 276:13483. doi:10.1074/jbc.m011392200.
45 Yamamoto, N., Sakai, F., Kon, S. *et al.* 2003. Essential role of the cryptic epitope SLAYGLR within osteopontin in a murine model of rheumatoid arthritis. *J. Clin. Investig.* 112:181. doi:10.1172/jci17778.
46 Christensen, B., Schack, L., Klaning, E. *et al.* 2010. Osteopontin is cleaved at multiple sites close to its integrin-binding motifs in milk and is a novel substrate for plasmin and cathepsin D. *J. Biol. Chem.* 285:7929.
47 Shao, Z., Morser, J. and Leung, L. L. K. 2014. Thrombin cleavage of osteopontin disrupts a pro-chemotactic sequence for dendritic cells, which is compensated by the release of its pro-chemotactic C-terminal fragment. *J. Biol. Chem.* 289:27146.
48 Lund, S. A., Giachelli, C. M. and Scatena, M. 2009. The role of osteopontin in inflammatory processes. *J. Cell Commun. Signal.* 3:311. doi:10.1007/s12079-009-0068-0.
49 O'Regan, A. 2003. The role of osteopontin in lung disease. *Cytokine Growth Factor Rev.* 14:479. doi:10.1016/s1359-6101(03)00055-8.
50 Nishimichi, N., Higashikawa, F., Kinoh, H. H. *et al.* 2009. Polymeric osteopontin employs integrin alpha9beta1 as a receptor and attracts neutrophils by presenting a de novo binding site. *J. Biol. Chem.* 284:14769. doi:10.1074/jbc.m901515200.
51 Nishimichi, N., Hayashita-Kinoh, H., Chen, C. *et al.* 2011. Osteopontin undergoes polymerization in vivo and gains chemotactic activity for neutrophils mediated by integrin alpha9beta1. *J. Biol. Chem.* 286:11170. doi:10.1074/jbc.m110.189258.
52 Christensen, B., Zachariae, E. D., Scavenius, C. *et al.* 2016. Transglutaminase 2-catalyzed intramolecular cross-linking of osteopontin. *Biochemistry* 55:294.
53 Addison, W. N., Masica, D. L., Gray, J. J. *et al.* 2010. Phosphorylation-dependent inhibition of mineralization by osteopontin ASARM peptides is regulated by PHEX cleavage. *J. Bone Miner. Res.* 25:695.
54 Barros, N. M., Hoac, B., Neves, R. L. *et al.* 2013. Proteolytic processing of osteopontin by PHEX and accumulation of osteopontin fragments in Hyp mouse bone, the murine model of X-linked hypophosphatemia. *J. Bone Miner. Res.* 28:688. doi:10.1002/jbmr.1766.
55 Hoac, B., Ostergaard, M., Wittig, N. K. *et al.* 2020. Genetic ablation of osteopontin in osteomalacic Hyp mice partially rescues the deficient mineralization without correcting hypophosphatemia. *J. Bone Miner. Res.* 35:2032.
56 Christensen, B., Kazanecki, C. C., Petersen, T. E. *et al.* 2007. Cell type-specific post-translational modifications of mouse osteopontin are associated with different adhesive properties. *J. Biol. Chem.* 282:19463.
57 Christensen, B., Karlsen, N. J., Jorgensen, S. D. S. *et al.* 2020. Milk osteopontin retains integrin-binding activity after in vitro gastrointestinal transit. *J. Dairy Sci.* 103:42.
58 Kariya, Y., Oyama, M., Kariya, Y. *et al.* 2021. Phosphorylated osteopontin secreted from cancer cells induces cancer cell motility. *Biomolecules* 11:1323. doi:10.3390/biom11091323.
59 Schytte, G. N., Christensen, B., Bregenov, I. *et al.* 2020. FAM20C phosphorylation of the RGDSVVYGLR motif in osteopontin inhibits interaction with the alphavbeta3 integrin. *J. Cell. Biochem* 121:4809.
60 Weber, G. F., Ashkar, S., Glimcher, M. J. *et al.* 1996. Receptor-ligand interaction between CD44 and osteopontin (Eta-1). *Science* 271:509. doi:10.1126/science.271.5248.509.
61 Katagiri, Y. U., Sleeman, J., Fujii, H. *et al.* 1999. CD44 variants but not CD44s cooperate with beta1-containing integrins to permit cells to bind to osteopontin independently of arginine-glycine-aspartic acid, thereby stimulating cell motility and chemotaxis. *Cancer Res.* 59:219.
62 Goodison, S., Urquidi, V. and Tarin, D. 1999. CD44 cell adhesion molecules. *Mol. Pathol.* 52:189. doi:10.1136/mp.52.4.189.
63 Raineri, D., Dianzani, C., Cappellano, G. *et al.* 2020. Osteopontin binds ICOSL promoting tumor metastasis. *Commun. Biol.* 3:615.
64 Raineri, D., Cappellano, G., Vilardo, B. *et al.* 2021. Inducible T-cell costimulator ligand plays a dual role in melanoma metastasis upon binding to osteopontin or inducible T-cell costimulator. *Biomedicines* 10:51. doi:10.3390/biomedicines10010051.
65 Inoue, M. and Shinohara, M. L. 2015. Cutting edge: role of osteopontin and integrin alphav in T cell-mediated anti-inflammatory responses in endotoxemia. *J. Immunol.* 194:5595. doi:10.4049/jimmunol.1500623.
66 Leavenworth, J. W., Verbinnen, B., Wang, Q. *et al.* 2015. Intracellular osteopontin regulates homeostasis and function of natural killer cells. *Proc. Natl Acad. Sci. U.S.A.* 112:494.
67 Leavenworth, J. W., Verbinnen, B., Yin, J. *et al.* 2015. A p85alpha-osteopontin axis couples the receptor ICOS to sustained Bcl-6

expression by follicular helper and regulatory T cells. *Nat. Immunol.* 16:96.
68 Zhao, K., Zhang, M., Zhang, L. *et al.* 2016. Intracellular osteopontin stabilizes TRAF3 to positively regulate innate antiviral response. *Sci. Rep.* 6:23771.
69 Dong, Q., Zhu, X., Dai, C. *et al.* 2016. Osteopontin promotes epithelial-mesenchymal transition of hepatocellular carcinoma through regulating vimentin. *Oncotarget* 7:12997. doi:10.18632/oncotarget.7016.
70 Kim, H. J., Lee, H. J., Jun, J. I. *et al.* 2009. Intracellular cleavage of osteopontin by caspase-8 modulates hypoxia/reoxygenation cell death through p53. *Proc. Natl Acad. Sci. U.S.A.* 106:15326.
71 Weber, G. F., Zawaideh, S., Hikita, S. *et al.* 2002. Phosphorylation-dependent interaction of osteopontin with its receptors regulates macrophage migration and activation. *J. Leukoc. Biol.* 72:752. doi:10.1189/jlb.72.4.752.
72 Nystrom, T., Duner, P., and Hultgardh-Nilsson, A. 2007. A constitutive endogenous osteopontin production is important for macrophage function and differentiation. *Exp. Cell Res.* 313:1149. doi:10.1016/j.yexcr.2006.12.026.
73 Grassinger, J., Haylock, D. N., Storan, M. J. *et al.* 2009. Thrombin-cleaved osteopontin regulates hemopoietic stem and progenitor cell functions through interactions with alpha9beta1 and alpha4beta1 integrins. *Blood* 114:49.
74 Heilmann, K., Hoffmann, U., Witte, E. *et al.* 2009. Osteopontin as two-sided mediator of intestinal inflammation. *J. Cell. Mol. Med.* 13:1162.
75 Shimizu, S., Okuda, N., Kato, N. *et al.* 2010. Osteopontin deficiency impairs wear debris-induced osteolysis via regulation of cytokine secretion from murine macrophages. *Arthritis. Rheum* 62:1329. doi:10.1002/art.27400.
76 Ge, Q., Ruan, C. C., Ma, Y. *et al.* 2017. Osteopontin regulates macrophage activation and osteoclast formation in hypertensive patients with vascular calcification. *Sci. Rep.* 7:40253.
77 Tan, Y., Zhao, L., Yang, Y. G. *et al.* 2022. The role of osteopontin in tumor progression through tumor-associated macrophages. *Front. Oncol.* 12:953283.
78 Uede, T. 2011. Osteopontin, intrinsic tissue regulator of intractable inflammatory diseases. *Pathol. Int.* 61:265. doi:10.1111/j.1440-1827.2011.02649.x.
79 Wang, K. X. and Denhardt, D. T. 2008. Osteopontin: role in immune regulation and stress responses. *Cytokine Growth Factor Rev.* 19:333. doi:10.1016/j.cytogfr.2008.08.001.
80 Renkl, A. C., Wussler, J., Ahrens, T. *et al.* 2005. Osteopontin functionally activates dendritic cells and induces their differentiation toward a Th1-polarizing phenotype. *Blood* 106:946. doi:10.1182/blood-2004-08-3228.
81 Kourepini, E., Aggelakopoulou, M., Alissafi, T. *et al.* 2014. Osteopontin expression by CD103- dendritic cells drives intestinal inflammation. *Proc. Natl Acad. Sci. U.S.A.* 111:E856.
82 Yang, M., Ma, C., Liu, S. *et al.* 2009. Hypoxia skews dendritic cells to a T helper type 2-stimulating phenotype and promotes tumour cell migration by dendritic cell-derived osteopontin. *Immunology* 128:e237. doi:10.1111/j.1365-2567.2008.02954.x.
83 Scutera, S., Salvi, V., Lorenzi, L. *et al.* 2018. Adaptive regulation of osteopontin production by dendritic cells through the bidirectional interaction with mesenchymal stromal cells. *Front. Immunol.* 9:1207.
84 Del Prete, A., Scutera, S., Sozzani, S. *et al.* 2019. Role of osteopontin in dendritic cell shaping of immune responses. *Cytokine Growth Factor Rev.* 50:19. doi:10.1016/j.cytogfr.2019.05.004.
85 Koh, A., da Silva, A. P., Bansal, A. K. *et al.* 2007. Role of osteopontin in neutrophil function. *Immunology* 122:466.
86 van der Windt, G. J., Hoogerwerf, J. J., de Vos, A. F. *et al.* 2010. Osteopontin promotes host defense during Klebsiella pneumoniae-induced pneumonia. *Eur. Respir. J.* 36:1337.
87 Paul, A. M., Acharya, D., Duty, L. *et al.* 2017. Osteopontin facilitates West Nile virus neuroinvasion via neutrophil "Trojan horse" transport. *Sci. Rep.* 7:4722.
88 Puxeddu, I., Berkman, N., Ribatti, D. *et al.* 2010. Osteopontin is expressed and functional in human eosinophils. *Allergy* 65:168. doi:10.1111/j.1398-9995.2009.02148.x.
89 Yang, H. W., Park, J. H., Jo, M. S. *et al.* 2022. Eosinophil-derived osteopontin induces the expression of pro-inflammatory mediators and stimulates extracellular matrix production in nasal fibroblasts: the role of osteopontin in eosinophilic chronic rhinosinusitis. *Front. Immunol.* 13:777928.
90 Morimoto, Y., Hirahara, K., Kiuchi, M. *et al.* 2018. Amphiregulin-producing pathogenic memory T helper 2 cells instruct eosinophils to secrete osteopontin and facilitate airway fibrosis. *Immunity* 49:134. doi:10.1016/j.immuni.2018.04.023.
91 Takahashi, A., Kurokawa, M., Konno, S. *et al.* 2009. Osteopontin is involved in migration of eosinophils in asthma. *Clin. Exp. Allergy* 39:1152. doi:10.1111/j.1365-2222.2009.03249.x.
92 da Silva, E. Z., Jamur, M. C. and Oliver, C. 2014. Mast cell function: a new vision of an old cell. *J. Histochem. Cytochem.* 62:698.
93 Nagasaka, A., Matsue, H., Matsushima, H. *et al.* 2008. Osteopontin is produced by mast cells and affects IgE-mediated degranulation and migration of mast cells. *Eur. J. Immunol.* 38:489. doi:10.1002/eji.200737057.
94 Ng, C. W., Tam, I. Y. S., Sam, S. W. *et al.* 2018. Immobilized osteopontin enhances adhesion but suppresses cytokine release of anti-IgE activated human mast cells. *Front. Immunol.* 9:1109.
95 Krasemann, S., Madore, C., Cialic, R. *et al.* 2017. The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. *Immunity* 47:566. doi:10.1016/j.immuni.2017.08.008.
96 Ginhoux, F., Greter, M., Leboeuf, M. *et al.* 2010. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 330:841. doi:10.1126/science.1194637.
97 Selvaraju, R., Bernasconi, L., Losberger, C. *et al.* 2004. Osteopontin is upregulated during in vivo demyelination and remyelination and enhances myelin formation in vitro. *Mol. Cell. Neurosci.* 25:707.
98 Squarzoni, P., Oller, G., Hoeffel, G. *et al.* 2014. Microglia modulate wiring of the embryonic forebrain. *Cell Rep.* 8:1271.
99 Li, Q., Cheng, Z., Zhou, L. *et al.* 2019. Developmental heterogeneity of microglia and brain myeloid cells revealed by deep single-cell RNA sequencing. *Neuron* 101:207. doi:10.1016/j.neuron.2018.12.006.
100 Sankowski, R., Bottcher, C., Masuda, T. *et al.* 2019. Mapping microglia states in the human brain through the integration of high-dimensional techniques. *Nat. Neurosci.* 22:2098.
101 Wlodarczyk, A., Holtman, I. R., Krueger, M. *et al.* 2017. A novel microglial subset plays a key role in myelinogenesis in developing brain. *EMBO J.* 36:3292.
102 Shin, Y. J., Kim, H. L., Choi, J. S. *et al.* 2011. Osteopontin: correlation with phagocytosis by brain macrophages in a rat model of stroke. *Glia.* 59:413.
103 Morisaki, Y., Niikura, M., Watanabe, M. *et al.* 2016. Selective expression of osteopontin in ALS-resistant motor neurons is a critical determinant of late phase neurodegeneration mediated by matrix metalloproteinase-9. *Sci. Rep.* 6:27354.
104 Tambuyzer, B. R., Casteleyn, C., Vergauwen, H. *et al.* 2012. Osteopontin alters the functional profile of porcine microglia in vitro. *Cell Biol. Int.* 36:1233. doi:10.1042/cbi20120172.
105 Rabenstein, M., Vay, S. U., Flitsch, L. J. *et al.* 2016. Osteopontin directly modulates cytokine expression of primary microglia and increases their survival. *J. Neuroimmunol.* 299:130. doi:10.1016/j.jneuroim.2016.09.009.
106 Ikeshima-Kataoka, H., Matsui, Y. and Uede, T. 2018. Osteopontin is indispensable for activation of astrocytes in injured mouse brain and primary culture. *Neurol. Res.* 40:1071. doi:10.1080/01616412.2018.1517995.
107 Jang, T., Savarese, T., Low, H. P. *et al.* 2006. Osteopontin expression in intratumoral astrocytes marks tumor progression in gliomas induced by prenatal exposure to N-ethyl-N-nitrosourea. *Am. J. Pathol.* 168:1676. doi:10.2353/ajpath.2006.050400.
108 Vay, S. U., Olschewski, D. N., Petereit, H. *et al.* 2021. Osteopontin regulates proliferation, migration, and survival of astrocytes depending on their activation phenotype. *J. Neurosci. Res.* 99:2822. doi:10.1002/jnr.24954.
109 Gliem, M., Krammes, K., Liaw, L. *et al.* 2015. Macrophage-derived osteopontin induces reactive astrocyte polarization

and promotes re-establishment of the blood brain barrier after ischemic stroke. *Glia* 63:2198. doi:10.1002/glia.22885.

110 Misawa, H., Hara, M., Tanabe, S. *et al.* 2012. Osteopontin is an alpha motor neuron marker in the mouse spinal cord. *J. Neurosci. Res.* 90:732.

111 Riew, T. R., Kim, S., Jin, X. *et al.* 2019. Osteopontin and its spatiotemporal relationship with glial cells in the striatum of rats treated with mitochondrial toxin 3-nitropropionic acid: possible involvement in phagocytosis. *J. Neuroinflammation* 16:99.

112 Sugiyama, Y., Oishi, T., Yamashita, A. *et al.* 2019. Neuronal and microglial localization of secreted phosphoprotein 1 (osteopontin) in intact and damaged motor cortex of macaques. *Brain Res.* 1714:52. doi:10.1016/j.brainres.2019.02.021.