Received: 8 November 2020 | Revised: 29 November 2020 | Accepted: 30 November 2020

DOI: 10.1002/rmv.2202

REVIEW

WILEY

Proteomes, kinases and signalling pathways in virus-induced filopodia, as potential antiviral therapeutics targets

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Summary

Filopodia are thin finger-like protrusions at the surface of cells that are internally occupied with bundles of tightly parallel actin filaments. They play significant roles in cellular physiological processes, such as adhesion to extracellular matrix, guidance towards chemo-attractants and in wound healing. Filopodia were recently reported to play important roles in viral infection including initial viral attachment to host cells, cell surfing, viral trafficking, internalization, budding, virus release and spread to other cells in a form that would avoid the host immune system. The detailed virus-host protein interactions underlying most of these processes remain to be elucidated. This review will describe some reported virus-host protein interactions on filopodia with the aim of identifying potential new anti-virus therapeutic targets. Exploring this research area may lead to the development of novel classes of anti-viral therapeutics that can block signalling pathways used by the virus to trigger filopodia formation. Successful compounds would inhibit initial virus attachment, formation of filopodia, expression of putative virus binding protein, extracellular virus trafficking, and budding.

KEYWORDS
filopodia, protein, viruses, therapeutics-targets

1 | INTRODUCTION

The most important cellular event that results in the production of filopodia is the coordinated polymerization of filamentous actin alongside cellular membranes. This is used by the cell to carry out important cellular physiological processes, including phagocytosis, cell migration, morphogenesis, embryogenesis, endocytosis and many others. Organization of filamentous actin into 3-dimensional assemblies which are constantly adjusted provide the working mechanism of filopodia to accomplish varying needs of the cell. Filopodia should be distinguished from lamellipodia both of which are structures seen at the prominent edge of a motile cell. A lamellipodium is a cellular protrusion which resembles a sheet; they are thin, measuring 0.1–0.2 μm, and branched actin network is seen to fill their internal hollow structures. On the other hand, filopodia are thinner measuring about 0.1–0.3 μm in length, they are longer

Abbreviations: ARP2/3, actin related protein 2/3 complex; Cdc42, cell division control protein 42 homologue; CD4, cluster of differentiation 4; CK2, casein kinase 2; CXCR-4, C-X-C chemokine receptor type 4; Dia1, diaphanous-related formin-1; Ena/VASP, enabled/vasodilator stimulated phosphoprotein; GTPases, guanosine triphosphatase; HaCaT, human epidermal keratinocyte line; HK, human kidney cell; HMEC1, human microvascular endothelial cell; IRSP53, insulin-receptor substrate P53; MARV, Marburg virus; MENA, mammalian ena; MV, mature virus; NSC23766, N6-[2-[5-(Diethylamino) pentan-2-ylamino]-6-methyl-4-pyrimidinyl]-2 methylquinoline-4,6-diamine; NWASP, neuronal Wiskott–Aldrich syndrome protein; PAR1, serine/threonine-protein kinase 1; PEST, sequences enriched with Pro, Glu, Ser, and Thr; PI3, phosphoinositide 3 (PI3)-kinase; Rac1, Ras-related C3 botulinum toxin substrate 1; RhoA, Ras homologue family member A; Rif, Rho in filopodia; ROCK, Rho-associated protein kinase; SARS-CoV-2, severe acute respiratory syndrome corona virus 2; TIAM, T-lymphocyte invasion and metastasis-inducing protein; TK, tyrosine kinase; VASP, vasodilator-stimulated phosphoprotein; VSVG, vesicular stomatitis virus G protein; WASP, neuronal Wiskott–Aldrich syndrome protein.

pseudopodia like structures inside which tight bundles of filamentous actin are arranged in parallel (extensively reviewed by Mattila and Lappalainen.[1] Both structures are generated when the end of the barbed rapidly growing filamentous actin is towards the cell membrane. These extension of filaments adjust the terminal end of the cell which subsequently results in cell motility.[2,3] Filopodia are sometimes found rooted or protruding from lamellipodial actin network.[4–6] They play important functions like adherence to outer cell matrix, wound healing, recognition and response to chemical stimulus, neuron functions and embryogenesis.[7,8] Recent findings showed increasing significance of these structures in viral infection; viruses were associated with filopodia in many cells, although, how these interactions contribute to infection remains poorly understood.[9] Viral binding to filopodia shows rapid surfing (highly ordered lateral movement) in the direction of cell membrane before internalization, disruption of this movement significantly reduced viral infection.[9]

Smith, et al.[10] noted that virus can rearrange cell cytoskeleton proteins; these follow cellular signals which subsequently result in filopodia formation. Upon virus attachment, reorganization of cytoskeleton plays an important role in virus adherence to the cytoplasmic membrane for internalization.[11,12] Viruses can travel along pre-formed filopodia after attachment in search for internalization receptors.[13] Moreover, it was recently suggested that viruses activate the formation of filopodia to achieved enhanced infection of host cells.[14,15] Endocytosis in most viruses occurs only after the virus has accessed and formed virus-internalization receptor complex on the filopodia.[16]

Despite extensive studies on filopodia and its proteomes, the biological functions of filopodia as it relates to virus infection, the mechanism with which virus induced their formation, the mechanisms of enhanced infection upon interaction with filopodia, and filopodial proteins involved in virus–filopodia interaction are still poorly elucidated. Here, we summarize the current understanding concerning the role of filopodia in viral infection, mechanism of virus induced filopodia, the signalling pathways and proteins involve in virus induced filopodia.

2 | PROTEOMES AND SIGNALING PROTEINS ASSOCIATED WITH FILOPODIA

To study filopodia–viral interaction there is a need to first review some of the important sets of proteins that are actively involved in the generation of filopodia in different types of cells and organism. In this review our discussion will be limited to well-studied proteins that are directly involved in filopodia stimulation, generation and maintenance. Most of these proteins regulate actin cytoskeleton and are localized to regulate filopodia formation.

2.1 | Myosins-X (myosin-10)

Myosins are very important protein family in filopodia biology. They are motor proteins which bind to actin, and play crucial role in cell motility, they are reported to play important role in vesicle trafficking and are also important in the formation of actin protrusions.[17] They demonstrate a fine directed movement along actin filament. Myosin-X is one of the most important members of the myosin family protein that strongly promotes formation of filopodia.[17] The exact role they play in filopodia formation is not fully understood. Myosin-X a member of unconventional group of myosin, consists structurally of three pH domains; a coiled-coil domain known to facilitate dimerization, Sequence enriched with Pro, Glu, Ser, and Thr domain involved in binding to microtubules, known as myosin tail homology 4 and the last domain, which are in addition to the major domain called actin motor domain three IQ motifs.[18] It has also been observed that overexpression of myosin-X triggers formation of filopodia in many cell types. Furthermore, this protein was observed to be localized to filopodia tips.[17] Other proteins associated to myosin family like myosin VIIa, VIIb and XV are essential in microvilli formation, a structure similar to filopodia.[19] Myosin-X moves along the length of filopodia shaft toward filament barbed end, and then uses retrograde actin flow to slide down, demonstrating the motor activity of the protein.[17,18] Myosin-X interaction with other proteins different from filamentous actin has also been reported, these includes integrins, vasodilator-stimulated phosphoprotein (VASP), netrin receptors, microtubules and phosphatidylinositol phosphates.[20–23] More information is still needed to fully elucidate the functions of myosin-X in the formation and function of filopodia. It was however reported that myosin-X transports important filopodial proteins to the dense tip of filopodia during active filopodia formation; these include enabled/vasodilator stimulated phosphoprotein (ENA/VASP) and integrins. And the motor domain of this protein is involved in the initiation of filopodia.[23] Furthermore, lateral movement of myosin-X at the periphery of cells was observed, this indicated the movement of barbed end of filamentous actin and the subsequent convergence of actin filament that results in filopodia formation.[24]

2.2 | Integrins are associated with myosin-X in filopodia formation

Integrins are heterodimeric molecular receptors which are very important in cell migration.[25] Integrins play crucial roles in the organization of actin by transducing the signals which activate regulatory pathways involved in this process.[26] Integrins are conveyed actively to the tip of filopodia by myosin-X during filopodia formation.[21] But whether the binding of integrin to myosin-X is required to regulate filopodia formation is not very clear.

Induction of lamellipodia nucleation which is an important event in filopodia formation was reported to be Rac1-dependent and was mediated by integrin-filopodia-adhesion.[27] Thus, Arjonan et al.[28] proposed that, though integrins are useful in filopodia dynamics, myosin-X can still induce the formation of filopodia independent of integrin, though they acknowledged the contribution of this protein in the function and stability of filopodia and lamellipodia especially in migrating cells.

2.3 | Formins

Formins are group of proteins characterized by having a conserved actin polymerizing formin homology2 domain, that is important in filopodia formation; they comprise about 15 Ras homologue (Rho) guanosine triphosphatase (GTPase) effector proteins.[29] Formins are important in many cellular processes, including cytokinesis, cell polarity and migration.[30] Formin triggers progressive elongation and nucleation of barbed-end of actin, which produces unbranched actin filament[29] to induce the formation of filopodia. Diaphanous-related formin-2 (Dia2) is the most important studied member of formin proteins in filopodia dynamics. Overexpression of Dia2 induces filopodia formation, and on the other hand, absence of Dia2 inhibits formation of filopodia in an experiment with melanoma cells.[31] Moreover, increased formation of filopodia correlated with up-regulation of compensatory Dia2 in an experiment with cells lacking diaphanous-related formin-1.[32] It is worth noting that, out of the 15 mammalian formin proteins, only few members are studied in detail for their functions, function of other members in filopodia formation remain to be elucidated.

2.4 | Enabled/vasodilator-stimulated phosphoprotein

The ENA/VASP-family are relatively large multifunctional actin-binding proteins that contribute significantly to filopodia formation in mammals and other organisms.[33] ENA/VASP were localized in mammalian cells at leading edge of filopodia and are useful in cell-cell contact.[34,35] They were thought to function in removal of capping protein during filopodia formation. However, findings with total internal reflection fluorescence microscopic studies and biochemical investigation have shown that, these proteins do not uncap barbed ends of actin filament, but rather demonstrate strong anti-capping activity during active rearrangement of actin in filopodia initiation.[36] Furthermore, ENA/VASP play important roles in anti-branching of actin, F-actin bundling, and enhancement of filament elongation, apart from inhibition of actin capping, which were important events in filopodia dynamics.[33,37] Functions and the role of this protein in filopodia formation need to be further elucidated, in vivo relevance of some of these studied activities also need to be established, as well as total description of ENA/VASP protein role in the formation of filopodia and its function.

2.5 | Rho GTPases (small GTPase protein family)

Rho GTPases belong to a protein family of (Mr~21,000) signalling G proteins, and a subgroup of Ras protein superfamily, they are functionally involved in regulation of actin cytoskelaton and cell morphology. For this review, cell division control protein 42 homologue (Cdc42), Ras-related C3 botulinum toxin substrate 1(Rac1) and Ras homologue family member A (RhoA) will be discussed, which are the best studied mammalian Rho GTPases.[38]

Ras homologue family member A

One of the most important functions of Rho protein has been observed in structural changes that lead to filopodia and lamellipodia formation, where Rho activates lamellipodia and filopodia formation in fibroblasts and other eukaryotic cells.[39] Differential equations were used by Sakumura, et al.[40] to develop a mathematical model for the activity of Rhos and their relation to cell motility.[40] The model includes RhoA, Cdc42 and Rac protein; in this model, actin de-polymerization and the extension of lamellipodia is correlated to Rac proteins, while blocking of actin de-polymerization and filopodia elongation was encouraged by Cdc42 and RhoA was significant in actin retraction.[40] Nobes and Hall[41] similarly, observed a promotion of lamellipodia formation by Rac1, filopodia formation by Cdc42 and focal adhesion and stress fibre formation by RhoA. Taken together these findings indicate that these proteins are critically involved in filopodia formation and its dynamics.

Cell division control protein 42 homologThese are also important well studied small GTPases involved in various cellular processes; it is also the most elucidated signalling pathway protein. They regulate the induction of actin related protein 2/3 complex (ARP2/3) complex-dependent nucleation of filamentous actin by activation of neuronal Wiskott–Aldrich syndrome protein (N-WASP) and Wiskott–Aldrich syndrome protein (WASP).[42,43] Branched lamellipodia actin network was believed to be nucleated by ARP2/3 complex. Interaction of Cdc42 with N-WASP and WASP protein also relieves auto-inhibited conformation of WASP protein. Cdc42 binds to phosphatidylinositol-4,5-bisphosphate which activates ARP2/3 complex.[44,45] Blockage of Cdc42 function blocked filopodia formation.[45]

Rho in filopodia

This is yet another member of Rho family GTPase, that is an important and potent filopodia stimulator.[46] They are reported to induce filopodia formation independent of other small GTPase and Cdc42, but reported to induce filopodia formation in association with Dia2.[45,46]

2.6 | Insulin-receptor substrate

Insulin-receptor substrate P53 (IRSp53) is another important filopodia protein which contains I-BAR-domain/IM domain. They are relatively large scaffolding proteins that normally bind the small GTPase Cdc42 and other proteins. Overexpression of this protein triggers formation of filopodia and lamellipodia.[47] A study shows that IRSp52 worked synergistically with mammalian ena under the control of Cdc42 to induced filopodia formation.[48] Bundling of filamentous

actin and membrane tabulation were induced by IRSp53, which is seen to directly facilitate formation of filopodia; IRSp52 was also reported to play part in engaging actin-regulatory proteins to the lamellipodia and filopodia.[49]

2.7 | Fascin

Fascin is an evolutionary conserved acting bundling protein known to regulate filopodia formation.[50] Expression of Fascin-1 in cells triggered cellular migration in vitro.[51,52] Fascin also plays significant roles in generation and maintenance of filopodial tight F-actin bundles. Fascin has also been demonstrated as the most significant actin filament-bundling proteins, which are directly linked to filopodia formation.[53] Fascin generates parallel and stiff filamentous actin bundles; even though they are not efficient preformed filament bundlers, they are implicated in bundling of loosely linked and polymerized actin filaments.[54] Dynamic, reversible relationship of actin bundles and fascin have been reported in filopodia and linked to dynamic filamentous actin crosslinking, which is a requirement for the efficient bundling of actin filament reaching filopodial tip. They are also important proteins in intra-filopodial trafficking and filopodial twisting. Resistance to fast perturbations are also mediated by fascin because fascin mediated crosslinks are mostly rigid.[50,55] Loss of filopodia were reported in mammalian cells experimentally depleted of fascin[50] indicating the importance of this protein in the formation of filopodia.

Many important actin-crosslinking proteins have also been reported in different cells, this includes; espin, α-actinin, filamin and fimbrin, they are flexible crosslinkers that are not directly important in filopodia.[50]

2.8 | Wiskott–Aldrich syndrome protein

Another important protein reported to be involved in filopodia activation is a member of protein/WASP family, known as Wiskott–Aldrich syndrome protein. This is a scaffold whose major function is conversion of small GTPase signals to the actin-related proteins 2 and 3 (Arp2/3). This is achieved through a pathway known as the convergent elongation model of filopodia initiation; induction of filopodia formation by Arp2/3 was reported to be related to enhanced activity of actin filament branching by this protein.[6,56]

3 | SIGNALING PATHWAYS AND KINASES OF FILOPODIA FORMATION AND THEIR ROLE IN VIRUS INFECTION

Tyrosine kinase (TK) and phosphoinositide 3 (PI3)-kinase signalling pathways are responsible for human papillomavirus (HPV) induced filopodia formation in human keratocyte (HKs) cells.[10] The roles of these kinases in viral induced filopodia was demonstrated by strong induction of filopodia observed in HK cells after exposure to HPV31 for 30 min. Wortmannin and genistein (inhibitors of PI3 and TK respectively), proportionally inhibited filopodia formation by HPV31 in viral infection assays, suggesting the involvement of both TK and PI3K in the regulation of processes that lead to the formation of filopodia.[10]

The usefulness of these signalling pathways in viral infection were investigated, complete and nearly complete infection inhibition was reported in cells treated with wortmannin and genistein respectively.[10]

Many other viruses also utilize different signalling pathways to activate filopodia for successful infection. Epstein–Barr virus and herpes simplex virus can activate Cdc42 GTPases and Rac1 to induced lamellipodia and filopodia in fibroblast cells and epithelial cells.[57] Virus activation of filopodia was also reported in dengue virus by Zamudio-Meza et al.[58] they were reported to have analysed signalling pathways known to regulate filopodia formation in Human microvascular endothelial cell (HMEC-1) cells infected with dengue virus-2. Treatment of these cells with specific inhibitors of Rac1 activation (i.e., N6-[2-[5-(Diethylamino) pentan-2-ylamino]-6-methyl-4-pyrimidinyl]-2 methylquinoline-4,6-diamine [NSC23766]) prior to virus infection, blocked filopodia formation, suggesting the importance of Rac1 in dengue virus activation of filopodia. However, activation of RhoA in these cells resulted in the formation of prominent stress fibres, after Rac1 pathway was blocked.[59] Inhibition of RhoA signalling pathways using Y-27632 does not seem to block filopodia formation. This demonstrates that Rac1 signalling pathway is involved in dengue virus infection. Blocking this signalling pathway ultimately results in virus infection reduction or virus infection inhibition,[58] suggesting potential therapeutic targets for this virus that has neither vaccine nor treatment yet.

Pull-down experiment in dengue virus infected cells, showed Rac1 activation to be induced just before the filopodia are produced and reaches high level before Cdc42 attained highest activity value. The possibility of cross-talk between Rac1 and Cdc42 signalling pathways which resulted in Rac1 upstream activation of Cdc42 was therefore suggested.[58] Pretreatment of cells with NSC23766, which specifically blocks Rac1 and T-lymphocyte invasion and metastasis-inducing protein interaction, suggest that dengue virus could trigger signalling by activating PI3K after binding to the integrin receptor on HMEC-1 cells, which results in the formation of filopodia. In this pattern, PI3K activation is reported to take place upstream of Rac1 activation,[58] signifying the importance of both signalling pathways in dengue virus induced filopodia formation in these cells. Clinical potential of compounds that block these pathways can be explored for possible therapeutic use in dengue virus infections.

Recent studies revealed down regulation of PAK1/2 and Rho-associated protein kinase1/2 kinases downstream of Rho/Rac/Cdc42 GTPases, Vimentin S39 and S56, stathmin S16 and S25 which are the phosphorylation target site for PARK1/2 kinases were also down regulated during severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) infection in Vero cells.[60] Interaction of SARS-CoV-2 Nsp-7 with RhoA has been implicated in the down regulation of these kinases during the virus infection.[61] On the other hand, strong up-

regulation was observed of casein kinase 2 (CK2) signalling pathway, this is evident by strong phosphorylation of the CK2 target proteins, like motor domain of myosin IIa.[62] Furthermore, SARS-CoV-2 Nsp2 protein interacts directly with a sub-unit protein of actin assembly-inducing WASH complex protein called strumpellin, strongly supporting the actin structural reorganization that leads to filopodia formation.[60] Taken together, there is strong evidence of altered cellular signalling pathways and kinases by SARS-CoV-2 infection of this cell, most of the targeted signalling were actin and cytoskeletal related with only one targeting cell cycle,[62] qualifying these kinases involved as a potential novel target for development of potent SARS-CoV-2 therapeutics.

4 | ROLE OF FILOPODIA IN ENHANCED VIRAL INFECTION

4.1 | Actin, myosin and other filopodia proteins in virus surfing and infection

The interaction of virus with the cellular filamentous actin that results in viral particles surfing, demonstrated a process that is actin motor-driven. The role of actin in this process has been experimentally proven by the addition of cytochalasin D, a blocker of barbed end actin filament. After its addition in viral infection experiment, viral surfing was inhibited, suggesting the significance of actin in viral surfing.[9] Lehmann, et al.[9] reported that treatment of the cell with cytochalasin D or sodium azide resulted in complete loss of directional motility, and diffuse random movement toward the cell body. This indicates that surfing might be an energy-dependent processes which is controlled primarily by actin cytoskeleton. Furthermore, myosin motors were involved in actin filament movement on filopodia and lamellipodia[63] by retrograde F-actin flow mechanism.[64] Research implicates different members of myosin in this important cellular event. Myosin II, a plus-end motor protein localized to lamellipodium and retraction fibres,[65] was implicated in viral surfing. This is also demonstrated by the addition of inhibitor compound blebbistatin to the cell in viral infection assay, and was reported to block viral surfing.[66] Addition of blebbistatin to cells also completely blocked avian leucosis virus (ALV), murine leukaemia virus (MLV), and vesicular stomatitis virus (VSV) from surfing the filopodia.

Virus surfing along lamellum of fibroblast cells was blocked in similar experiments, which ascertained that viral surfing over the surface of this cellular protrusion is also facilitated by the myosin II-actin machineries.[9] Host cell infection by vaccinia virus can be inhibited by treatment with blebbistatin.[14] A study shows diffuse perinuclear redistribution of myosin II in a cell treated with blebbistatin.[9] From the highlighted studies it can be seen that viral motility on the filopodia and lamellipodia are promoted by myosin II. Myosin II also affects retrograde filamentous actin flow in filopodia, concluding that the major ATPase implicated in virus surfing is myosin II and qualifying myosin II activities as a potential virus therapeutic target with broader consequences.

Myosin-X plays vital roles in the transportation of Marburg virus on host cell filopodia before subsequent internalization. Schudt et al. reported that even though the signalling, adaptor and motor protein that mediate the transportation of nucleocapsids of Marburg virus (MARV NC) in the cell cytoplasm is not fully elucidated, the velocity of the propelled MARV on different surfaces of the cell and on the filopodia, implicated differential regulation of actin-motor proteins.[67] Myosin-V was good candidate, looking at the speed of the virus on filopodia, because the speed of 200 to 1,000 nm/s was associated with plus end directed myosin-V activity, and the virus was propelled at similar speed.[68] It was further suggested that minus end-directed myosin-VI is involved in MARV surfing, for the fact that myosin-VI also uses speeds of 300–400 nm/s to transport its cargo.[69] Myosin-X mediates slower MAR virus movement (84 ± 36 nm/s).[17] Taken together Myosin-X, myosin-V and myosin VI, were crucially important for virus surfing. Attenuation of these proteins negatively affected or completely blocked virus surfing, and subsequently affected infection and hence, may serve as potential anti-virus drug or therapeutic targets.

4.2 | Cell surfing observed in many viruses precedes virus internalization

Virus surfing has been reported in many viruses and it is an important process required for virus infection. Virus surf filopodia in the search for an internalization hot-spot or virus receptor/co-receptor, which upon encounter, forms a virus-host receptor complex, this complex was observed to be transported via actin cuticle and become internalized. This was demonstrated in an assay involving MLV, where modified MLV containing viral capsid poly-protein precursor (Gag) labelled with yellow fluorescence protein and MLV capsid containing envelope glycoprotein of ALV were fused with ALV receptor Tva (forming virus-receptor complex) on the surface of filopodia, and surf along HEK 239 cells before internalization.[70] Filopodia surfing was also reported in human immunodeficiency virus (HIV) upon encounter with the HIV receptors C-X-C chemokine receptor type 4 (CXCR4) and cluster of differentiation 4. This was further demonstrated in particles bearing the VSV envelope protein on the filopodia of rat fibroblasts.[9] Furthermore, progressive surfing along filopodia toward cytoplasmic membrane was observed in Vesicular stomatitits virus G protein-containing viruses, this continued until the particle disappeared from the microscopic plane of confocal microscopy, indicating virus internalization.[9] In another demonstration, colocalisation of clathrin with the viruses within fifteen minutes after exposure was reported. Recruitment of clathrin to the surfing viruses occurs as soon as the virus particle reaches the plasma membrane. Interestingly, this movement continued as the clathrin is recruited. These findings showed that viruses move along filopodia to reach cell surface for endocytic hot spots.[9] Internalization of particles after surfing was also reported in human papillomavirus 31 (HPV31) on A431 HKs and human epidermal keratinocyte line HKs cells. In these cells, HPV31 travelled along filopodia towards cytoplasmic

membrane, indicating the role of filopodia surfing in facilitated uptake of the HPV31 from extracellular matrix.[10] Furthermore, it has been reported that formation of filopodia in a cell facilitate enhanced virus infection, this was achieved via one of the several ways among which is, filopodial retraction, in which a distal viral particle is transferred to the cell body via filopodia for internalization. Or by a mechanism called lateral curling; here the virus particle which just attached to the filopodia tip is quickly and immediately internalized by filopodia bending backward to the cell body and the particle is internalized.[10] In this way virus particles are internalized even more quickly than in a normal viral particle surfing.

Another form of viral motility on filopodia was observed in endocytic viruses like Semliki Forest virus and VSV, where the virus is transported directly to clathrin coated pits by inducing directed and fast viral trafficking along filopodia to reach the cell body for entry into cells, in an efficient infectious pathway.[9] Taken together, it is obvious that virus surfing can be a promising anti-virus therapeutic target, with potential to block initial virus infection or reinfection.

5 | VIRAL INTERACTION AND MOTILITY ON FILOPODIA ENHANCES INFECTION

Viruses transport along filopodia during the attachment that result in virus internalization has been reported in many enveloped viruses,[9,14] and non-enveloped viruses.[10,71] The particle motility on filopodia is defined as positive when the movement is directed to the cell cytoplasm, and as negative when the particles is moving away from the cell cytoplasm to filopodia tip. Lehmann, et al.[9] reported initial random movement of MLV particles, which later changed to directional surfing toward cytoplasm at various speed. Upon virus attachment to the filopodia, particles were observed to rapidly move along the length of filopodia toward the cell body, and more than 90% of the particles were internalized shortly after this event.

In a study by Mercer and Helenius,[14] viral association with filopodia was required for infection of variola virus, a poxvirus containing DNA and an envelope viruses and the causative agent of smallpox. During replication of this virus, two types of infectious viral particles are produced; extracellular enveloped virus and intracellular mature virus(MV). Intracellular mature virus attached to filopodia and surfed to the cytoplasmic membrane of the host cell; this movement was uninterrupted, and on reaching the cytoplasmic membrane spherical bleb extruded at a point where virus had contact with the membrane. Further blebs were also formed and lasted for seconds, bleb retraction occur after the accumulation of actin on the membrane. Virus entry coincided with reassembly of cortical actin and bleb retraction. When blebbistatin, a chemical known to block myosin II–dependent blebbing[72] was used to treat these cells, infection was reduced by 65% by preventing the formation of MV-induced blebs.[14] This clearly indicates the role of viral association with filopodia for productive entry and infection, and its novelty as a virus therapeutic targets.

Furthermore, filopodia tip was recently reported to be the point through which MARV budding takes place.[73,74] MARV is released into the surrounding environment via filopodia.[73,75] Similarly, MARV NCs were reported to bud out from the tip and the side of the filopodia in addition to the surfing through the interior of filopodia.[73,74] MARV utilized this strategy to spread to the neighbouring cell without been affected by the hostile host environment immune system and proteases.[67] Release of other virus via filopodia is well documented. Virus egress via filopodia has been reported in retroviruses.[9,76] Furthermore, the budding of HIV-1 particles from filopodia has also been reported in infected dendritic cells.[76,77]

HIV-1 upon expression of Nef protein was reported to induce filopodia formation during infection of T-lymphocytes. Expressed HIV-1 Nef protein interacts with Arp2/3 to promote formation of unbranched actin-filament which subsequently results in filopodia formation.[78]

Filopodia serve a vital role in HIV-1 infection by enhancing cell-to-cell spread of the virus, communication and exchange of virus and cellular materials between neighbouring lymphocytes and bystander macrophages through filopodia-bridges, a nanotube like cellular architecture that remotely connects neighbouring cells.[79,80]

Nsp2 protein of SARS-CoV-2 directly interacts with strumpellin a sub-unit of actin assembly-inducing WASH protein complex, this interaction results in cytoskeletal modifications that lead to the formation of filopodia.[60] SARS-CoV-2 induces rapidly increasing filopodial protrusion during virus infection in human colon epithelial cells.[60]

Furthermore, using higher resolution electron microscopy, M protein of SARS-CoV-2 clusters along the shaft of the already formed filopodia, indicating virus assembly on this structure. Virus particles line up along the filopodia tips and shaft, in a format suggesting virus egress via filopodia. Partial colocalization of SARS-CoV-2 N protein with CK2 in assembled virus and virus budding along the filopodia shaft were also observed.[60] Taken together, this finding indicated that SARS-CoV-2 utilized filopodia for virus egress, virus budding, cell-to-cell spread, and virus assembly. Targeting filopodia and its machineries through drug design, therapeutics or vaccines could be a promising approach toward control of this virus. The up-regulation of annexin II expression, a newly discovered dengue virus 2 interacting protein, was observed upon filopodia formation caused as a result of dengue virus 2 exposure to Vero cells.[81] Formation of filopodia results in the translocation of annexin II to the surface of Vero cells for virus interaction and internalization.[81] This further stressed the potential of targeting filopodia and its machineries in the control of this virus. This was reviewed extensively by Aliyu, et al.[82]

6 | SUMMARY, CONCLUSION AND FUTURE DIRECTIONS

One of the promising cellular events not fully explore for virus–host interaction is filopodia–viral interaction; there is a paucity of data and the published data mostly concentrate on imaging studies.

Filopodia play a significant role in viral infection; they facilitate initial viral attachment to cells, they serve as a transportation medium for the virus to reach the cell surface, they are involved in viral trafficking, cell surfing and virus budding, egress and spread. Filopodia enhance virus infection by many physical strategies including lateral curling and filopodia retraction.

Exploring filopodia proteomics and signalling dynamics may potentially lead to the development of a novel class of viral therapeutics that can work, for example, to block some signal pathways that the virus uses to trigger filopodia formation. Such a compound could target inhibition or reduction of initial attachment of virus particles to the cell. Novel classes of therapeutics might also target blockage, expression or activation of some important proteins expressed upon filopodia formation, which are used by the virus for infection. They may inhibit filopodia formation, viral transport to cell surface, subsequent internalization and productive infection. If formation of filopodia is inhibited, internalized virus particles cannot bud out or be released to infect other cells.

ACKNOWLEDGMENT

We want to acknowledge the support from the Ministry of Education Nigeria, TETFUND Research Grant Number: BUK/R/T-EPIC/BIO/F2 Bayero University Kano (BUK), for their support toward the successful execution of this research.

CONFLICT OF INTEREST

The authors have no competing interest.

AUTHORS CONTRIBUTION

Conceptualization of idea, Isah Abubakar Aliyu, Abdulhadi Sale Kumurya, Jamilu Bala Abubakar and Hassan Yahaya. Methodology and literature search; Isah Abubakar Aliyu, Jamilu Bala Abubakar and Hayatu Saidu. Format and organization; Isah Abubakar Aliyu and Hayatu Sa'id. Writing-Original Draft; Isah Abubakar Aliyu, Hassan Yahaya and Jamilu Bala Abubakar. Writing-Review, supervision & Editing; Isah Abubakar Aliyu and Abdulhadi Sale Kumurya.

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How to cite this article: Aliyu IA, Kumurya AS, Bala JA, Yahaya H, Saidu H. Proteomes, kinases and signalling pathways in virus-induced filopodia, as potential antiviral therapeutics targets. *Rev Med Virol*. 2021;31(5):e2202. doi:10.1002/rmv.2202