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The many faces of EMMPRIN - roles in neuroinflammation**Smriti M. Agrawal, and V. Wee Yong**

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Abstract

The central nervous system (CNS) is a relatively immune-privileged organ, wherein a well-instated barrier system (the blood-brain barrier) prevents the entry of blood cells into the brain with the exception of regular immune surveillance cells. Despite this tight security immune cells are successful in entering the CNS tissue where they result in states of neuroinflammation, tissue damage and cell death. Various components of the blood-brain barrier and infiltrating cells have been examined to better understand how blood cells are able to breach this secure barrier. Proteases, specifically matrix metalloproteinases (MMP), have been found to be the common culprits in most diseases involving neuroinflammation. MMPs secreted by infiltrating cells act specifically upon targets on various components of the blood-brain barrier, compromising this barrier and allowing cell infiltration into the CNS. Extracellular matrix metalloproteinase inducer (EMMPRIN) is an upstream inducer of several MMPs and is suggested to be the master regulator of MMP production in disease states such as cancer metastasis. EMMPRIN in the context of the CNS is still relatively understudied. In this review we will introduce EMMPRIN, discuss its ligands and roles in non-CNS conditions that can help implicate its involvement in CNS disorders, showcase its expression within the CNS in healthy and disease conditions, elucidate its ligands and receptors, and briefly discuss the emerging roles it plays in various diseases of the CNS involving inflammation.

EMMPRIN (CD147) is a member of the immunoglobulin (Ig) super family, comprised of two Ig-like domains which have a strong homology with both Ig V κ domain and the β -chain of the major histocompatibility complex (MHC) class II antigen (Figure 1). It is a cell surface glycoprotein expressed on numerous cell types . Originally identified in lung carcinoma cells as a secreted factor capable of stimulating collagenase activity in human fibroblasts, EMMPRIN has been identified independently in several different model systems resulting in a long list of acronyms for this molecule including tumor collagenase stimulatory factor (TCSF) , M6 (Human) , Neurothelin, 5A11 and HT7 (chicken) , OX47 and CE9 (rat) , and basigin and gp42 (human and mouse) . EMMPRIN was most recently shown to induce the production of several MMPs, resulting in its renaming to extracellular matrix metalloproteinase inducer, EMMPRIN .

EMMPRIN Structure and Homology

The full-length human EMMPRIN is comprised of 269 amino acid residues, wherein position 25 to 200 on the protein sequence comprise the extracellular domain; 77 residues from position 25-101 are the extracellular Ig-like domain I (ECI); and 95 residues from position 106 to 200 are the extracellular Ig-like domain II (ECII) (Figure1). The extracellular region contains three N-linked glycosylation sites, associated with 5-35 kDa glycosylation (glycan) content . The MMP induction site has been identified on the ECI domain of EMMPRIN and N-glycosylation at the Asn site (residue 44 in both mouse and human) of this domain is considered crucial in the MMP-stimulating activity (Figure 1). The single transmembrane domain sequence is completely conserved among species, and the presence of a charged residue, glutamic

acid, in the middle of this domain suggests possible association of this molecule with other transmembrane proteins

Human EMMPRIN is expressed as two differentially spliced isoforms and is characterized by the presence of two extracellular immunoglobulin-like domains, and a single transmembrane domain possessing a charged amino acid and a short cytoplasmic tail . A recently discovered retina specific isoform with 3 Ig domains has been renamed basigin-1, and the prototypical isoform, possessing two immunoglobulin domains, has been renamed basigin-2 . Both isoforms are variably glycosylated, resulting in significant alterations in EMMPRIN function and protein interactions. A 22 kDa active form of EMMPRIN is shed from cell surfaces by MMP-14 and secreted .

CNS tissue and cellular distribution of EMMPRIN in development and adult

EMMPRIN (identified as neurothelin) is present on chicken endothelial cells at the blood-brain barrier and also on pigment epithelial cells forming the blood-eye barrier . During chicken CNS development, EMMPRIN is expressed in neuroblasts, but disappears from neurons upon differentiation, becoming restricted to capillary endothelial cells in the adult brain . In contrast to chicken, in mouse and rat, EMMPRIN is differentially expressed in various sub-regions of the CNS suggesting that in mammals EMMPRIN has diverse functions other than contributions to the blood-brain barrier . EMMPRIN expression has been reported in the olfactory system, hippocampal formation, septal area, amygdala, thalamic anterior nuclei, hypothalamus, mesencephalic tegmentum, entorhinal cortex, and cingulate gyrus EMMPRIN is also expressed in the

retinal neuronal layers, the Vth layer of the cerebral neocortex, Purkinje cells of the cerebellum, several nuclei of the brain stem, and the gray matter of the spinal cord .

In adult mammalian CNS, EMMPRIN is thought to influence synapse formation , neuron–glial interactions as well augments the outgrowth of astrocyte processes. Astrocytes represent an important lactate source for neurons. They constitutively exhibit a high glycolytic rate and release large amounts of lactate . Monocarboxylate transporters (MCTs) are involved in mediating lactate transport and three MCT subtypes have been described on astrocytes . Two members of the MCT family of proteins, MCT1 and MCT4, are found to use EMMPRIN as a chaperone for their expression at the plasma membrane ; in this manner, the association of MCT with EMMPRIN is thought to affect development of neuron-glial interactions in the drosophila retina .

Roles of EMMPRIN, ligands and receptors: Lessons from non-CNS systems

Early observations of EMMPRIN expression in tumors have shed light on its role as an MMP inducer. EMMPRIN was initially identified following the result that interstitial collagenase (MMP-1) production was induced during the co-culturing of tumor cells and fibroblasts . Further studies revealed that EMMPRIN was capable of inducing the expression of several other MMPs, including MMP-2, MMP-3, MMP-9 and MMP-11 . It was also determined that either EMMPRIN expressing tumor cells or conditioned media from the same cells were equally capable of inducing MMP production in co-cultured fibroblast cells (Figure 2), indicating that both membrane bound and soluble EMMPRIN are equally capable of inducing MMP production . It has also been determined that EMMPRIN, similar to other Ig containing molecules, interacts

homotypically, and that this homotypic interaction, and its N-linked glycosylation, are both crucial for its role as an MMP inducer .

The signaling pathway used by EMMPRIN to stimulate MMPs is poorly understood. Previous studies reported that EMMPRIN stimulates MMP-1 or -2 using the p38 MAPK pathway , but stimulates MMP production in breast cancer cells using the phospholipase A₂ and 5-lipoxygenase pathways . Another potential signaling pathway may involve ErbB2.

Although an EMMPRIN specific receptor has not yet been identified, and besides the homotypic binding of EMMPRIN noted above, immunoprecipitation and immunofluorescence co-localisation experiments have identified two integrins ($\alpha3\beta1$ and $\alpha6\beta1$) that associate with EMMPRIN at points of cell-cell contact (Figure 1). These integrins, which are shown to preferentially bind to laminins that serve as the major constituents of the basement membrane, have been implicated in cell adhesion, metastasis and signaling. Through the association of EMMPRIN with integrin, it is possible that EMMPRIN may regulate the integrin/laminin association and in doing so, regulate basement membrane formation, cell adhesion, MMP induction, chemotaxis and proliferation. It is shown that within cultured insect cells, EMMPRIN/integrin interactions are essential for lamellipodia formation and within retinal cells. Disruption of the EMMPRIN–integrin interaction results in aberrant distribution of organelles, including mitochondria, nuclei and rough endoplasmic reticulum

There are likely undiscovered binding partners for EMMPRIN. Recently, platelet glycoprotein VI (GPVI) was shown to be a novel adhesion receptor for EMMPRIN. Prestimulated human platelet cells showed significantly enhanced rolling (but not

enhanced firm adhesion) on immobilised EMMPRIN-Fc, and pretreatment with anti-EMMPRIN or anti-GPVI blocking antibodies significantly reduced this rolling .

Other Substrates or binding partners of EMMPRIN

EMMPRIN has been shown to associate with various proteins, resulting in its varied functions. Both the transmembrane and cytoplasmic domains of EMMPRIN are thought to be critical for protein-protein interactions within the plasma membrane .

Cyclophilins are members of the immunophilin family of peptidyl-prolyl cis–trans isomerases and have been proposed to function as chaperones and mediators of protein folding . Recently, several reports implicated cyclophilins as regulators of EMMPRIN surface expression following the observation that cyclosporine A (CsA), which binds cyclophilins, adversely affected EMMPRIN surface expression . Aside from their functioning as protein chaperones, cyclophilins also mediate chemotaxis of neutrophils, eosinophils and T cells when secreted from cells in response to inflammatory stimuli (Figure 3A). Recently, EMMPRIN has been identified as being essential for cyclophilin-mediated signaling , wherein binding of extracellular cyclophilin A to EMMPRIN resulted in the activation of the ERK pathway and chemotaxis of cells. Interestingly, the association of cyclophilin A and EMMPRIN is further supported by findings that increased levels of cyclophilin A in synovial fluid and increased EMMPRIN levels on reactive neutrophils in rheumatoid arthritis patients correlated with the severity of disease (Figure 3B). Furthermore, cyclophilin A is incorporated into HIV-1 virions and has been shown to play a role in early viral infection . EMMPRIN has been shown to facilitate HIV-1 infection through its interaction with viral associated cyclophilin A, as explained

later on (Figure 3C).

Caveolin-1 has been shown to directly associate with the ECII domain of EMMPRIN. This association begins within the Golgi apparatus of the cell, with caveolin-1 specifically binding to and escorting the lower glycosylated forms of EMMPRIN to the plasma membrane, thus preventing the formation of the self-aggregating, highly glycosylated species of EMMPRIN which is known to be responsible for MMP production. Caveolin-1 is a negative regulator of EMMPRIN, wherein the direct association of Cav-1 with the second Ig domain of EMMPRIN was found to decrease EMMPRIN clustering. This hypothesis was supported by the generation of an EMMPRIN mutant lacking the second Ig domain, which was incapable of associating with caveolin-1, as well as following the RNAi knockdown of caveolin-1, both of which resulted in impaired MMP production (Tang and Hemler, 2004).

In breast cancer cells, thrombin is shown to cleave osteopontin; the cleaved osteopontin product is shown to bind to cyclophilin C and to EMMPRIN (Figure 3D). This in turn activates the Akt1 and 2 pathways, eventually resulting in MMP-2 activation and release from breast cancer cells. It is reported that EMMPRIN stimulates hyaluronan production by colon carcinoma and mammary carcinoma cells and that the effect of EMMPRIN on anchorage-independent growth is dependent on hyaluronan. Furthermore, EMMPRIN stimulates cell survival pathway signaling in a hyaluronan-dependent manner. These substrate interactions in part contribute to the complexity associated with the functions of EMMPRIN.

EMMPRIN null mice

EMMPRIN null mice are rarely born due to the embryonic loss around the time of implantation. It is estimated that only about 30% of embryos are born, of which 50% of these surviving pups die of interstitial pneumonia within the first week of life. Both males and females EMMPRIN null homozygotes are sterile, with impaired spermatogenesis observed in males, and the failure of fertilization and implantation in female mice. Retinal degeneration leading to dysfunction is also detected in EMMPRIN null mice. Only the two above phenotypes are a direct result of the lack of the bsg gene, the other observed phenotypes are attributed to linkage disequilibrium. Additionally, EMMPRIN null mice show various disorders related to neural behavior such as poor performance in learning and memory, decreased sensitivity to irritating odors, and increased sensitivity to electric foot shock. Moreover, EMMPRIN is expressed in the normal CNS and EMMPRIN null mice have several behavioral and sensory defects.

A role for EMMPRIN in disease states including of the CNS

Due to its MMP inducing capability EMMPRIN is a candidate for early upstream regulation of various diseases including tumors and inflammatory diseases, not all of which involve MMPs. EMMPRIN has been shown to play a role in leukocyte activation and migration, wherein circulating immune cell populations (including activated T and B lymphocytes, dendritic cells, monocytes and macrophage) highly express EMMPRIN. The mRNA pool from activated T cells revealed a 3.75-fold increase in EMMPRIN transcription. Aside from leukocyte activation, EMMPRIN augments tissue repair and remodeling and thus is a key player in various major diseases.

Low oxygen conditions exist in the brain during a stroke and during these conditions of hypoxia and ischemia, cells within the tissues become dependent upon

glycolysis for their energy metabolism. Lactic acid, a byproduct of glycolysis, is transported by the association of EMMPRIN and lactate transporters MCT-1 and MCT-4 . Upregulation of MCT and EMMPRIN expression is reported under ischemic conditions in both neuronal and cardiac cells . As well, EMMPRIN in conjunction with cyclophilin A has been reported to protect neurons from oxidative stress and ischemia . Although the EMMPRIN/cyclophilin A association and related signaling may provide protection to neurons from ischemic damage, the induction of MMPs through increased EMMPRIN expression may promote ischemic related pathology by increasing tissue destruction.

Alzheimer's disease is a neurodegenerative disease characterized by the errant production of amyloid β peptides that is believed to lead to the formation of the characteristic amyloid plaques and neurofibrillary tangles within the neocortical and subcortical regions of the brain resulting in disease pathology. The γ secretase complex regulates production of these amyloid β peptides (reviewed in . Recently, EMMPRIN was shown to be a regulatory component of the γ secretase complex . Depletion of EMMPRIN from the γ secretase complex by utilizing siRNA in cell lines resulted in an increase in amyloid β peptide production but had no effect on the expression levels of other complex components or complex substrates suggesting that EMMPRIN may regulate γ secretase activity in Alzheimer's disease.

Systemic lupus erythematosus (SLE) is a prototypical systemic autoimmune disease, characterized by T and B cell dysfunctions and many abnormalities of intracellular signaling pathways, including the enhanced secretion of MMP-9 by PBMCs. Upon examination, EMMPRIN was overexpressed on CD3⁺ T lymphocytes from SLE

patients when compared with CD3⁺ T lymphocytes from healthy donors. Monoclonal anti-EMMPRIN antibodies, were able to inhibit activation of T lymphocytes from SLE patients, suggesting a role for EMMPRIN in SLE pathology.

Cyclophilin A incorporation into the virions of HIV-1 is shown to be significantly enhanced in the early steps of HIV-1 infection. Virus-associated cyclophilin A is shown to co-immunoprecipitate with EMMPRIN from infected cells and blocking antibodies to EMMPRIN inhibited HIV-1 entry as evidenced by the delay in translocation of the HIV-1 core proteins from the membrane and inhibition of viral reverse transcription. The interaction between HIV-1-associated cyclophilin A and EMMPRIN on target cells significantly enhances infection by HIV-1 (Figure 3D). These results suggest that HIV-1 entry depends on an interaction between virus-associated cyclophilin A and EMMPRIN on a target cell, and EMMPRIN, therefore, appears to be a cofactor that mediates activity of virus-associated cyclophilin A and is required for efficient infection by HIV-1. EMMPRIN is also shown to enhance HIV-1 replication in a signaling-independent fashion through specific events mediated by the cytoplasmic domain of the protein

EMMPRIN in Multiple Sclerosis

There is currently no direct reference to EMMPRIN in the classic inflammatory disease of the CNS, multiple sclerosis (MS). In this regard, we have recently examined EMMPRIN expression in MS as well as in an animal model of the disease, experimental autoimmune encephalomyelitis (EAE). We show an increase in EMMPRIN levels in CNS samples from MS patients (Figure 5 B) where EMMPRIN levels are upregulated both on endothelial cells (arrow) as well as cells within the CNS (Figure 5 B, inset)

whereas EMMPRIN staining is restricted to endothelial cells of blood vessels in healthy controls (Figure 5A). In brief, in MS and EAE, MMPs are known to play detrimental roles, specifically in the breach of the blood brain barrier as well as in the degradation of myelin within the CNS. EMMPRIN is known to be an inducer of MMPs, and may have a role in the MMP induction both at the level of the blood-brain barrier in MS and EAE (Figure 4A), as well as within the CNS in the MMP mediated myelin destruction (Figure 4B). Various other roles for EMMPRIN are possible in autoimmune diseases such as MS; EMMPRIN may play an early role in leukocyte activation and polarization within peripheral lymphoid organs, as well as within the blood vessels of the CNS, wherein EMMPRIN interaction with integrins may affect the cell adhesion crucial in T-cell migration through the blood-brain barrier (Figure 4A).

The monocarboxylate transporter, MCT-1, is expressed on astrocytes. On these cells, MCT-1 transports lactate out of the cells into the surrounding, providing energy for neighboring cells such as neurons. EMMPRIN has been shown to be crucial in the proper lactate transport via MCT-1 by astrocytes. In MS and EAE, EMMPRIN may also play a protective role in the CNS parenchyma, where it binds to MCT-1 on astrocytes to allow the transport of lactate to the neighbouring neurons and oligodendrocytes (Figure 4B). This proposed function of EMMPRIN is currently being investigated in our laboratory.

EMMPRIN based therapy

Various anti-EMMPRIN based therapies have been utilized to target EMMPRIN mediated diseases. In a mouse model of asthmatic inflammation, it was shown that cyclophilins induce EMMPRIN-dependent chemotaxis of activated CD4⁺ T cells in vitro.

In vivo treatment with anti-EMMPRIN function blocking antibody significantly reduced the accumulation of CD4⁺ T-cells in lung tissues, suggesting a novel mechanism whereby asthmatic lung inflammation may be reduced by targeting cyclophilin-EMMPRIN interactions . Utilizing a specific anti-EMMPRIN function blocking antibody, Damsker and colleagues demonstrated that pro-inflammatory leukocytes lose their ability to migrate in response to cyclophilin A. Furthermore, they showed that mice treated with anti-EMMPRIN antibody reduced the development of collagen-induced arthritis by > 75%, suggesting a role for EMMPRIN in promoting the recruitment of leukocytes into joint tissues.

Tumor specific EMMPRIN peptides have been sequenced and identified for many years. Some of these are utilized as activity-blocking peptides (emp#2) in *in vitro* experiments ; and others such as HAb18G/CD147 (AP-9), constructed against the EC1 domain of EMMPRIN has been utilized *in vitro* and *in vivo* to block EMMPRIN activity, and specifically blocking the MMP inducing function of EMMPRIN . HAb18G/CD147 is shown to be a hepatocellular carcinoma associated antigen. In hepatocellular carcinoma, iodine (¹³¹I) metuximab (Licartin); a novel ¹³¹I-labeled HAb18G/CD147-specific monoclonal antibody F(ab')₂ fragment, was found to be pharmacologically safe and clinically efficacious in phase I/II trials . Metuximab injections were shown to be efficient in murine anti-hepatocellular carcinoma and the humanized (HAb18-huscFv)₂-Fc was shown to be a more efficient antibody fragment with less immunogenicity and additional roles in cell cytotoxicity .

Resveratrol (3,4',5 trihydroxystilbene), a naturally-occurring molecule known as a phytoalexin, is synthesized by plants in response to attacks by fungi and bacteria; it is

also known to possess an array of cardioprotective effects. Recently, studies have shown resveratrol to protect against the metabolic changes associated with hypercaloric diets in mice with induced insulin resistance, hyperglycemia, and dyslipidemia . Both resveratrol and a PPAR γ agonist, pioglitazone, were recently shown to significantly inhibit EMMPRIN expression and MMP-9 activity in a concentration-dependent manner . The effects of pioglitazone and resveratrol were reversed by pretreatment of THP-1 cells with a PPAR γ antagonist, GW9662, suggesting that resveratrol may down-regulate EMMPRIN and MMP-9 through PPAR γ activation. .

Conclusions

EMMPRIN is an early inducer of MMPs, it has a varied distribution and binds not only to itself in a homotypic cis pattern, but also to other ligands such as cyclophilin, MCT-1 and integrins. Binding to these various partners makes EMMPRIN-mediated functions varied and accounts for the multiple roles it plays in various diseases. Targeting EMMPRIN in inflammatory diseases may aid in reducing the destruction caused by infiltrating EMMPRIN positive immune cells.

Figure legends

Figure 1. Schematic of EMMPRIN structure and functions attributed to its various domains.

EMMPRIN is composed of two extracellular domains (EC1 and EC2), a transmembrane domain (TD), and a cytoplasmic domain (CD). Each EC loop is hinged by disulphide bonds (s-s), and made up of a specific number of amino acids (a.a). Sites of glycosylation are marked by curved lines on EC1. Specific sites are reserved on the EMMPRIN molecule for binding with various ligands; these are represented for each domain. Abbreviations: a.a. – amino acid; MCT-1 – monocarboxylate transporter-1. Adapted from

Figure 2. Mechanisms by which EMMPRIN mediate invasiveness of cancer cells

Membrane associated EMMPRIN expressed on tumor cells binds homotypically with EMMPRIN on stromal cells, or through soluble EMMPRIN, causes the release of MMPs that facilitate tumor invasiveness. Adapted from

Figure 3. EMMPRIN interaction with cyclophilin in disease

EMMPRIN interacts with cyclophilin (cyp) A, B and C to result in immune cell chemoattraction in arthritis and HIV, adhesion to ECM, and osteopontin (OPN) binding that then mediates MMP-2 activation and secretion.

Figure 4. Specific activities of EMMPRIN help mediate the infiltration of leukocytes into the CNS and the production of neuropathology.

In neuroinflammatory diseases such as MS, EMMPRIN may play multiple roles. In the periphery, it may alter cell activation and polarization; at the level of the blood-brain barrier, it may augment cell adhesion and migration; and within the CNS itself it may alter CNS pathology via its MMP inducing function.

Figure 5. Increase in EMMPRIN expression in MS CNS inflammatory lesions compared to healthy controls.

Double immunofluorescence staining reveals EMMPRIN staining (green) to be restricted to blood vessels (CD31 positive) in healthy control CNS samples (A, 100X magnification). In MS CNS samples (B, 200X magnification), EMMPRIN staining is highly upregulated on endothelial cells of blood vessels (arrow) and also on leukocytes infiltrating the CNS (CD45 positive, inset).

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Figure 1.

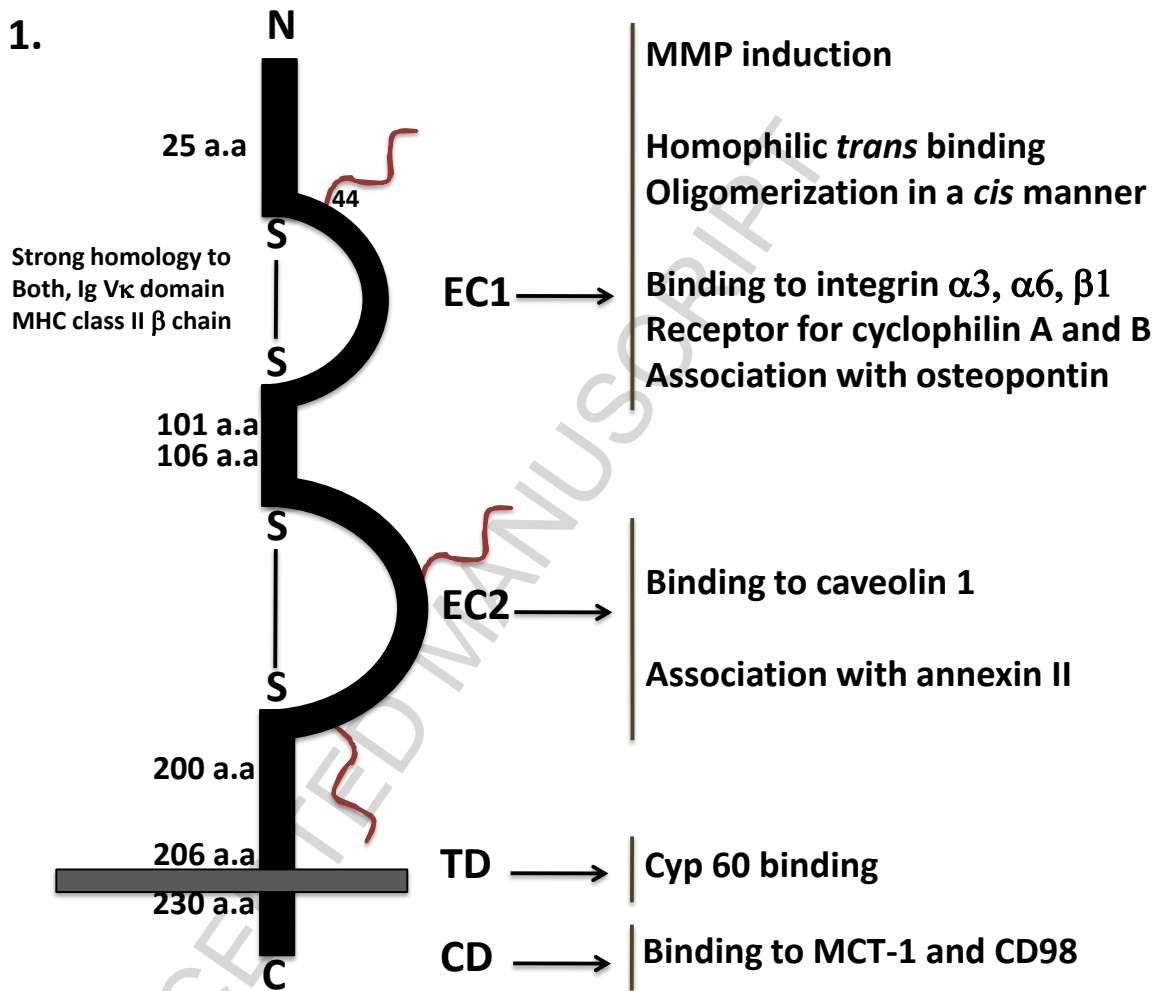


Figure 2.

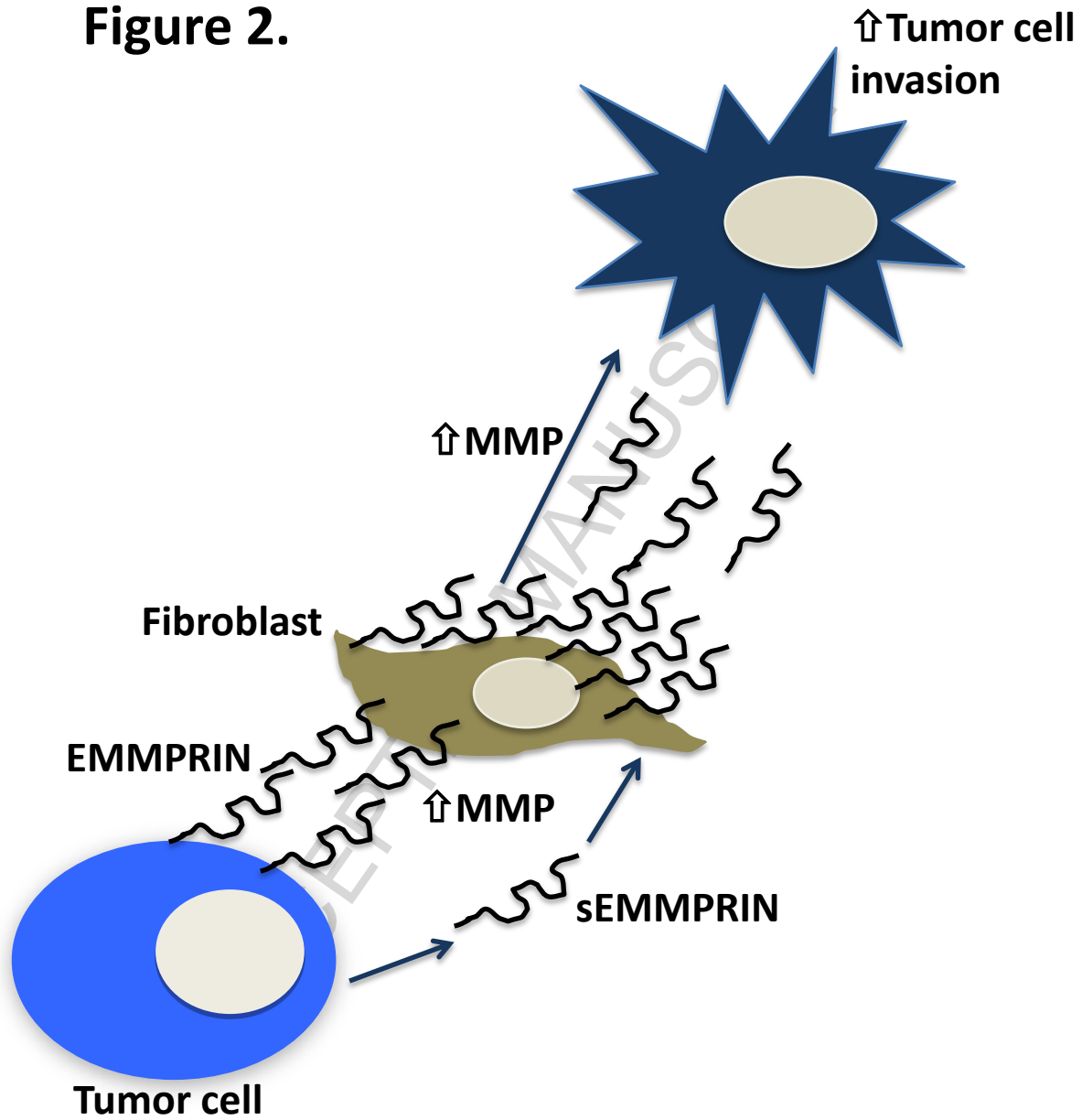


Figure 3.

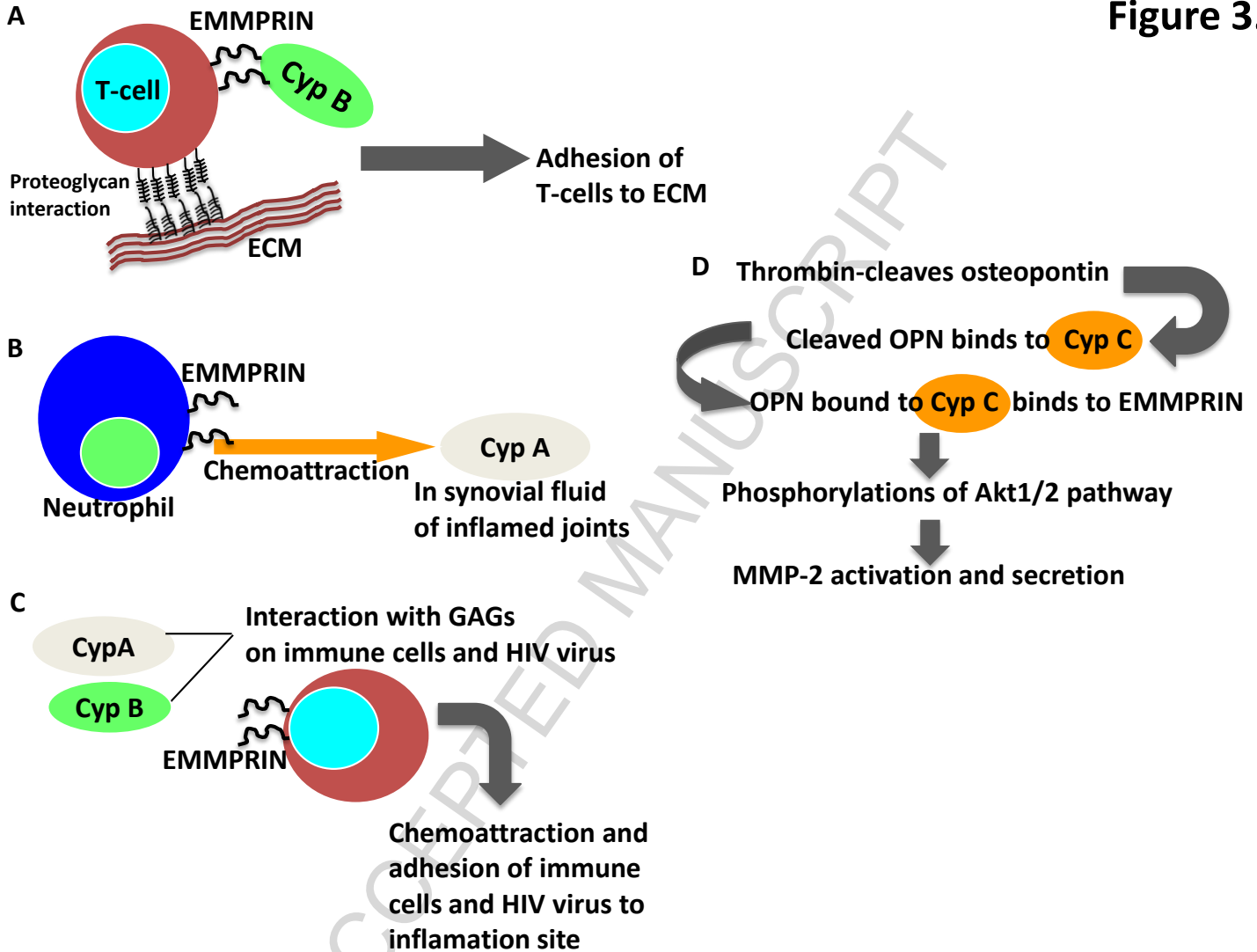


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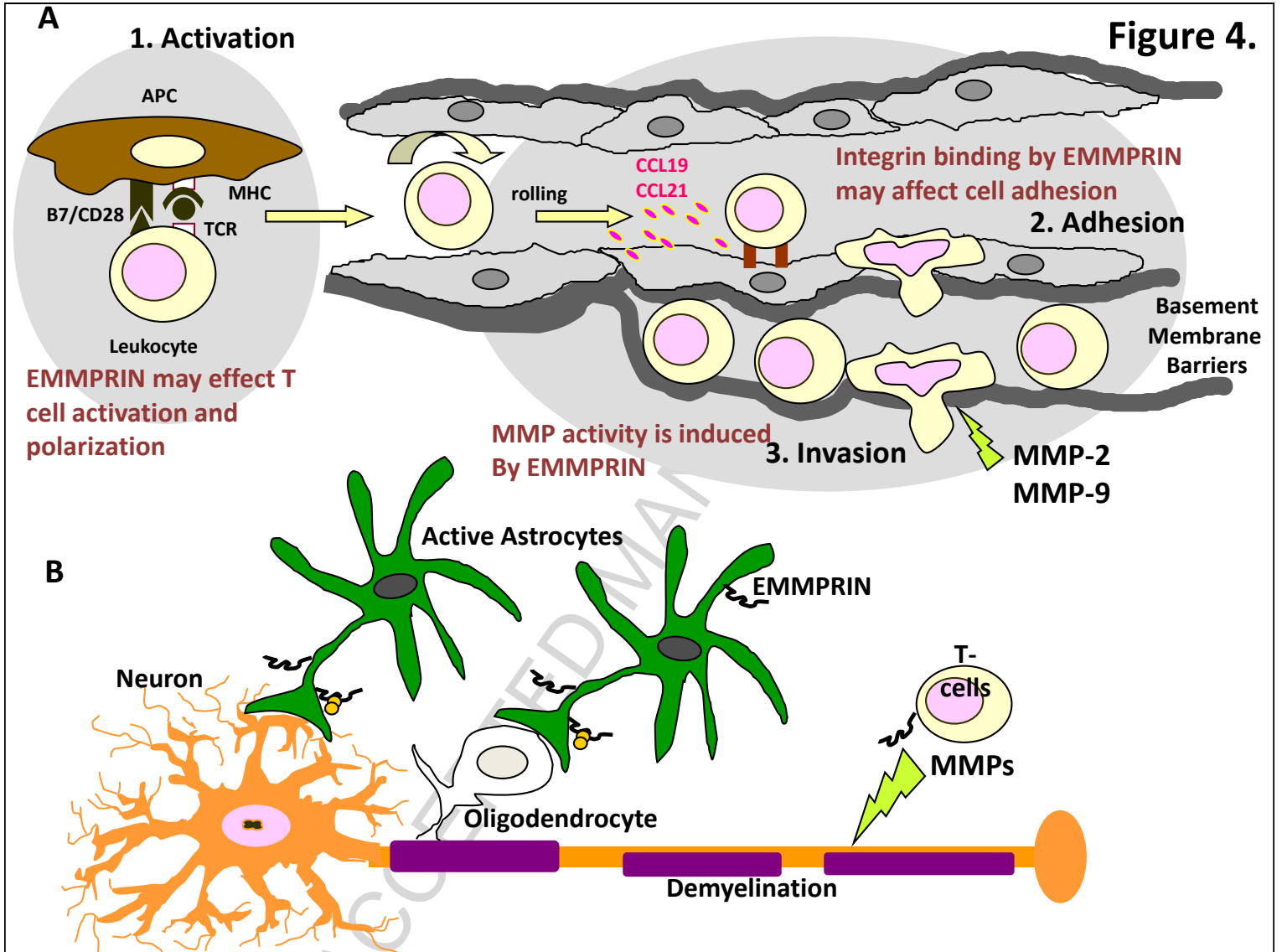
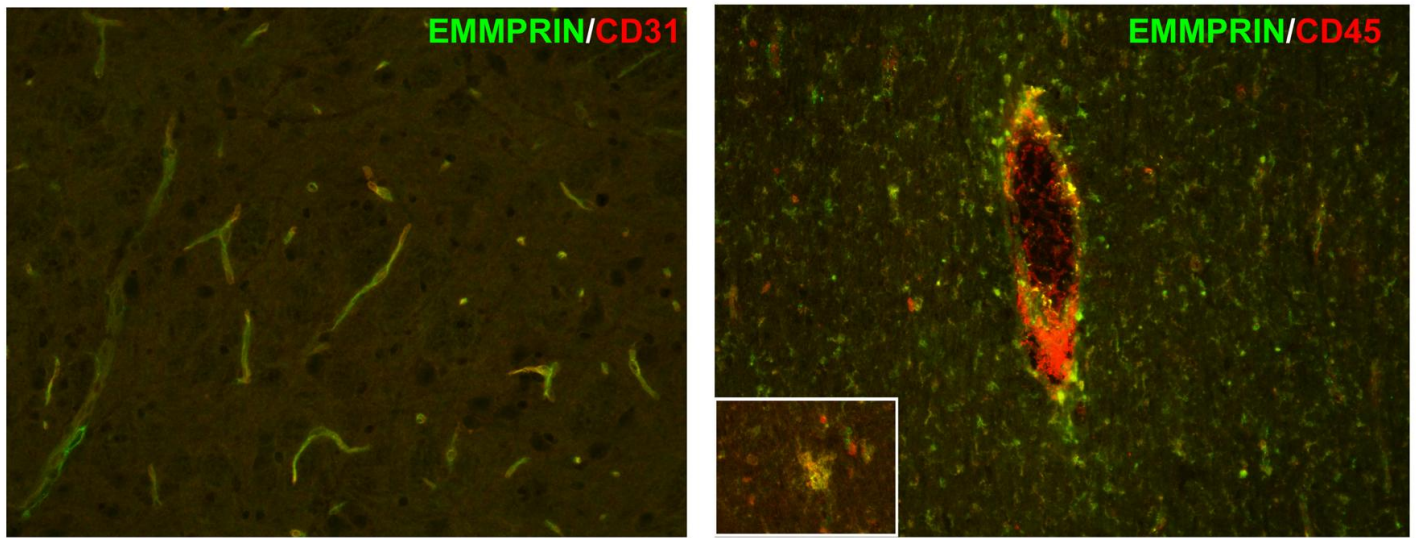


Figure 5.



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