Minireview

Biomedical potential of mammalian spectraplakin proteins: Progress and prospect

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Impact statement

Spectraplakins are a highly conserved group of proteins which have the rare ability to bind to each of the three major cytoskeletal networks. The mammalian spectraplakin MACF1/ACF7 has proven to be instrumental in many cellular processes (e.g. signaling and cell migration) since its identification and, as such, has been the focus of various research studies. This review is a synthesis of scientific reports on the structure, confirmed functions, and implicated roles of MACF1/ACF7 as of 2019. Based on what has been revealed thus far in terms of MACF1/ACF7's role in complex pathologies such as metastatic cancers and inflammatory bowel disease, it appears that MACF1/ACF7 and the continued study thereof hold great potential to both enhance the design of future therapies for various diseases and vastly expand scientific understanding of organismal physiology as a whole.

Abstract

The cytoskeleton is an essential element of a eukaryotic cell which informs both form and function and ultimately has physiological consequences for the organism. Equally as important as the major cytoskeletal networks are crosslinkers which coordinate and regulate their activities. One such category of crosslinker is the spectraplakins, a family of giant, evolutionarily conserved crosslinking proteins with the rare ability to interact with each of the three major cytoskeletal networks. In particular, a mammalian spectraplakin isotype called MACF1 (microtubule actin crosslinking factor 1), also known as ACF7 (actin crosslinking factor 7), has been of particular interest in the years since its discovery; MACF1 has come under such scrutiny due to the mounting list of biological phenomena in which it has been implicated. This review is an overview of the current knowledge on the structure and function of the known spectraplakin isotypes with an emphasis on MACF1, recent studies on MACF1, and finally, an analysis of the potential of MACF1 to advance medicine.

Keywords: Cytoskeleton, F-actin, microtubule, cell migration, cell polarity, spectraplakin

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Introduction

The cytoskeleton is to the cell as bones, ligaments, and tendons are to a vertebrate organism. Ever since the discovery of the earliest identified cytoskeletal element, actin, in the 1940s,[1] new components have been discovered at an exponential rate; at present, several dozen cytoskeleton affiliated proteins are known, three of which are major cytoskeletal networks—actin, intermediate filaments (IFs), and microtubules (MTs)—and the remainder of which are ancillary factors which stabilize intracellular infrastructure or otherwise regulate these filaments (e.g. plectins and spectrins).[1,2]

These proteins serve a plethora of other purposes within the cell, when combined to form the cytoskeleton, in both prokaryotes[3–5] and eukaryotes.[1,2] The cytoskeleton primarily influences a cell's shape and internal environment which, in turn, determines many of a cell's physiological characteristics and behaviors.[6] For example, modifications of the cytoskeleton are believed to be required for cellular maturation and lipid deposition in adipocytes[7,8] and in neurons, some have proposed that the actin cytoskeleton and its regulatory proteins are essential to the structural stabilization of dendritic spine modifications that are believed to maintain long-term

memories.[9,10] Also notable is the fact that F-actin is indispensable to the process of neuronal migration.[10] The existence of a common thread between these mostly unrelated processes in the form of cytoskeletal structure serves to underscore its far-reaching importance to a cell, and ultimately the organism to which the cell belongs. Given the intimate relationship between the cytoskeleton and various underlying processes of the cell, the cytoskeleton seems to be filled with untapped potential relevant to both medicine and research.

What should not be understated are the contributions of the aforementioned ancillary factors which contribute significantly to the many functions of the cytoskeleton. One category of such factors is a group of proteins known as the spectraplakins; spectraplakins are a family of cytolinker proteins that are conserved in metazoans and possess the rare ability to bind to actin, IFs, and MTs. This affinity for the three major cytoskeletal networks allows spectraplakins to coordinate their activities.[11] The function and necessity of these proteins in the organisms in which they exist have been studied extensively, revealing that they are involved in a vast nexus of cellular processes.[12] This paper will focus on this subgroup of cytoskeleton-associated proteins, particularly on one isotype of the mammalian spectraplakins which is encoded by the *MACF1/ACF7* gene and known as ACF7 or MACF1.[11] This protein is referred to as MACF1 in this review.

Spectraplakins overview

The name spectraplakin is a portmanteau formed by the combination of the words "spectrin" and "plakin."[11,12] Spectrins and plakins are, like the protein family that is named after them, cytolinker proteins that participate in the maintenance of a cell's plasma membrane integrity or the organization of cytoskeleton adhesion complexes, respectively.[13,14] In the context of erythrocytes, spectrins underlie the plasma membrane of the cell as tetrameric or higher oligomeric proteins and confer upon the cell resistance to mechanical forces.[13] Plakins, on the other hand, are giant proteins which connect cytoskeletal elements to each other and junctional complexes (e.g. desmosomes) to regulate polarization, adhesion, and migration.[14] Spectraplakins, first identified in the 1990s,[15] are a group of cytolinker proteins with seven main domains which may be attributed to either spectrins or plakins giving them the unique ability to bind to all three cytoskeletal elements (actin, MTs, and IFs),[11,15,16] allowing them to act as an intermediary between these filaments within the cell.[15] Moreover, the typical size of a spectraplakin is also considered highly irregular as isoforms may be over 8000 amino acid residues in length and may have a quaternary structure predicted to be as large as 0.4 μm across.[11,12] The size and versatile binding activity of a spectraplakin makes it a key component of cytoskeletal coordination and the overall structural integrity of tissues; it is for these reasons that the spectraplakin protein family and the genes that encode them have been studied for decades as either the subject of or as an adjuvant to research on tissue structure.

The *spectraplakin* family of genes are believed to have evolved from the *spectrin* family and are highly conserved evolutionarily within metazoan animals[11,17]; as a result of this conservation, genes for these proteins are found within *Caenorhabditis elegans, Drosophila melanogaster, Danio rerio, Mus musculus,* and humans.[17] At this point, there are five known *spectraplakin* genes found across the metazoan division, each of which have the ability to yield multiple products due to alternative splicing.[15]

Spectroplakins in *C. elegans*

C. elegans, one of the most basal metazoans, has only one spectraplakin gene known as *Vab-10* which resides on the first chromosome and is considered an ortholog of *MACF1*.[18] The *Vab-10* gene in *C. elegans* has a total of 32 exons[18] which are combined to form two major isoform categories, Vab-10A and B, with a common 5′ start site and ending in either exons 16–17 or 18–32, respectively.[11,18] Other isoforms resulting from alternative splicing affecting exons 5, 9, 16, 21, 22, 23, and 27 may be possible, though it is unclear whether these isoforms are produced *in vivo* or not.[18] Vab-10A has proven essential for epidermis–extracellular matrix attachment, while Vab-10B maintains the connection between the apical, basal epidermis, and plasma membranes during morphogenesis.[11] In addition to providing interstitial support, Vab-10 proteins may also take part in the maintenance of dopaminergic neurons because its knockdown (KD) leads to the degradation of this subtype of neurons.[18,19] Because Vab-10B is the closest homolog of an isoform of MACF1 known as MACF1b, this observation greatly supported a human genome-wide association study that revealed MACF1b as a risk candidate in Parkinson's disease.[18]

Spectroplakins in *D. melanogaster*

In *D. melanogaster*, there exists an ortholog for *MACF1* known as *Short stop* or *Shot.*[17] As is the case with *C. elegans*, *D. melanogaster* has been used extensively as a model system for deciphering the function of proteins such as spectraplakins to develop concepts that can guide investigations in higher organisms.[11,15,17] *Shot* appears to be expressed in nearly all tissues of *D. melanogaster* and as a result has been previously studied in a variety of biological contexts, revealing significant variation in domain requirements and mechanisms.[17] Shot may exist as one of 22 annotated isoforms which may be classified as A,B,C, or D-type based on the makeup of their actin-binding domain (ABD) which in turn is formed by two calponin homology domains (CHDs), one with a high actin affinity and another with little or no actin affinity. A and B-type Shot isoforms possess a full ABD (CH1 and CH2), C-type Shot lacks a high actin affinity and instead possesses only CH2 which has little to no actin affinity, a D-type isoform simply lacks either.[17] A large number of studies indicate that Shot has many identified purposes related to cytoskeletal regulation in nervous system constituents much like mammalian spectraplakins, prominent examples of these roles are its contributions to axon growth and pathfinding.[17,19]

Spectroplakins in vertebrates

As is the case with their invertebrate counterparts, the spectraplakin proteins of vertebrate organisms play pivotal roles in the function of integumentary and nervous systems among others.[11] However, unlike these counterparts *spectraplakin* genes exist in pairs in vertebrate model systems excluding zebrafish, which has only one *spectraplakin* gene known as *Magellan*,[15,20] One should note that based on a 2014 report by Antonellis *et al.*, it appears that the zebrafish *spectraplakin* gene, *Magellan,* may have a paralog on chromosome 16 due to an antediluvian duplication event.[21] For many years, the exact purposes of each of the mammalian genes, *MACF1* and *BPAG1* have been interrogated *in vivo* using either knockout (KO) or KD models in *mice*.[11,19] A full *MACF1* KO results in animals who do not live past gastrulation[11]. In contrast, a globalized KO of *BPAG1* seems to produce animals who can survive until weaning.[20] Interestingly, *MACF1* loss-of-function does not cause embryonic lethality in zebrafish.[22] Beyond this, conditional KOs of *MACF1* in the nervous system leads to neuronal migration and organization defects,[23] and when KOs of *MACF1* in skin they lead to blistering or delayed recovery.[11,23] The implicated roles of the mammalian spectraplakins have led to the discovery that *MACF1* produces at least six protein isoforms,[14,23] while *BPAG1* produces seven, each with a presence in a large number of tissues.[14] As is the case with each of the other observed categories of spectraplakin isoforms, mammalian spectraplakins isoforms are some combination of the multiple domains coded within the gene[14] as outlined in Figure 1. Though the tissue distribution of each of these isoforms is known, some attributes such as the exact intracellular localization remain uncharacterized.[14,24]

The existence of *spectraplakin* genes throughout the metazoan clade and ultimately *MACF1* and *BPAG1* in mammals has been attributed to evolutionary conservation rather than independent development due to a large amount of apparent sequence homology.[16,25,26] Both the functions of spectraplakin isoforms and *spectraplakin* genes themselves in humans have been assayed to decipher their roles in the pathogenesis of certain diseases.[25] Based on the differences between the phenotypes produced by their respective KOs it seems that, though related by virtue of sharing certain structural features,[11] MACF1 and BPAG1 likely occupy distinct roles within the vertebrate organism. The remaining sections of this review will focus on what recent studies have revealed about the architecture, functions, products, and clinical potential of the mammalian spectraplakin MACF1 as well as the gene that encodes it.

Key functional domains of spectroplakin MACF1

Given the copious isotypes and respective functions of spectraplakins, their genotypic and proteomic infrastructure has been closely examined in tandem with their actual purposes. As mentioned previously, spectraplakins as a group are highly conserved evolutionarily, and one or more iterations thereof is found in each member of the clade metazoan,[11] in each of these orthologs a commonality is seen in the form of seven key functional domains.[14,16] Overall seven different types of functional domains have been found in the six MACF1 isoforms: the ABD domain, with CH1 and CH2 subcomponents; the plakin domain (PD); the spectrin repeat rod domain; two EF-hand (EFH) motif domains; the G2R domain; the plectin repeat domain (PLRD); and the plakin repeat domain (PRD).[23]

ABD domain

Conserved in four of the six known MACF1 isoforms is the aforementioned ABD,[23] which is also found in the drosophila homolog, Shot, and has similar dissociation constants in each case.[11] In MACF1 isoforms that possess an ABD this domain may be composed of only one (CH2) or both (CH1 and CH2) of the CHDs that have the ability to bind F-actin.[11,17,27]It should be noted that though CH1 binds actin more tightly than CH2, the ABD displays a higher actin avidity when these subdomains are joined.[11,23] CH1 and CH2 domains consist of only alpha helices connected to one another by short loop sequence and adopt a closed conformation with substantial contact between CH1 and CH2.[11,24] Although the exact conformation for this domain during F-actin binding is currently unknown, data derived from other spectrin family proteins strongly suggest that this domain undergoes a dramatic conformational change when bound to F-actin.[24] Other studies suggest that the ABD of MACF1 also confers the ability to interact with other protein classes such as the tetratricopeptide repeat domains of rapsyn, as part of MACF1's role in anchoring acetylcholine receptors.[28]

Plakin domain

Also residing at the N-terminus of MACF1 is the PD; this domain is one of the defining characteristics of the spectraplakin superfamily and is shared with members of the plakin superfamily.[23,29] The PD consists of six to nine spectrin-like repeats arranged in a tandem array with the fifth repeat containing an embedded Src-homology-3 protein–protein interacting domain.[24] Moreover, the secondary structure of this domain is characterized by a high alpha-helical content.[23] This structure contributes heavily to the function of the PD, typically proteins which contain a PD bind to membrane-associated junctional proteins[11] and in and of itself this domain is responsible for binding to adhesion and signaling molecules (e.g. BPAG2 and Erbin).[23] Collectively, crystallography and protein sequence alignment analysis show that the PD most likely originated from spectrin repeats evolutionarily.[11,23] In addition to the likely lineage put forth by structural analysis, the nonexistence of *plakin* genes in *D. melanogaster* and *C. elegans* despite these species having spectraplakin homologs suggests that the plakin protein family evolved from spectraplakins.[11]

Spectrin repeats domain

Spectrin repeats are typical structures belonging to the spectrin family.[11,13] MACF1 contains 23 dystrophin-like

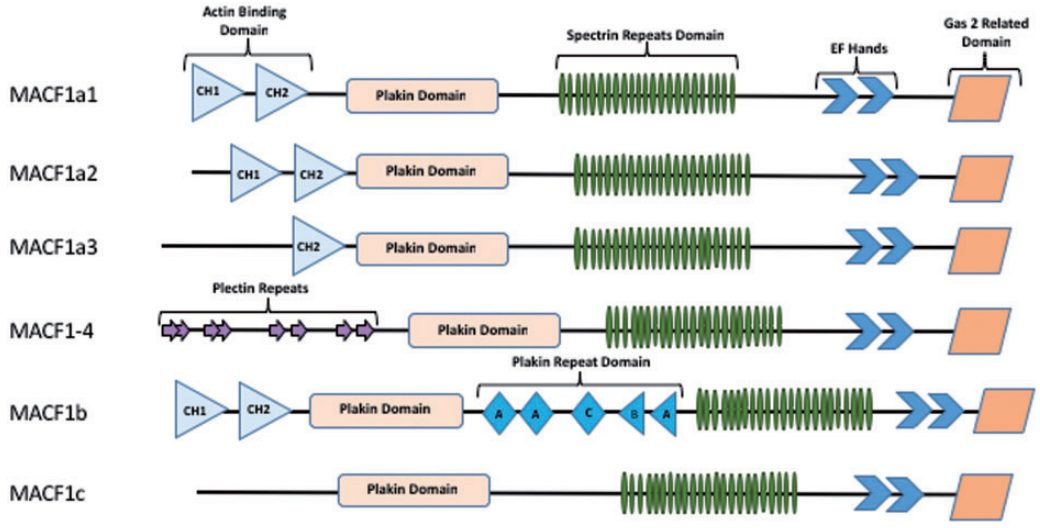

Figure 1. The domain composition of each of the six known isoforms of MACF1. Seven unique functional domains are known to exist: the actin binding domain (composed of CH1 and CH2 subcomponents), the plakin domain, the spectrin repeats rod domain (with 23 alpha-helical spectrin repeats), the EF hand motifs, the GAS2-related domain, the plectin repeats domain, and the plakin repeats domain (comprised of distinct subcomponent types, A,B and C). Note: half diamonds shown in the plakin repeat domain indicate a partial subcomponent.

spectrin repeats,[23] each comprised of between 110 and 120 residues which assume an antiparallel coiled-coil secondary structure.[23,24] This motif, in turn, forms an extended rod-like structure that acts as a spacer between the N and C termini, granting the entire protein flexibility and allowing it to respond to mechanical stress with elasticity.[23,24] In addition to providing physical separation between the functional domains that exist at the termini of a complete protein,[23] this domain also functions in the structural and signaling activities of spectraplakin family members.[18,24] In MACF1, spectrin repeats associate with signaling proteins involved in the Wnt signaling pathway (e.g. Axin proteins, LRP6, and GSK3β)[24] as well as with spectrin-associated protein 3 (CAMSAP3), which is a MT minus-end-binding calmodulin-regulated protein.[14] Unlike plakins, spectrin genes exist in the genomes of both *D. melanogaster* and *C. elegans*.[13] Stemming from this, the spectraplakin family of proteins is believed to have developed from the spectrin family.[11]

EFH calcium-binding domain

The *spectraplakin* exons that form the EFH are encoded at the 3′ end of *MACF1* and are located near the C-terminus of a given MACF1 isoform; this region is considered highly conserved among spectraplakin proteins with a sequence identity of about 60% across *D. rerio*, *Drosophila*, *C. elegans*, *and Homo sapien* homologs.[27] In addition to being a key spectraplakin component, EFH is found in pure spectrin proteins as well as several unrelated protein classes where it serves the purpose of direct calcium ion binding and sensing.[11,30] Within an MACF1 isoform, there are heterogeneous pairs of EFHs, wherein the proximal pair is calcium-dependent and the distal one is calcium-independent[11]; structurally, the EF1-EF2 domain consists of two EFHs which are each, in turn, comprised of a helix-loop-helix motif that may each bound a calcium ion.[27] In spectrin, the binding of a calcium ion by the calcium-dependent proximal hand changes the domain from a closed to an open conformation.[11,27] This domain's MT engagement is enhanced by the G2R in the mammalian spectraplakin BPAG1 but its ablation seems to have no effect on MT binding in MACF1.[14,23] Though this domain does not seem to be crucial to MT binding in MACF1 recent structural biology reports theorize that it may have a role in sampling the MT lattice for equivalent binding sites when some are sterically blocked by other MT-associated proteins.[27] Interestingly, some muscle-specific spectrin family members tend to lack the ability to bind calcium ions through their EF hands; this mechanism likely developed to avoid tissue destabilization upon the calcium ion fluctuations seen in muscle tissues.[11]

GAS2-related domain

As previously mentioned, the G2R is closely associated with the EFH in function and proximity,[11] but unlike the EFH, it is restricted to the spectraplakin members of the spectrin and plakin protein families.[23] However, this domain is not entirely restricted to spectraplakins and in fact is found in growth arrest-specific protein 2 as well as GAS2-related proteins 17 and 22.[11] Located at the C-terminal of a spectraplakin, it is made up of about 57 amino acid residues and is connected to the EF hands by a flexible linker.[14] Lane *et al.*[27] showed that this domain contains a very unique zinc-coordinating structure called an alpha/beta sandwich. This means that the isolated domain has an N-terminal alpha helix followed by a five-stranded, anti-parallel beta-pleated sheet followed by a C-terminal alpha helix which is positioned anti-parallel to the N-terminal alpha helix as a secondary structure, the tertiary structure consists of alpha helices that pack against either side of the beta-pleated sheet hence the "sandwich" descriptor. Distal to the G2R's zinc binding site is a conserved basic region which is a G2R MT binding determinant.[27] The G2R, like the EFH, is related to MT binding but, unlike the EFH it is

the primary actor in this process.[14,23] In fact, functional analyses have shown that G2R of MACF1 is sufficient for MT network colocalization.[14] The differences in the roles of the G2R and the EFH in the process of MT binding are consistent with the fact that the G2R is conserved throughout the MT-binding Gas 2 protein family, while the EFH is not.[27]

Plakin repeat domain (PRD)

The PRD is a structural feature unique to the plakin superfamily.[24] This domain is globular and contains a central core region that is itself comprised of four and a half copies of the 38 amino acid plakin-repeat motif known collectively as the plectin repeat.[14,24] The 171 residues of the plectin module form a beta-hairpin secondary structure and is immediately followed by two antiparallel alpha helices.[11,14] Further, three separate subtypes of PRDs have been identified, these being types A, B, and C; also, these are found at the C-terminal of most plakins and the internal region of some spectraplakins.[14] The differences between the PRD subtypes are structural, though a basic groove that functions in IF binding is conserved in each.[14,31] Within the mammalian spectraplakins, the numbers of PRDs are variable.[11] Four distinct isoforms of BPAG1 contain the PRD, in the case of BPAG1e, there are two of these domains, one B-type and one C-type, while BPAG1b1, 2, and 3 each have a single PRD.[14] In MACF1b, the only MACF isoform to contain the PRD,[24] there are five of these domains including two complete A-type, one complete C-type, one incomplete B-type, and one incomplete A-type.[14,23] In MACF1b, the two complete A-type have been shown to serve a role in targeting MACF1b to the Golgi complex via the N-terminal of its consecutive A-type subdomains.[23] Primarily, the role of the PRD is to bind to IFs and stabilize their structure within the cell by co-associating with other structural elements of the cell.[14,24] The mechanism by which the PRD manages to interact with IFs, in the context of envoplakin, was identified by Fogl *et al.*[32] by solving its crystal structure. Herein, a basic groove on PRD possesses the ability to accommodate acidic patches within IF proteins and in addition to this, residues within the groove mediate targeting of a plakin to vimentin and keratin IFs.[32] Interestingly, though *Drosophila* does not possess cytoplasmic IFs, one isoform of the drosophilid spectraplakin, known as Shot B, contains conserved plectin repeats.[11] These plectin repeats are not organized into sets of four and a half tandem repeats and as such likely do not form a PRD globular domain structure[11,24]; this suggests that plectin repeats may hold some functionality outside of interacting with IFs.

Plectin repeats domain

The plectin repeats domain (PLRD) is a structure unique to the MACF1-4 isoform of MACF1.[14,23] MACF1-4, like the MACF1c isoform, totally lacks an ABD at its N-terminus but unlike MACF1c, has a set of eight PLRDs in its place.[14,23] This isoform was first identified by Gong *et al.* in 2001.[33] Each of these spectrin repeats is encoded by a single large exon, exon 38. Each of these repeats contains 45 residues whose predicted secondary structure is an alpha helix.[33] Studies on plakins that contain PLRDs show that this domain plays a role in the process of IF binding, which may hold true for the PLRDs of MACF1-4, but which are yet to be confirmed by current data and requires additional study.[23]

MACF1 isoform distribution

Taken together, the collective functions of these domains speak to the diverse contributions of spectraplakins, particularly the MACF1 isotype, to cellular infrastructure. As previously mentioned, spectraplakins are relatively large proteins which come from large genes[16,26,33]; MACF1 is no exception to this, the gene which encodes it has no less than 102 exons and spans over 270 kb on human chromosome 1p32 (or mouse chromosome 4).[23,33] Six separate isoforms of MACF1 that result from alternative splicing have been observed *in vivo*. These are: MACF1a1, MACF1a2, MACF1a3, MACF1-4, MACF1b, and MACF1c.[23] The *in vivo* tissue distributions of each of these isoforms are non-homogenous with most being broadly expressed while being primarily concentrated in certain areas[11,23] as summarized in Table 1. The differences in expression of the various MACF1 isoforms have been determined in postnatal and adult *mice*.[23,34]

MACF1a1, MACF1a2, MACF1-4, and MACF1b each exhibit broad expression.[23] MACF1a1 shows predominance in the skin, kidney, and stomach, while MACF1a2, an isoform whose mRNA is identical apart from the 5′ region, is highly enriched in the brain, spinal cord, lung, heart, kidney, and skeletal muscle.[23,34] MACF1a3 and MACF1c are expressed in the nervous system; the former is predominant in the central nervous system rather than being ubiquitous in the organism.[11,23] Finally, both MACF1b and MACF1-4 are broadly expressed; the latter having an especially high concentration in the lungs, heart, pituitary gland, and placenta.[23] Beyond this, differences in transcript detection also result from the stage of development; for instance, MACF1a1 mRNA is detectable in embryos from day seven and a half to day 10·5 of embryonic development while MACF1a2 mRNA does not become detectable until embryonic day 10·5.[23]

Biomedical potential of MACF1

Spectraplakins have been shown to function extensively in the coordination and stabilization of the cytoskeleton[11,15] as well as in the proper development and maintenance of body systems[23,24] and have even been proven essential to organismal survival.[23] Recent studies have more closely investigated the real significance of MACF1 and its isoforms in a variety of cellular and organismal processes. It appears that these studies have uncovered many novel responsibilities of MACF1, whereby it holds either intrinsic or extrinsic roles in disease pathogenesis.[23] It currently has many alleged functions with new ones being identified frequently; based on the nature of what has been discovered already, it stands to reason that at least in theory an understanding of MACF1 has serious relevance to the future of

Table 1. Different isoforms of MACF1 and their tissue distribution.

Isoform	Tissue distribution	Domain composition	References
MACF1a1	Broadly expressed with predominance in skin, kidney, and stomach	ABD (CH1, CH2), PD, Spectrin repeats domain, EFH, and G2R.	14,23
MACF1a2	Broadly expressed with high level in brain, spinal cord, muscles, pancreas, kidney, liver, and lung.	ABD (CH1, CH2), PD, Spectrin repeats domain, EFH, and G2R.	14,23,34
MACF1a3	Predominant in brain and spinal cord. Also found in skin lung and kidney.	Incomplete ABD (CH2 only), PD, Spectrin repeats domain, EFH, and G2R.	14,23
MACF1-4	Broadly expressed with high level in heart, lung, pituitary gland and placenta	PLRD, PD, Spectrin repeats domain, EFH, and G2R.	14,23
MACF1b	Broadly expressed	ABD (CH1, CH2), PD, PRD, Spectrin repeats domain, EFH, and G2R.	14,23
MACF1c	Broadly in nervous system	PD, Spectrin repeats domain, EFH, and G2R.	14,23,34

medicine. In this section, the potential of research on the nature and dynamics of MACF1 to advance the treatment and predicted outcome of disease will be discussed. This section will highlight recent studies that have identified new and significant roles of MACF1 as well as their implications.

Disease pathology

In 2014, the first confirmed case of human disease caused by a mutation in the *MACF1* gene was reported in a 12-year-old child who presented a number of skeletal muscle problems which included both pain and globalized hypotonia; this mutation (elucidated by genomic analysis) was a single region duplication on chromosome 1p34·3, which mapped to a large portion of the *MACF1* gene and deleteriously affected each of the four major splice products of this gene in the subject. Further testing revealed that this mutation caused markedly reduced MACF1 expression which, in turn, resulted in significant changes to the infrastructure and motility of both endothelial and satellite cells of the skeletal muscle, a novel condition which was later designated "spectraplakinopathy type 1".[23] This revelation brought into focus the importance of MACF1 to human physiology and served as the theoretical basis of many subsequent studies on the purposes of MACF1. More recently, other groups have implicated MACF1 and defects thereof in various cancers [14,23,34,35] as well as inflammatory bowel disease (IBD).[36,37]

Implications in cancer

The main reason that the behavior of many cytoskeletal components has been studied in relation to human cancers is that the cytoskeleton undergoes many changes during the processes of both invasion and metastasis.[14] In addition, the link between *MACF1* expression and the process of metastasis has been supported by functional analyses.[38] Duhamel *et al.* published a report in 2018 on the relationship between HectD1, a previously characterized E3 ubiquitin ligase, MACF1, and the epithelial-to-mesenchymal transition that elucidated the involvement of this factor in the process of metastasis.[34] This group showed that MACF1 was the only MT plus-end tracking protein strictly required for cellular motility in the breast cancer cell line MDA-MB-231. MACF1 contributes to directed motility and is required for accurate cell migration. Going further, Duhamel *et al.* showed that MACF1 promoted an epithelial-to-mesenchymal transition and enhanced invasion and colony-forming capacity in representative breast cancer cell lines. When applied in a nude mouse allograft model, a majority of mice failed to show signs of metastatic progression to the lungs for up to six weeks following with treatment of MACF1 mRNA-depleted cell transplantations, and these assays showed that *MACF1* expression had a direct relationship to cell invasion both *in vivo* and *in vitro*.[38]

A 2017 study conducted by Afghani *et al.*[39] found a similar relationship between glioblastoma and *MACF1* expression based on functional assays with two established brain cancer cell lines and patient-derived lines. This group showed that *MACF1* is expressed diffusely and at the periphery in 40% of grade IV glioblastoma tissue samples, while there is none in normal glial cells and that its levels were elevated within grade II–IV astrocytomas as compared to normal astrocytes.[39] These results showed that *MACF1* is much more predominantly expressed in high-grade brain tumors, pointing to the conclusion that it is a potential biomarker. The subsequent use of *MACF1*-specific inhibitory RNA treatment on immortalized glioblastoma cell lines, as well as some established patient-derived xenograft mouse models, produced inconsistent results, however. In U251 cells, siRNA silencing of *MACF1* leads to the loss of viability and decreased cell migration, while in A172 cells, neither effect was observed.[39] Furthermore, shRNA silencing of *MACF1* in T4105 and T4302 xenograft lines significantly inhibited growth of these cell lines, while in SF295 and GBM76 cells, cell growth inhibition were less dramatic.[39] Down-regulation of Wnt-signaling pathway components was also observed in cells treated with inhibitory RNAs,[39] suggesting that the reduction of this pathway is related to the observed anti-tumorigenic effects of silencing *MACF1*.[24,39] Finally, the group combined *MACF1* KD in each of the cell lines with selected chemotherapeutic drugs to determine whether *MACF1* expression contributed to acquired tumor resistance. Interestingly, a combination of temozolomide and *MACF1* silencing resulted in a roughly two-fold viability decrease in glioblastoma cells as compared to those subjected separately to one treatment or the other.[39] These results seem to suggest that the reduction

of *MACF1* expression acts synergistically with temozolomide treatment.[39]

Together, the findings of these reports strongly suggest that MACF1 has a sizable role in a variety of metastatic cancers. Though the observation of this phenomenon has, thus far, been primarily restricted to high-throughput genomic and proteomic approaches which provided highly circumstantial evidence,[38,40] the *in vitro* and *in vivo* studies put forth by both Afghani *et al.* and Duhamel *et al.* lend credence to the notion of a relationship between MACF1 and many cancers.[38,39] Zhao *et al.*[35] assert that *MACF1*-specific micro RNAs could potentially suppress the metastasis and invasion of cancer cells and provide a list of 50 endogenous micro RNAs predicted to interact with MACF1 mRNA. Bearing in mind the results of numerous *MACF1*-specific shRNA and siRNA silencing assays,[14,35,38] it logically follows that antisense RNAs, at least in theory, represent a potentially exploitable mode of counteracting the aberrantly high expression of *MACF1* in order to suppress metastasis[38,39] and even enhance the effects of existing therapies[39] This would depend upon whether either could be induced in or delivered to affected tissues from an exogenous source. However, one should note that, at least currently, RNA therapies are not a perfect solution and still often suffer from low success rates due to heterogeneous drug penetration, rapid excretion, and delivery issues;[41] the last being especially problematic because a *MACF1* KD in an unintended wild-type cell can lead to abnormalities in its form or function.[23,42]

Regulatory RNAs aside, tracking the expression of *MACF1* and its interactors may also hold the key to many clinical advances.[38] Correlative data constructed using patient-derived samples show that decreased expression of *HectD1*, an E3 ubiquitin ligase that mediates the degradation of MACF1, is associated with both increased metastasis and cisplatin resistance in mouse models and that unusually elevated *MACF1* expression is predictive of poor clinical outcome.[38] Moreover, in the future, *MACF1* expression beyond a certain threshold could act as a biomarker for many cancers including glioblastoma[39] and colorectal cancers,[40] meaning that it could potentially be an additional parameter in diagnosis.

Intestinal barrier defects

The idea of MACF1 causation in other disease states is not unheard of, in fact, KO mouse models have shown that this factor is critical to maintaining an appropriate level of paracellular permeability in the mucosal epithelium of the intestine.[23,34] In 2019, Shi *et al.*[36] investigated how intestinal barrier defects induced by *MACF1* KO interact with a high-fat diet using a mouse model. This interaction was of interest due to the fact that a high-fat diet is associated with microbiota dysbiosis, metabolic disorders, inflammatory responses, and an increase in intestinal permeability.[36] In order to assay this interaction, four groups of eight-week-old mice (one conditional *MACF1* KO fed a standard diet, one conditional *MACF1* KO fed a high-fat diet, one floxed *MACF1* fed a standard diet, and one floxed *MACF1* fed a high-fat diet) were fed on either a standard diet or a high-fat diet for 16 weeks and subjected to a battery of tests during and afterward to assess how this variable affects their gut function. The results showed that *MACF1* conditional KO (cKO) mice fed on a standard diet not only gained 16.1% less weight but also had smaller livers, less white adipose tissue in their fat pads, a higher amount of lipid in their feces, and lower plasma triglyceride levels when compared to floxed mice fed the same diet.[36] This appears to show that *MACF1* cKO may lead to defective lipid uptake.[36] Each group of cKO mice also exhibited elevated rates of epithelial apoptosis and increased intestinal permeability compared to floxed *MACF1* mice.[36] Histological analysis of the intestines of the cKO mice fed a high-fat diet showed a higher than normal amount of submucosal swelling and inflammatory response.[36] Complementary plasma analysis showed high levels of tumor necrosis factor alpha which is associated with a higher susceptibility to the inflammatory response[36]; of note is the fact that all of the cKO mice seemed to have an especially high number of goblet cells that were larger than they are typically. [36] Changes in the community infrastructure of the gut biota in cKO mice were also observed.[36]

MACF1 defects in the intestine have been similarly examined with relation to abnormalities in wound healing response by Ma *et al.* in a study published in 2017.[37] This group used a combination of *in vitro* and *in vivo* techniques to measure the contributions of MACF1 to wound closure, tight junction dynamics, and the impact of its loss on an organism's susceptibility to developing ulcerative colitis. [37] The study showed that the loss of *MACF1* and MT disruption both inhibits tight junction disassembly upon extracellular calcium removal,;[37] this implicates MACF1's interaction with the MT network in the rapid tight junction disassembly seen in wild-type cells after extracellular calcium depletion.[37] In addition, loss of *MACF1* had a profoundly negative effect on cellular motility in wound healing assays. These results were recapitulated *in vivo*;[37] *MACF1* cKO mice showed significantly decreased migration rates in their intestinal epithelia and a significant delay in repair of oligocellular wounds.[37] Ultimately, compared to wildtype littermates, *MACF1* cKO mice experienced a quintupled mortality rate following DSS exposure, a significant increase in the rate of colitis, and higher amounts of both submucosal swelling and inflammatory cell infiltration in DSS-induced colitis. [37] This outcome is consistent with the correlation of *MACF1* expression levels and ulcerative colitis in human patients.[37]

IBD encompasses two chronic conditions, Crohn's disease, and ulcerative colitis, characterized by recurrent episodes of inflammation in the gastrointestinal tract;[43] this condition has many potential consequences including increased incidence of colitis-associated colorectal cancers.[44] IBD is lifelong and often debilitating; but, unfortunately there are few answers as to why it occurs.[37,43] However, Ma *et al.* provide very important insight into the pathogenesis of IBD by showing that MACF1-mediated coordinated cytoskeletal dynamics contributes greatly to normal cell adhesion during intestinal wound repair and that its dysfunction results in a vulnerability to IBD.[37] Though these findings do not amount to an

exhaustive explanation of IBD pathogenesis, they do elucidate an important mechanism by which MACF1 contributes to a related process and corroborates data derived from human patients that correlates MACF1 level with the progression and development of ulcerative colitis.[37] Additionally, this study provides a serious basis for future IBD research that may focus on other pathways in which MACF1 has an established role and in turn provide more answers as to how IBD develops.[37] Of note is the fact that despite the lack of a definitive answer as to how and why IBD occurs, there are many theories.[45] Among the most prominent of these is that intestinal immune imbalance resulting from an abnormal relationship between host and microbiota may lead to IBD.[45] Changes in overall microbiota community structure resulting from *MACF1* loss, shown by Shi *et al.*,[36] link this theory to MACF1 activity in that it shows coincidence between *MACF1* loss, colitis, and microbiota irregularities. Overall, it appears that more closely examining MACF1 activity may be an important stepping stone to a better understanding of the pathogenesis of IBD because it has established roles in many other cellular processes[14,17–19,23] and, in the future, may lead to a complete understanding of this disease, its interactors, and causes.

Other pathologies associated with MACF1 abnormalities

Though more data exist on the relationship between MACF1 and the pathologies discussed in the previous section (cancers and IBD) due to both the magnitude and pervasiveness of MACF1's regulation of the cytoskeleton, its dysfunction has, unsurprisingly, also been shown to at least potentially contribute to an already large and still growing number of other disorders.[42] In addition to those mentioned previously, MACF1 irregularity is linked to the pathogenesis of osteoporosis,[42] schizophrenia,[46] and as of most recently a spectrum of major brain deformity phenotypes in humans(e.g. lissencephaly, brain stem dysplasia and hypoplasia).[47]

The connection between osteoporosis and MACF1 was first identified in mice as a result of a bone mesenchymal stem cell-specific KO.[42] The loss of *MACF1* in this cell type leads to an osteoporosis phenotype in which the mice displayed both reduced osteogenesis and low bone mass.[42] A firm association between these observations and osteoporosis in humans exists in the form of expression data derived from human patients which showed low levels of *MACF1* mRNA.[42] Regarding schizophrenia, its possible link to MACF1 was extrapolated from performing exome sequencing on 45 schizophrenic patients along with their 90 unaffected parents as controls; this showed that the *MACF1* gene, as well as the other candidates identified, often displayed *de novo* variants, had an enriched co-expression profile in the prenatal prefrontal cortex, and showed some overlap with genes previously linked to schizophrenia.[46] Unlike the previous two cases, the link between *MACF1* mutations and developmental abnormalities of the brain described by Dobyns *et al.*[47] was discovered in humans due to a multicenter review which uncovered several cases of a rare form of lissencephaly in unrelated patients followed by the analysis of available genomic data. [47] Ultimately, this group identified recurrent heterozygous missense variants involving three of the four zinc-binding residues of the G2R domain of MACF1 which appear to be causative in defective neuronal migration and axon pathfinding. Taken together, these cases support the multisystemic importance of MACF1 activity and demonstrate ways in which it is relevant to medicine. Though these cases were not beyond reproach in that each had only a relatively small sample size[42,46,47] they provide at least a preliminary basis to pursue MACF1 as either a biomarker for these diseases or potential therapeutic target in the future.

Summary

The cytoskeleton and its associated factors are vital components of the cell which are intertwined in function just as they are in structure. A family of cytoskeleton-associated proteins known as the spectraplakins contributes a great deal to each of these attributes; the five known members are conserved across the metazoan clade and have shared functions. The giant cytoskeleton crosslinker MACF1/ACF7 is one of the two mammalian spectraplakins and, like its counterparts, has many roles in a diverse array of functions during the processes of signaling, development, cell migration, and potentially others. The large number of cellular processes associated with the *MACF1* gene is due to the large number of structurally different isoforms that result from alternative splicing events. The six isoforms of MACF1 known to exist differ from one another by their domain composition. Both the structure and responsibilities of each of the seven possible domains have been at least partially characterized, some outside the context of MACF1 and instead based on data extrapolated from other closely related proteins.

Moreover, MACF1 has roles in many diseases including Parkinson's disease, cancers, and IBD. Correlative and functional studies of the past decade have strongly suggested that either the dysfunction or dysregulation of *MACF1* leads to disease pathogenesis, indicating that MACF1 may be used as a biomarker, or is at least a special risk candidate in many instances. In the case of many different forms of metastatic cancers, expression data derived from patient cells show that abnormal levels of *MACF1* expression are a commonality and correlate strongly with clinical outcome. shRNA silencing screens have revealed that MACF1 is required for cell motility in model breast cancer cell lines; additionally, *MACF1* KD leads to a significant reduction in the migration and invasion of these cell lines both *in vivo* and *in vitro.* As for IBD, the loss of *MACF1* in the intestine was found to lead to a special vulnerability to colitis, defective nutrient absorption, and abnormalities in the healing process. Also, the *MACF1* expression level has been strongly correlated with colitis-associated colorectal cancers. *MACF1* expression could act as a potential biomarker that could aid in the diagnosis of these diseases. Based on these studies, some groups suggest that *MACF1*-specific antisense oligo therapy could, in theory,

be a viable clinical intervention to deter the progression of many cancers; applicable to both cancers and IBD.

Even given what has been deciphered thus far, there are many questions about MACF1 which remain. For instance, the structure of the PRD and conformation of the ABD during F-actin binding as they exist in MACF1 isoforms has not been fully parsed out; thus, some structural and mechanistic details of MACF1's domains remain unknown. The PLRD also needs to be further studied to determine its full range of activity and whether or not it holds the same functionality as it does in plakins. Structure aside, a better understanding of how MACF1 interacts with certain diseases is still needed, and what is known currently is not enough to fully exploit MACF1 in a clinical setting.

Parkinson's disease's genetic etiology and mechanistic causation have only been linked to *MACF1* in humans by *C. elegans* KD studies and correlative data from a small number of human cadavers, and thus a more robust interrogation of how and why this association exists is necessary. Concerning cancers, although silencing *MACF1* expression is known to suppress a metastatic phenotype, which isoform is responsible has not yet been identified. Finally, as for IBD, a large amount of information is needed to identify the signaling cascade in the process of *MACF1*-KO-induced inflammatory colitis.

Search strategy criteria

Data for this Review were identified by searches of ScienceDirect, Google Scholar, PubMed, and references from relevant articles using the search terms "MACF1," "ACF7," "Cytoskeleton," Spectrins," Plakins," "RNA," "Cancer," "IBD," and "Spectraplakin." Only articles published in English between 2000 and 2019 were included.

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