high specificity could also be utilized for the development and optimization of more affordable and accessible assays for alpha-synuclein in the peripheral tissues and blood as a biomarker for Lewy body disease in the brain.

DECLARATION OF INTERESTS

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Unveiling TMEM106B: SARS-CoV-2's secret entrance to the cell

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The lysosomal membrane protein TMEM106B functions as a proviral factor in SARS-CoV-2 infection, though it was not known how. In this issue of *Cell*, Baggen et al. demonstrate that TMEM106B serves as an ACE2-independent receptor for SARS-CoV-2 entry by promoting the fusion of the viral membrane with the lysosomal membrane.

The COVID-19 pandemic was caused by widespread transmission of SARS-CoV-2, a single-stranded RNA virus from the *Coronaviridae* family. Numerous host factors crucial for SARS-CoV-2 infection have been identified recently.[1,2] One such factor is the cell-surface receptor angiotensin-converting enzyme 2 (ACE2), which plays a critical role in viral entry by interacting with the viral spike (S) protein. Binding of ACE2 triggers conformational changes in spike, leading to its cleavage by the protease TMPRSS2 on the cell surface or lysosomal proteases such as cathepsins after endocytosis. This cleavage exposes the S2 fusion peptide, resulting in the fusion of the viral membrane with the plasma or endolysosomal membrane and the subsequent release of the viral genome into the cytosol.

In this issue of *Cell*, Baggen et al. investigate the role of another host factor, TMEM106B, in SARS-CoV-2 infection.[3] TMEM106B is a type II lysosomal membrane protein, consisting of an N-terminal cytosolic domain, a transmembrane helix, and a glycosylated C-terminal lumenal domain (Figures 1A and 1B). At the lysosomal membrane, TMEM106B interacts with the V-ATPase

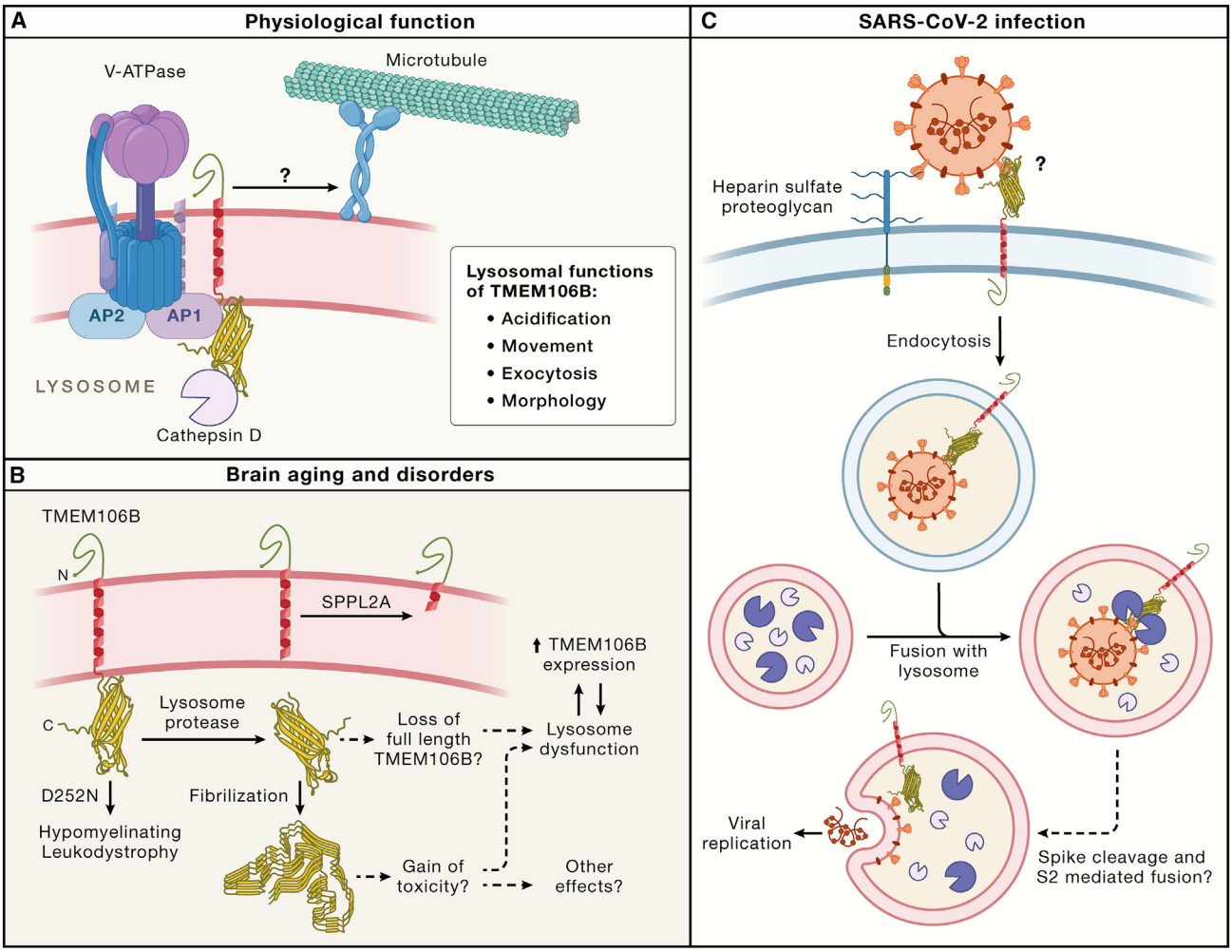

Figure 1. Physiological and pathological functions of TMEM106B

(A) TMEM106B is a type II transmembrane protein and regulates several aspects of lysosomal functions, including lysosomal acidification, movement, morphology, and exocytosis. It interacts with V-ATPase subunit ATP6AP1 and lysosomal protease cathepsin D.

(B) TMEM106B is tightly linked to brain aging and brain disorders. The D252N mutation in the lumenal domain of TMEM106B causes hypomyelinating leukodystrophy. Increased expression of TMEM106B has been associated with a higher risk of neurodegenerative diseases. In addition, the lumenal domain of TMEM106B is cleaved to form amyloid fibrils during aging and neurodegeneration. The N-terminal fragment could be further processed by the intramembrane protease signal peptide peptidase-like 2A (SPPL2A). Both increased levels of TMEM106B and fibril formation could lead to lysosomal dysfunction.

(C) During SARS-CoV-2 infection, TMEM106B acts as a proviral host factor by binding to the spike (S) protein to promote the fusion of the viral membrane with the lysosomal membrane after endocytosis and allow the release of the viral genome into the cytosol. Heparin sulfate proteoglycan (HSPG) might serve as a co-receptor for SARS-CoV-2. TMEM106B might trigger a conformational change in spike to facilitate cleavage of spike by lysosomal cathepsins, exposing the S2 fusion peptide. Additionally, TMEM106B might function at the cell surface to facilitate SARS-CoV-2 entry. Dotted lines indicate hypotheses rather than experimentally proven results.

subunit ATP6AP1 and the lysosomal protease cathepsin D, regulating critical lysosomal functions such as acidification, movement, morphology, and exocytosis (Figure 1A).[4] Research during the past decade has shown a tight association between TMEM106B and brain disorders (Figure 1B).[4,5] Certain single-nucleotide polymorphisms in TMEM106B associated with increased expression have been linked to a higher risk of neurodegenerative diseases, including frontotemporal dementia, amyotrophic lateral sclerosis (ALS), Parkinson's disease, and Alzheimer's disease (Figure 1B).[4,5] A mutation in the lumenal domain of TMEM106B (D252N) leads to hypomyelinating leukodystrophy, a myelination disorder.[4,5] Furthermore, the lumenal domain of TMEM106B can be shed by lysosomal proteases,[6] and fibrils consisting of TMEM106B's C-terminal lumenal domain accumulate in the brains of aged individuals and patients with various neurodegenerative diseases.[5]

Intriguingly, TMEM106B has recently been identified as a proviral host factor for SARS-CoV-2 infection through genome-wide CRISPR screens,[7,8] adding another twist to TMEM106B's biology. However, its specific function in SARS-CoV-2 infection remained elusive. Previous studies have indicated that TMEM106B functions independently of ACE2.[7] Baggen et al. further find that the enhanced infectivity of

a SARS-CoV-2 variant with the E484D substitution within spike's receptor binding domain (RBD) is associated with increased binding to TMEM106B.[3] To gain further insights into the function of TMEM106B, Baggen et al. solve the crystal structure of TMEM106B lumenal domain, revealing a seven-bladed beta-sandwich fold. Additionally, they determine the complex structure of spike with TMEM106B using cryoelectron microscopy (cryo-EM) and demonstrate that the interaction involves residues 443–495 of spike and M210 and F213 of TMEM106B.[3] Importantly, this binding interface overlaps with the ACE2 binding site on spike. However, ACE2 interacts with spike with a several hundred-fold higher binding affinity than TMEM106B, indicating that ACE2 is the preferred receptor for SARS-CoV-2.[3]

Going further, Baggen et al. demonstrate that pretreatment with several monoclonal antibodies targeting TMEM106B's lumenal domain effectively prevents SARS-CoV-2 infection in cells expressing low levels of ACE2. The antibodies with the highest efficiency localize on both the cell surface and within the lysosome, raising the question of whether TMEM106B could facilitate SARS-CoV-2 endocytosis, viral release from the lysosome, or both. The authors find that SARS-CoV-2 can still attach to the cell surface even in cells lacking ACE2, TMEM106B, and heparan sulfate—a known SARS-CoV-2 receptor—suggesting the existence of additional cell-surface receptors for SARS-CoV-2 binding. Furthermore, TMEM106B ablation does not affect the endocytosis and lysosomal delivery of the virus. However, in the absence of both ACE2 and TMEM106B, the virus cannot undergo fusion with the lysosomal membrane. In cell-cell fusion assays, co-expression of spike, TMPRSS2, and either ACE2 or TMEM106B on the cell surface induces cell fusion, indicating that TMEM106B may promote membrane fusion similarly to ACE2.

Based on these data, Baggen et al. proposed a model in which TMEM106B facilitates the fusion of the viral membrane with the lysosomal membrane during endocytosis-dependent SARS-CoV-2 infection, independent of ACE2 (Figure 1C).[3] This model explains how SARS-CoV-2 can infect cells and replicate in organs with low ACE2 levels, such as non-airway cell types derived from the intestines and brain. This work has opened many new and exciting avenues for research. For example, the precise mechanism by which TMEM106B promotes the fusion of the virus with the lysosome membrane has yet to be elucidated. Does TMEM106B binding trigger conformation changes in spike, allowing it to be recognized by the proteases? Is the interaction between spike and TMEM106B affected by pH? Given the important role of the endolysosome in SARS infection,[1] could TMEM106B indirectly affect SARS-CoV-2 infection by regulating lysosomal activities, in addition to functioning as a spike receptor? Could TMEM106B also act at the plasma membrane to facilitate membrane fusion and viral entry because TMEM106B promotes spike-mediated fusion of TMPRSS2-overexpressing cells in the cell-cell fusion assay?

Beyond TMEM106B's function in SARS-COV-2 infection, many other questions remain to be answered regarding its physiological and pathological functions. While several TMEM106B binding partners have been identified, we have merely scratched the surface of understanding how TMEM106B precisely regulates lysosomal activities (Figure 1A). For instance, while its impact on lysosomal pH and interaction with ATP6AP1, a V-ATPase subunit, has been established,[5] how this interaction explicitly regulates V-ATPase function is unknown. Recent structural predictions suggest that TMEM106B could be part of a family of lipid-transfer proteins.[9] However, the absence of a prominent hydrophobic groove in the crystal structure raises questions about its lipid-binding capabilities. Furthermore, the mechanisms by which TMEM106B regulates lysosomal movement along microtubules remain to be dissected. In the context of aging and neurodegeneration, the triggers for TMEM106B cleavage and subsequent amyloid fibril formation and the impact of these fibrils on cellular pathways are yet to be determined (Figure 1B). To unravel these mysteries, future studies employing structural, biochemical, cell biological approaches, and *in vivo* models are imperative. Nevertheless, the antibodies developed by Baggen et al. offer a valuable tool for exploring the multifaceted roles of TMEM106B and hold promise as potential therapeutics for treating SARS-CoV-2 infections.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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