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Vimentin as a target for the treatment of COVID-19

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ABSTRACT

We and others propose vimentin as a possible cellular target for the treatment of COVID-19. This innovative idea is so recent that it requires further attention and debate. The significant role played by vimentin in virus-induced infection however is well established: (1) vimentin has been reported as a co-receptor and/or attachment site for SARS-CoV; (2) vimentin is involved in viral replication in cells; (3) vimentin plays a fundamental role in both the viral infection and the consequent explosive immune-inflammatory response and (4) a lower vimentin expression is associated with the inhibition of epithelial to mesenchymal transition and fibrosis. Moreover, the absence of vimentin in mice makes them resistant to lung injury. Since vimentin has a twofold role in the disease, not only being involved in the viral infection but also in the associated life-threatening lung inflammation, the use of vimentin-targeted drugs may offer a synergistic advantage as compared with other treatments not targeting vimentin. Consequently, we speculate here that drugs which decrease the expression of vimentin can be used for the treatment of patients with COVID-19 and advise that several Food and Drug Administration-approved drugs be immediately tested in clinical trials against SARS-CoV-2, thus broadening therapeutic options for this type of viral infection.

INTRODUCTION

The COVID-19 has triggered a global public health crisis with more than 10 million infected people up to date (30 June 2020) worldwide and a global incidence rate which is still growing.[1] SARS-CoV-2 infection causes an explosive immune response, characterised by a cytokine storm, a subsequent progression to lung injury and an acute respiratory distress syndrome leading to death.[2 3] Despite many promising therapeutic options for the treatment of COVID-19, anti-SARS-CoV-2 drugs or vaccines are still under investigation.[4]

Vimentin, known as a cytoskeletal protein belonging to the intermediate filament (IF) family, plays an important role in stabilising intracellular architecture through its mechanical role in cell plasticity and organelle anchoring, in the metabolism of lipids and in cell remodelling through its interaction with signalling molecules and components of gene regulatory networks.[5–10] At cellular level, vimentin has been shown to be implicated in cell proliferation, stiffness, adhesion, migration, differentiation, senescence and apoptosis.[7–10] Vimentin also plays a role in inflammation and immune responses, as well as in the epithelial to mesenchymal transition (EMT); the latter, in turn, being involved in the opening of epithelial barriers and cell migration.[11 12]

Strikingly, intracellular vimentin has been shown to be implicated in the processes of virus fusion, replication and assembly (for a review, see Ramos *et al*[8]). Vimentin was additionally found to be located outside of the cell, where it serves as an attachment site for viral proteins, in the majority of cases favouring viral binding/entry.[13–16] One possible exception was reported for the human papillomavirus type 16 (HPV-16) infection, in which vimentin actually had the opposite effect.[17] In this case, the authors reported that cell surface vimentin interfered with viral binding to its receptor, thus having a protective effect against viral infection. However, the role of vimentin for this specific virus is debated and contradictory reports have been published. As evidence to the contrary, Jacobs *et al*[18] experimentally reduced the level of vimentin on the surface of cervical cancer cells by exposing the cells to the hookworm *Nippostrongylus brasiliensis*, with the result of decreasing the internalisation of the HPV-16 in these cells.[18] One of the proposed mechanisms underlying this observation was the production of T helper-derived cytokines (eg, interleukin (IL)-4) associated with the helminth infection, which could, in turn, have modified the expression of HPV receptors in cancer cells.

During the submission period of the present manuscript in which we propose vimentin as a potential therapeutic target against viral infection, another two papers were written advocating the same hypothesis.[8 9] Vimentin was also been previously proposed as a potential therapeutic target in cancer treatment and other disease conditions such as fibrosis. The upregulation of vimentin typically observed in oncogenic transformation and its involvement in cancer cell migration highlighted the possibility that vimentin is not only a marker but also a player in the development of cancer (reviewed in Refs 10 and 19). Therefore, molecules such as withaferin A, FiVe1 and simvastatin were used to perturb the expression and/or the assembly of vimentin filament and led to the downregulation of soluble vimentin, accompanied by decreased fibrosis, the disruption of mitotic cells, filament disorganisation and cell death.[20–22]

This paper summarises the scientific evidence indicating that reducing the expression of vimentin could be a powerful therapeutic option for COVID-19, since it could hamper SARS-CoV-2 infection and, at the same time, decrease the immunological response that is ultimately responsible for the often lethal acute respiratory distress syndrome. This hypothesis can be readily tested through clinical trials since Food and Drug Administration (FDA)-approved pharmacological treatments targeting vimentin for other pathologies are already available.

Vimentin is a co-receptor for SARS-CoV and possibly for SARS-CoV-2

ACE 2 (ACE2) is a cellular receptor for the SARS-CoV spike protein.[23] However, it has been shown that ACE2 is not sufficient to make host cells susceptible to infection[24–26] and vimentin has been proposed as a co-receptor for the entry of SARS-CoV into cells.[13] Indeed, the authors reported that the SARS-CoV virus enhances cell surface vimentin expression and, importantly, showed the existence of a direct interaction between vimentin and the SARS-CoV spike protein during viral entry.[13] Moreover, SARS-CoV and SARS-CoV-2 share similar spike protein sequences and the same cell entry route.[27] Interestingly, it has been recently reported that two proteins that are well known to be involved in the entry of SARS-CoV-2 into the cell—ACE2 and TMPRSS2—are both expressed by two cell types, that is, lung type II pneumocytes and nasal goblet secretory cells[28]; it is worth noting these two cell types also express high levels of vimentin.[28–30] In addition, a potential interaction between vimentin and the SARS-CoV-2 spike protein is revealed in a SARS-CoV-2 protein–protein interaction map (see supplemental data).[31]

Vimentin starts being expressed in the early stages of embryonic development by highly plastic precursor cells, whereas in postnatal life it is found in fibroblasts, endothelial cells, smooth muscle cells and in the lining epithelial cells of the lung, gut and other mucosae.[29 30 32 33] Here vimentin expression is quickly increased in response to viral infection and inflammatory stimuli[34–36] since vimentin belongs to the 'immediate early genes' family, a group of genes which are quickly activated in the presence of infection and inflammation. In this condition, due to its translocation to the cell surface, vimentin works as a co-receptor or attachment site for several viruses, including the Japanese encephalitis virus,[37] the porcine reproductive and respiratory syndrome virus,[38] the enterovirus 71,[39] cowpea mosaic virus,[14] dengue virus[16] as well as the coronavirus.[13] Interestingly, an upsurge of vimentin expression accompanies, and favours, viral entry of the majority of viruses including SARS-CoV[13] with the exception of HPV-16 in which viral entry is actually hampered by an increase in vimentin expression.[17] Consistently, interfering with vimentin expression or treating cells with the neutralising 'anti-vimentin' antibodies attenuates some types of viral infection.[8 9 18 37 38] However, it is unknown if vimentin is involved in the infection of the Middle East respiratory syndrome coronavirus (MERS-CoV) which uses dipeptidyl peptidase 4 as the host cell receptor.[40]

Vimentin is involved in different steps of viral replication or assembly since it colocalises with viral capsid proteins in the endoplasmic reticulum of the infected cells,[41] cooperating to produce virus particles.[42] It is reported that vimentin is implicated—through the control of lysosomal trafficking and reduced translocation of viral ribonucleoproteins—in the progression of the influenza A virus, acting as a restriction factor for viral replication.[43 44] In addition, vimentin influences the hepatitis C virus (HCV) and Theiler's virus replication through its interaction with virus core proteins.[45 46] A strong reduction of HIV-1 replication was reported in vimentin knockdown cells, showing that vimentin is necessary for viral replication.[47] Vimentin contributes to EMT, a process by which epithelial cells, including those lining the lung mucosa, lose their polarity and adhesion. EMT thus confers migratory and invasive properties to the cells in a variety of pathological conditions such as viral infections, angiogenesis, chronic autoimmune inflammatory diseases, inflammatory bowel diseases, chronic obstructive pulmonary disease, cystic fibrosis and cancer. Overall, EMT promotes the creation of a suitable environment for viral infection and inflammation.

Vimentin expression is regulated by a plethora of transcription factors such as NF-κB (nuclearfactor-kappa B), Stat3 (signal transducer and activator of transcription 3), ZBP-89 (Kruppel-type zinc-finger family transcription factor), Smads (smallmothers against decapentaplegic transcription factors), AP-1 (activator protein 1), Sp1 (proximal specificity protein 1) and PEA3 (polyomaenhancer activator 3)—see figure 1. In response to viral infection or other insults, vimentin expression quickly increases. There are other transcription factors, such as Snail, Twist, ZEB2 and Slug,[12 48–52] that regulate vimentin expression during EMT so that the overexpression of vimentin and N-cadherin in epithelial cells leads to the loss of the epithelial phenotype.

In addition, vimentin expression is also regulated epigenetically through the chromatin modifications of its promoter.[53] These modifications have been studied in cancer cells since they promote EMT—a crucial event in the formation of tumour metastases. In particular, the methylation level of the vimentin promoter inversely correlates with the expression of vimentin in gastric

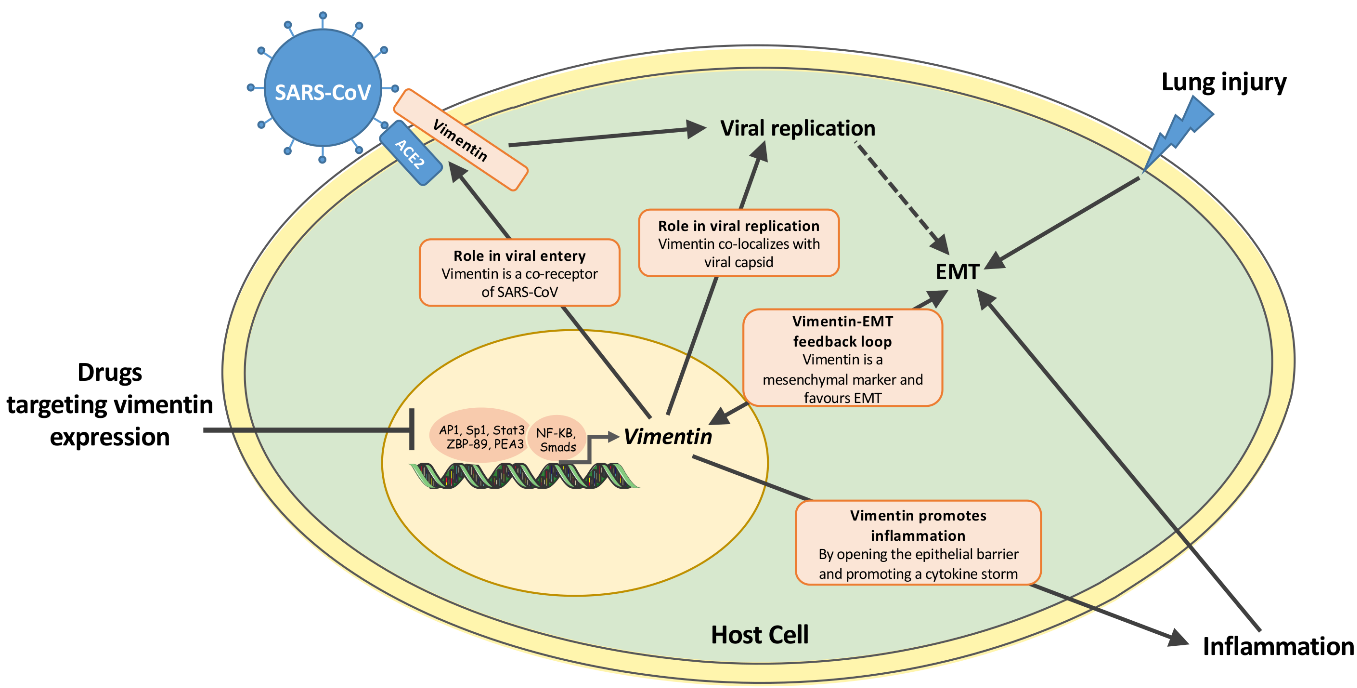

Figure 1 Schematic representation of the hypothetical role of vimentin in the crucial steps of viral infection and in the inflammation leading to lung injury. Drugs inhibiting vimentin expression in the nucleus of infected cells offer the advantage of a synergic effect as compared with other treatments not targeting vimentin. This is due to the fact that the vimentin protein is involved in: (1) viral entry[13–16]; (2) viral replication[8]; (3) endothelial to mesenchymal transition (EMT)[12] and (4) inflammation.[11 56–60] Therefore, decreasing the expression of vimentin would contribute to hampering viral infection and, in parallel, reducing the inflammatory response ultimately leading to COVID-19 lung injury, acute respiratory distress syndrome and fatalities. Worth noting, several transcription factors (including NF-kB, AP1 and Sp1) are implicated in the regulation of vimentin expression.[34 48–52] The figure includes material from SMART Servier Medical Art (https://smart.servier.com/) under a Creative Commons (CC) license 3.0.AP1, activator protein 1; NF-κB, nuclear factor-kappa B; PEA3, polyoma enhancer activator 3; Smads, small mothers against decapentaplegic transcription factors; Sp1, proximal specificity protein 1; Stat3, signal transducer and activator of transcription 3; ZBP-89, Kruppel-type zinc-finger family transcription factor

cancer.[54] Finally, vimentin expression is also regulated by post-transcriptional mechanisms including that of non-coding microRNAs.[12]

Vimentin actively participates in inflammation and immune response

Targeting vimentin could have additional effects against the progression of COVID-19 by directly modulating the inflammatory response of patients with COVID-19. Indeed, a subgroup of these patients suffer from a cytokine storm syndrome, that is, an excessive cytokine production sustained by a positive feedback loop producing an inflammatory response, often leading to respiratory failure from acute respiratory distress syndrome.[2 3]

The NLRP3 inflammasome (nucleotide-binding and oligomerisation domain-like receptor protein 3 inflammasome) is an intracellular multiprotein complex that triggers the immune response of the host against pathogens including viruses and is implicated in acute lung injury.[55] It has been demonstrated that vimentin is a key regulator of the NLRP3 inflammasome by direct interaction.[11] Important events in lung inflammation and the onset of injury are the increase in IL-1β levels, the permeability of the endothelial–alveolar epithelium barrier and irreversible fibrosis. The relevance of vimentin in these responses was demonstrated in animal models in which the inflammatory response of vimentin-knockout mice to lipopolysaccharide (LPS), bleomycin or asbestos treatment was considerably lower, entailing a less significant lung injury.[11] Leucocyte adhesion to vascular endothelium and platelets is an early step in acute inflammatory response and attenuation of this adhesion may be beneficial in acute lung injury. By increasing circulating IL-1β—the major proinflammatory cytokine— vimentin is responsible for activating and recruiting inflammatory cells. In this context, it was shown that the vimentin IFs of both endothelial cells and lymphocytes form an anchoring structure between these two cell types.[56] Further evidence of the importance of vimentin for leucocyte extravasation comes from the report that the transendothelial migration of blood T and B lymphocytes is markedly reduced in vimentin-deficient lymphocytes and endothelial cells.[56] On the other hand, the presence of vimentin on the platelet and endothelial cell surface also serves as an adhesive receptor for the von Willebrand factor (VWF) and causes the binding of platelets to the subendothelial collagen and the subsequent intravascular generation of thrombin.[57 58] The latter being responsible for microthrombus formation and contributing to a non-haemostatic effect, that is, the inflammatory response.

The inhibition of the vimentin–VWF interaction, by anti-vimentin antibodies, is effective in interfering with the VWF-mediated platelet adhesion to different matrices.[57 58] The administration of exogenous recombinant human vimentin, binding specifically to P-selectin, stops leucocyte adhesion to platelets and endothelium by blocking P-selectin interaction with P-selectin glycoprotein ligand, ultimately decreasing endotoxin-induced acute lung injury.[59] The oxidised form of membrane-bound vimentin is a marker of senescent cells,[60] which, as such, produce a plethora of potentially harmful proinflammatory cytokines, chemokines and growth factors. Virus incubation times in senescent cells is longer, as observed in the case of HPV-16[61] and studying coronavirus infection in aged cells has been proposed, since this important issue for this virus is still unexplored.[9]

As shown above, vimentin is expressed by many cell types, it has multiple cellular localisations, it plays multiple roles in cell behaviour by interacting with a plethora of molecular partners. The complexity of the functions exercised by vimentin, especially as concerns viral infection, is exemplified by contradictory reports in the literature showing opposite roles for vimentin, depending on the type of virus and the specific partners involved in its direct or indirect interactions. In the following paragraph and in table 1, we report and comment on a list of vimentin-targeting drugs, which are globally known to downregulate vimentin expression, since we think that such a downregulation would be beneficial. However, we decided to provide only a few significant case by case comments. Each single drug, depending on the type of virus and host cell type concerned, should be further investigated.

Drugs targeting vimentin could combat coronavirus-related effects

Based on the fact that vimentin is important for viral infection and the associated inflammatory response, we have searched the literature for compounds targeting vimentin so as to select drugs that may treat COVID-19. Some of these drugs are as common and as easily available as melatonin, which makes our hypothesis immediately verifiable through clinical trials on COVID-19 specifically.

Some of the 49 compounds that we have identified (table 1) are completely new molecules that have recently started to be studied, others have never been employed in clinical practice although they are referenced by the FDA, while others are already being tested in clinical trials. However, all these molecules in table 1 affect vimentin levels, as demonstrated by western blot and/or real-time PCR analysis. Several of these compounds have been identified and characterised in in vitro experiments only, while others have been validated in vivo against cell growth and dispersion in cancer, and for their capacity to inhibit EMT which, as mentioned, amounts to fighting inflammation.

Interestingly, a significant fraction (16 out of 49) of the drugs listed in table 1 have been reported to play a role in acute lung injury and are briefly discussed below for this reason. *Melatonin* markedly reduces pulmonary injury and decreases the infiltration of macrophages and neutrophils into the lungs of LPS-treated mice, by inhibiting the NLRP3 inflammasome, in a model of acute lung injury.[62] Worth noting, it has already been speculated that melatonin would be beneficial in patients with COVID-19.[63] *Niclosamide*, a cheap antihelminthic drug, is effective against several viruses such as SARS-CoV, MERS-CoV, Zika virus, HCV and human adenovirus.[64] In particular, niclosamide has been shown to be an inhibitor of SARS-CoV 3CL protease, involved in viral replication.[64] Endogenous *hydrogen sulfide* participates in the regulation of important biological processes in the respiratory tract, such as airway tone, pulmonary circulation, cell proliferation and apoptosis, fibrosis, oxidative stress and inflammation.[65] Since hydrogen sulfide exerts a broad-spectrum antiviral activity, as well as having an anti-inflammatory action, the controlled release of hydrogen sulfide from chemical donors has been proposed as a treatment in lung diseases.[65] *Simvastatin* has protective vascular effects in the lungs since it improves the function of the endothelial barrier. The efficacy of simvastatin against LPS-induced lung injury involves the stabilisation of the cell cytoskeleton and adherent junctions.[66] The ability of *triptolide* to inhibit the proinflammatory NF–κB signalling pathway makes it another promising therapeutic agent for acute lung injury.[67] *Sinomenine* attenuates septic shock-dependent acute lung injury, probably thanks to the inhibition of inflammation and oxidative stress.[68] *Tanshinone IIA* inhibits the production of proinflammatory factors in LPS-induced acute lung injury through the regulation of calcium in pulmonary interstitial macrophages.[69] *Ginsenoside Rg3* attenuates LPS-induced acute lung injury by decreasing the production of proinflammatory factors and increasing the synthesis of anti-inflammatory cytokines, through the activation of the PI3K/AKT/mTOR pathway downstream of the Mer receptor.[70] *Icariin* reduces acute lung injury by enhancing the expression of the glucocorticoid receptor alpha in lung tissues and by inhibiting the expression of p65, c-Jun, Stat3, IL-6 and tumour necrosis factor-alpha.[71] The therapeutic window of *Valproic acid* is narrower but interesting, since it is effective in a mouse model of Gram-negative bacteria-induced pneumonia.[72] *Apigenin C-glycosides* inhibit acute inflammation and apoptosis by suppressing the activation of the TLR4/TRPC6 signalling pathway in a murine model of acute lung injury.[73] *Inositol derivatives* present in surfactant preparations diminish the activation of key inflammatory pathways in lung diseases.[74] *Pterostilbene 4′-β-glucoside*, the glycosylated form of the antioxidant pterostilbene, diminishes intracellular and mitochondrial reactive oxygen species production, thus reducing the inflammatory response to LPS.[75] *Osthole* exerts

Table 1 Compounds downregulating vimentin expression

Name of molecule	Type	Effects	Modes of action	Models	Reference
17-DMCHAG	Geldanamycin derivative	Antimigratory; antiproliferative	Inhibits HSP90; induces the proteasome-dependent degradation of androgen receptor	Human prostate cancer cells; tumour-bearing mice	79
Alpha-lipoic acid*	Acid; antioxidant	Inhibitor of EMT; antiproliferative	Inhibits TGF-ß; activates AMPK and downregulates mTOR-S6 signalling	Human thyroid cancer cells; tumour-bearing mice	80
Apigenin*†	Flavonoid derivative	Antiapoptosis; pro-proliferative; antifibrotic	Decreases miR-34a	Mouse mesothelial peritoneal cells	81
Berberine*	Alkaloid derivative	Inhibitor of EMT; antiproliferative	Decreases PI3/AKT, Ras-Raf-ERK and TGF-ß1 signalling	Mouse neuroblastoma cells	82
Bergamottin	Furanocoumarin derivative	Inhibitor of EMT; antimigratory	Decreases PI3K/AKT/mTOR, TGF-ß signalling	Human lung adenocarcinoma cells	83
Beta-asarone	Phenylpropanoid derivative	Inhibitor of EMT; antimigratory	Decreases hnRNP A2/B1, MMP-9, p-STAT3 expression	Human glioma cells	84
Beta-lapachone	Quinone derivative	Inhibitor of EMT; antimigratory; proapoptosis	Induces apoptosis through caspase-3, -8 and -9 activation and poly(ADP-ribose) cleavage; decreases MMP-2 and MMP-9	Mouse colon cancer cells; tumour-bearing mice	85
BHX	Pyrazoline derivative	Inhibitor of EMT; antimigratory; proapoptosis	Inhibits Wnt/β-catenin signalling	Human breast cancer cells; tumour-bearing mice	86
BMS345541	Selective I kappa B kinase inhibitor	Inhibitor of EMT; anti-inflammatory	Inhibits NF–κB/RelA pathway	Human epithelial cells; polyinosinic–polycytidylic acid-treated mice	87
Chrysin*	Flavonoid derivative	Inhibitor of EMT; antimigratory	Reduces MMP-2 activity; induces zona occludens protein-1 and occludin expression	Human renal epithelial cells; db/db mice	88
Chrysotobibenzyl		Inhibitor of EMT; antimigratory	Decreases integrins (β1, β3, αv), p-FAK, p-AKT, caveolin-1 expression	Human lung cancer cells	89
Cisplatin	Anticancer	Inhibitor of EMT; antimigratory; antiproliferative	Reduces YAP activity	Human colon cancer cells; tumour-bearing mice	90
Compound 11	Selective class I HDAC inhibitor	Inhibitor of EMT; antiproliferative	Decreases p-AKT, p-ERK activity	Human colorectal cancer cells; tumour-bearing mice	91
Curcumin*	Polyphenolic compound	Inhibitor of EMT; antimigratory; antiproliferative	Decreases TGF-ß/Smad2/3 signalling	Human thyroid cancer cells	92
Cypripedin	Phenanthrenequinone derivative	Inhibitor of EMT; antimigratory	Decreases AKT/GSK-3β signalling	Human lung cancer cells	93
D-4F	Apolipoprotein A-I mimetic	Inhibitor of EMT; antifibrotic	Inhibits TGF-ß1	Human alveolar epithelial cells	94
Dehydroepiandrosterone	Steroid	Inhibitor of EMT; antimigratory	Decreases N-cadherin and Snail expression	Human breast cancer cells; tumour-bearing mice	95
Dictamnine	Alkaloid	Inhibitor of EMT; antiproliferative; proapoptosis	Decreases mTOR/p70S6K/eIF4E and MAPK signalling	Human lung cancer cells	96
Ginsenoside Rg3*†	Steroidal saponin	Inhibitor of EMT; antimigratory; proapoptosis	Inhibits LncRNA colon cancer-associated transcript 1 expression and PI3K/AKT signalling; inhibits MAPK and NF–κB signalling	Human colorectal cancer cells; lung cancer cells; tumour-bearing mice	97 98
Hydrogen sulfide*†	–	Inhibitor of EndMT; decrease ER stress; vasodilator; cardioprotective	Decreases Smad2 and Src signalling	Human umbilical vein endothelial cells	99

Continued

Table 1 Continued

Name of molecule	Type	Effects	Modes of action	Models	Reference
Hydroxygenkwanin	Flavonoid derivative	Antimigratory; antiproliferative	Activates p21 signalling	Human oral squamous cell carcinoma	44
Icariin*†	Flavonoid derivative	Antimigratory; antiproliferative; proapoptosis	Inhibits PI3K, AKT and MEK/ERK signalling; decreases miR-625-3p and MMP-9 expression	Human thyroid cancer cells	100
Icariside II	Flavonol glycoside	Inhibitor of EMT; antimigratory	Inhibits NF-κB and AKT/GSK-3β signalling	Human lung cancer cells; tumour-bearing mice	101
Inositol*†	Lipid	Inhibitor of EMT; antimigratory	Inhibits PI3K/AKT and NF-κB signalling	Human breast cancer cells	102
Melatonin*†	Hormone	Inhibitor of EMT; antimigratory; antiproliferative	Inhibits PI3K/AKT signalling; decreases MMP-2, MMP-9, NF-κB p65 expression	Human ovarian cancer cells; gastric cancer cells; tumour-bearing mice	103 104
Metapristone*	RU486 derivative	Antimigratory; antiproliferative; proapoptosis	Decreases AKT and ERK phosphorylation and Bcl-2; upregulates total p53 and Bax expression	Mouse skin melanoma cells	105
Metformin*†	Biguanide derivative; antidiabetic	Inhibitor of EMT; antimigratory	Downregulates HIF-1alpha, CAIX, miR-34a, SNAIL1 and ZEB1; upregulates miR-200a, miR-200c and miR-429	Human cervical squamous cancer cells; human colorectal cancer cell	106 107
Moscatilin	Bibenzyl derivative	Inhibitor of EMT; proapoptosis	Decreases ERK and AKT activity; downregulates caveolin-1 level	Human lung cancer cells	108
N-acetylcysteine*	Antioxidant	Reduce fibrosis; anti-inflammatory	Enhances antioxidant enzyme activities; downregulates oxidising enzymes' expression	Crystalline silica-induced pulmonary fibrosis mice model	109
Niclosamide*†	Antihelminthic	Inhibitor of EMT; antimigratory	Inhibits Wnt/β-catenin signalling	Human oral squamous cell carcinoma	110
Norcantharidin	Cantharidin derivative	Inhibitor of EMT; antimigratory	Inhibits αvβ6-ERK-Ets1 signalling	Human colon cancer cells	111
Osthole*	Coumarin derivative	Inhibitor of EMT; antimigratory; antiproliferative; proapoptosis	Decreases MMP-2, MMP-9, Smad-3, Snail-1, Twist-1 expression	Human renal cell carcinoma	112
PAC*	Curcumin analogue	Inhibitor of EMT; antimigratory; antiproliferative	Inhibits JAK2/STAT3, AKT/mTOR and MEK/ERK signalling; inhibits STAT3/cyclin D1 pathway	Human colorectal cancer cells; tumour-bearing mice	113
Palbociclib	Selective CDK4/6 inhibitor	Inhibitor of EMT; antimigratory	Inhibits c-Jun/COX-2 signalling	Human breast cancer cells; tumour-bearing mice	114
Pargyline	Lysine-specific demethylase 1 inhibitor	Inhibitor of EMT; antimigratory	Reduces prostate-specific antigen	Human prostate cancer cells; tumour-bearing mice	115
Physcion	Anthraquinone derivative	Inhibitor of EMT; antimigratory	Activates ROS/AMPK/GSK3β signalling; inhibits SOX2	Human colorectal cancer cells	116
Pterostilbene*†	Antioxidant	Inhibitor of EMT; antimigratory	Reduces Src/Fak signalling; upregulates miR-205 expression	Human breast cancer cells; tumour-bearing mice	117
Salidroside*	Glucopyranoside derivative	Inhibitor of EMT; antimigratory; antiproliferative	Downregulates miR-891b expression; inhibits PI3K/AKT/mTOR and NF-κB signalling	Wilms' tumour cells; mouse prostate cancer	118
Selenium*†	Metal	Inhibitor of EMT	Downregulates genes involved in cellular migration, inflammation and mesenchymal markers; upregulates genes involved in epithelial markers	Human prostate	119

Continued

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Table 1 Continued

Name of molecule	Type	Effects	Modes of action	Models	Reference
Simvastatin*†	Statin	Inhibitor of EMT; antifibrotic	Decreases Toll-like receptor 4 and NF-κB signalling	Human biliary epithelial cells	120
Sinomenine*†	Alkaloid	Antimigratory; antiproliferative	Downregulates miR-23a expression; inhibits PI3K/AKT and JAK/STAT signalling	Human prostate cancer cells	121
Swainsonine	Indolizidine alkaloid	Antimigratory; antiproliferative; proapoptosis	Downregulates miR-92a, inhibits PI3K/AKT/mTOR signalling	Human glioblastoma cells	122
Tanshinone IIA*†	Lipophilic compound	Inhibitor of EMT; antimigratory; antiproliferative	Inhibits STAT3-CCL2 signalling	Human bladder cancer cells	123
Tetramethylpyrazine nitrone	Tetramethylpyrazine derivative	Reduces brain infarction; preserves neurological function	Decrease expression of neuroinflammatory markers	Cynomolgus macaques brain ischaemic stroke model	124
Tetrandrine	Alkaloid	Inhibitor of EMT; antimigratory	Reduces glioma-associated oncogene family zinc finger 1 (Gli-1) expression	Human bladder cancer cells	125
Toosendanin	Alkaloid	Inhibitor of EMT; antimigratory; antiproliferative	Inhibits AKT/mTOR signalling	Human pancreatic cancer cells; tumour-bearing mice	126
Triptolide*†	Diterpenoid derivative	Antifibrotic	Inhibits TLR4-induced NF-κB/IL-1β immune pathway; inhibits NF-κB/TNF-α/VCAM-1 inflammatory pathway; downregulates TGF-β1/α-SMA/vimentin fibrosis pathway	Diabetic rats	127
Valproic acid*†	Acid	Inhibitor of EMT; antiproliferative	Downregulates Smad4 expression; upregulates transcriptional intermediary factor-1γ (TIF1γ) expression	Human prostate carcinoma cells; tumour-bearing mice	128 129
Wogonin*	Flavonoid derivative	Inhibitor of EMT; antimigratory	Inhibits IL-6/STAT3 signalling pathway	Human alveolar adenocarcinoma cells	130

A selection of compounds downregulating vimentin expression and their major cellular and molecular effects. Some of these molecules already have FDA approval, thus can be immediately used to set up clinical trials against COVID-19.
*Indicates the compounds have been referenced by the FDA; the latter group of drugs is briefly discussed in the text.
†Indicates the compounds that have been reported to play a role in acute lung injury.
AMPK, AMP-activated protein kinase; ANG, Angiotensin; Bax, Bcl-2–associated X; Bcl-2, B-cell lymphoma 2; CCL2, Chemokine ligand 2; 17-DMCHAG, 17-(6-(3,4-dimethoxycinnamamido)hexylamino)-17-demethoxy-geldanamycin; eIF4E
, Eukaryotic translation initiation factor 4E; EMT, epithelial to mesenchymal transition; EndMT, endothelial to mesenchymal transition; ER, endoplasmic reticulum; ERK, Extracellular signal-regulated kinase; FAK, Focal adhesion kinase; FDA, Food and Drug Administration; GSK-3, Glycogen synthase kinase-3; HDAC, Histone deacetylase; HIF, Hypoxia Inducible Factor; hRNP, Heterogeneous Nuclear Ribonucleoprotein; HSP, Heat Shock Protien; IL, Interleukin; JAK2, Janus kinase 2; LncRNA, Long noncoding RNAs; MAPK, Mitogen-activated protein kinase; MMP, Matrix metallopeptidase; mTOR, Mechanistic target of rapamycin; NF-κB, nuclear factor-kappa B; PEA3, polyoma enhancer activator 3; PI3K, Phosphatidylinositol 3-kinase; ROS, Reactive oxygen species; SMA, Smooth muscle actin; Smad, small mothers against decapentaplegic transcription factor; STAT3, Signal transducer and activator of transcription 3; TGF, Transforming growth factor; TLR4, Toll-like receptor 4; TNF, Tumor necrosis factor; VCAM, Vascular cell adhesion molecule; YAP, Yes-associated protein.

beneficial effects on bleomycin-induced pulmonary fibrosis in rats by modulating the ACE2/ANG-(1–7) axis and by inhibiting lung inflammation.[76] *Selenium* restores the antioxidant capacity of the lungs and reduces inflammatory responses, thus improving lung mechanics.[77] All the previous examples concerned infection-induced lung injury, while lung injury may also be caused by the mechanical stress caused by forced ventilation. To specifically address this type of damage, Tsaknis *et al*[78] set up an elegant experimental model, exercising high-pressure ventilation on ex vivo lung preparations thereby increasing their microvascular permeability, oedema and microhaemorrhages; in this model, pretreatment with *metformin* decreased the severity of the damage preserving alveolar capillary permeability. Additional FDA-approved drugs that have already been used in clinical trials, but have not been discussed here, are metapristone, N-acetylcysteine, chrysin, berberine, salidroside, dehydroepiandrosterone and cisplatin. It is worth noting that many of the compounds above have been tested against cancer and reported to inhibit cell growth and EMT, but will not be discussed in this article. High-throughput screening in cell culture systems using a reporter system driven by the vimentin promoter will identify additional novel drugs for their capacity to specifically decrease vimentin transcription. In addition, alternative therapeutic interventions based on the downregulation of vimentin can be designed, such as the administration of antisense oligonucleotides, anti-vimentin RNA, humanised neutralising antibodies or dominant-negative peptides capable of blocking the interaction between vimentin and viral proteins.

CONCLUSIONS

Vimentin functions as a co-receptor for SARS-CoV—and likely for SARS-CoV-2—thus contributing to viral infection. Evidence of the significant role played by vimentin in virus-induced infection comes from the following observations: vimentin expression increases during viral infection in several clinical settings, while the absence of vimentin in knockout mice makes them more resistant to inflammation and acute lung injury than wild-type mice. Therefore, vimentin can be a target for the treatment of COVID-19-related pneumonia. It is a matter of fact that drugs that have proven efficient against viral infection downregulate vimentin expression. While the correlative nature of this evidence does not yet prove a causative role for vimentin downregulation in diminishing viral infection, it indicates that establishing whether drugs which decrease vimentin expression can be used for the treatment of patients with COVID-19 is the point at issue. Since vimentin has a twofold role in the disease, not only being involved in the viral infection but also in the associated life-threatening lung inflammation, the use of vimentin-targeted drugs may offer a synergistic advantage as compared with other treatments not targeting vimentin. In particular: (1) the decreased amount of vimentin on the cell surface would decrease its interaction with SARS-CoV-2 spike protein, thus interfering with viral entry; (2) the decreased amount of vimentin within the cell would affect virus replication; (3) the lesser amount of vimentin within inflammatory cells would exert negative effects on the inflammasome. This in turn would avoid the cytokine storm and curb the arrival of infiltrating inflammatory cells; (4) lower vimentin expression in epithelial cells and fibroblasts would decrease EMT and fibrosis. Taken together, all these considerations strongly suggest that vimentin represents a valuable therapeutic target at the early stages of the SARS-CoV infection, may help avoid the progression to serious complications and indicate that several FDA-approved drugs (such as melatonin, niclosamide, selenium, hydrogen sulfide, inositol) should be tested in clinical trials against SARS-CoV-2.

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