On the consequences of cell fusion in COVID-19

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Abstract

This commentary proposes how the coronavirus SARS-CoV-2 can contribute to clinical manifestations of COVID-19s by producing syncytia.

Keywords: cell fusion, syncytia, hybrids, viruses, coronaviruses, SARS-CoV-2, COVID-19, blood coagulation cascade, thrombosis, cancer

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A hallmark of severe COVID-19 is the abundance of syncytia, the products of fusion between two or more cells, in the lungs of the patients (Buchrieser *et al.*, 2020; Bussani *et al.*, 2020; Braga *et al.*, 2021). These syncytia were attributed to the ability of spike, a protein encoded by SARS-CoV-2, to fuse cells to each other, and prompted a search for already approved drugs that prevent this fusion. In a recent report (Braga *et al.*, 2021), some of such were found to inhibit TMEM16F, a protein that has two activities: a calcium-activated ion channel that regulates chloride secretion, and a scramblase, a lipid transporter that relocates phosphatidylserine (PS) to the cell surface (Scudieri *et al.*, 2015) in a process known as PS externalization.

PS externalization is required for cell fusion in many systems (Brukman *et al.*, 2019; Zhang *et al.*, 2020), which has explained why inhibiting a scramblase prevented the formation of spike-induced syncytia. However, the report has concluded that although PS externalization "is required for plasma membrane fusion, chloride secretion might have relevance in COVID-19 pathogenesis" (Braga *et al.*, 2021). This assumption, that the scramblase activity merely helped to identify the ion channel activity as a potential therapeutic target, reflects a common opinion that syncytia made in the body by infectious viruses are inconsequential.

To evaluate this assumption let us consider how syncytia and cell fusion that creates them might be involved in COVID-19.

Cell fusion as a cause of conditions associated with the activation of the blood coagulation cascade

COVID-19 is associated with widespread thrombosis (Hanff *et al.*, 2020). Discovering syncytia in COVID-19 patients led to a suggestion that "the fusogenic properties of the MERS-CoV- and SARS-CoV-2-infected cells might be linked to the pathogenesis of thrombosis" (Bussani *et al.*, 2020).

What could this link be?

I would like to suggest as a candidate the scramblase activity induced by spike-mediated fusion because PS externalization resulting from this activity not only enables cell fusion but also controls the rate limiting steps of the blood coagulation cascade (Zwaal, Comfurius and Bevers, 1998; Grover and Mackman, 2018; Smith and Morrissey,

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Terminology and abbreviations

Cell fusion: a process of merging two or more cells into one by merging their plasma membranes.

Fusogen: an agent, often a protein such as SARS-CoV-2 spike, capable of fusing cellular membranes. Viral fusogens fuse the viral envelope to the plasma membrane of the target cell and can fuse plasma membranes of adjacent cells to each other.

Syncytium (plural **syncytia**): a multinucleated cell produced by the fusion of two or more cells. The term comes from Greek *syn* "together" and *kytos* "box, or cell".

Heterokaryon: a syncytium produced from more than one cell type, say, a pneumocyte fused to an epithelial progenitor or a leukocyte.

Homokaryon: a syncytium produced from cells of the same type, as would be the case with the fusion of two or more pneumocytes.
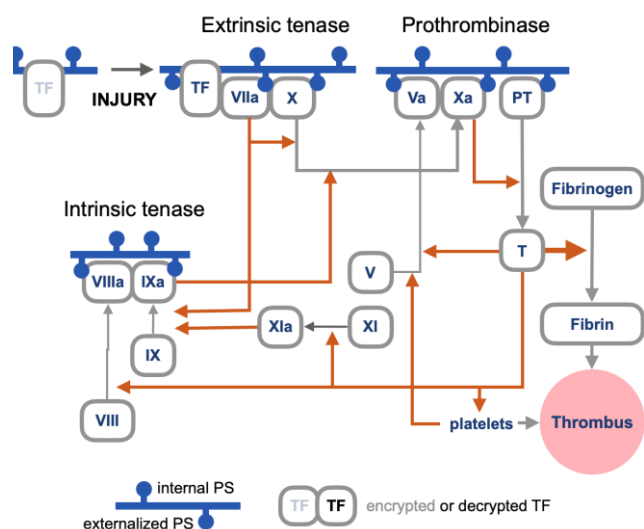
Cell hybrid: Mononuclear offspring of syncytia, produced once a syncytium undergoes mitosis. For example, hybridomas are made by fusing leukocytes with plasmacytoma cells to obtain hybrids that produce monoclonal antibodies.

PS (phosphatidylserine): the most abundant anionic (negatively charged) membrane lipid. In live cells, PS is actively moved to the cytoplasmic side of plasma membrane.

Scramblases: Proteins, such as TMEM16F, that randomize, or scramble, the asymmetric distribution of PS across the membrane, a process known as **PS externalization**.

2019) (Fig. 1). In agreement with this role, a deficiency of TMEM16F, the scramblase induced by spike, is responsible for Scott syndrome, a bleeding disorder (Suzuki *et al.*, 2010).**Viruses and the coagulation cascade.** Viral infections activate the coagulation cascade primarily by triggering the assembly of extrinsic tenase, a complex between Tissue Factor (TF) and Factor VIIa, on the outer surface of the cellular membrane (Mackman *et al.*, 2020) (Fig. 1). Full activation of the tenase requires PS externalization (Antoniak and Mackman, 2014), which, in synergy with TF binding, increases Factor VIIa activity by eight orders of magnitude (Bom and Bertina, 1990; Smith, Travers and Morrissey, 2015).

Since FVIIa and its precursor FVII are always present in the blood, the limiting steps for fully activating FVIIa are PS externalization and the presence of TF capable of binding and activating FVIIa. Accordingly, TF has been scrutinized as a potential target for COVID-19 therapy (Cañas *et al.*, 2021).

Figure 1. An outline of the blood coagulation cascade

Blood coagulation cascade is a network of proteases, their precursors, cofactors, cells, enzymes, feedbacks, and feed-forwards whose complexity and still unresolved questions make this outline by necessity rudimentary, with the primary goal to illustrate where the proteins that require binding to externalized PS (phosphatidylserine) for activation are in the network.

Most proteins involved in coagulation are called factors and are labeled by Roman numbers, such as Factor X or FX (hence enzymes that process FX are *ten*ases). For simplicity, here the letter F is omitted. Activated factors are labeled with an a, as in FXa. Orange arrows represent proteolytic activity, grey arrow show a transition between forms. Blue horizontal lines represent cellular membrane with the cell surface facing down. Accordingly, the pinheads of externalized PS also face down. Note that most PS is actively relocated to face the cytoplasm unless the cell dies or the distribution is randomized by lipid scramblases.

As discussed in the text, the primary trigger of coagulation induced by viral infections is the extrinsic tenase (top left), which is a complex of TF (Tissue Factor) and FVIIa assembled on externalized PS in the presence of calcium ions. This tenase produces FXa to activate enough thrombin for generate the components of the intrinsic tenase, which increases FXa production and thus thrombin to yield enough fibrin for making a thrombus, which is a meshwork of polymerized and cross-linked fibrin with entrapped blood cells, primarily platelets, that is large and stiff enough to obstruct a blood vessel.

Note that TF is encrypted, that is unable to activate FVIIa, until it is de-encrypted by externalized PS (reviewed in (Grover and Mackman, 2018)).

TF is regulated by controlling its expression, by tissue factor pathway inhibitor (TFPI), and by priming TF through a process known as de-encryption. The primary candidate for the de-encrypter is externalized PS [Grover 2018]. How PS externaliza-

tion is induced in TF-expressing cells is unclear (Smith and Morrissey, 2019).

The hypothesis. The remarkable synergy of TF and externalized PS in activating FVIIa and the report that spike-induced syncytia externalize PS (Braga *et al.*, 2021) together suggest a hypothesis that these syncytia can be a platform for assembling fully active extrinsic tenase capable of triggering blood coagulation (Fig. 2).

Whether this activation would result in clinically significant thrombosis would depend on whether syncytia express TF, which is likely because endothelial cells express TF during viral infections, and because the lungs are abundant in other cell types that express this protein (Grover and Mackman, 2018), whether syncytia come in contact with blood, whether they are large or abundant enough to assemble sufficient number of tenase complexes, which can be tested using available clotting assays, and the state of the coagulation system in a patient. Since even one thrombus can cause problems, the contribution of syncytia to COVID-19 could be clinically relevant.

Fusion at a distance. SARS-CoV-2 spike, as other viral fusogens, can fuse cells by two mechanisms. In the first, known as fusion from within, an infected cell begins to produce viral components, including spike, which is transported to the plasma membrane and can fuse it to the membrane of an adjacent cell if that cell has a spike receptor. Another mechanism, fusion from without, is executed by viral particles or lipid vesicles studded with spike that serve as a bridge between two cells (Gallaher and Bratt, 1972; Theuerkauf *et al.*, 2021) (Fig. 2). In this case whether the virus is infectious or inactivated is irrelevant, as its ability to produce spike in the cell is not required.

Since SARS-Cov-2 infection induces the release of extracellular vesicles (also called microvesicles) in patients with moderate or severe COVID-19 (Rosell *et al.*, 2021), and that SARS-Cov-2 spike can fuse cells if delivered on membrane vesicles (Theuerkauf *et al.*, 2021), the site of thrombosis caused by syncytia can differ from that of infection.

Thrombosis by death. Syncytia might also contribute to thrombosis by mechanism unrelated to the induction of a scramblase. A syncytium upon its demise would uncover a patch of the thrombogenic basement membrane. Since even a single 20 micron fiber of collagen is sufficient to trigger platelet-dependent clotting (Zhu, Tomaiuolo and
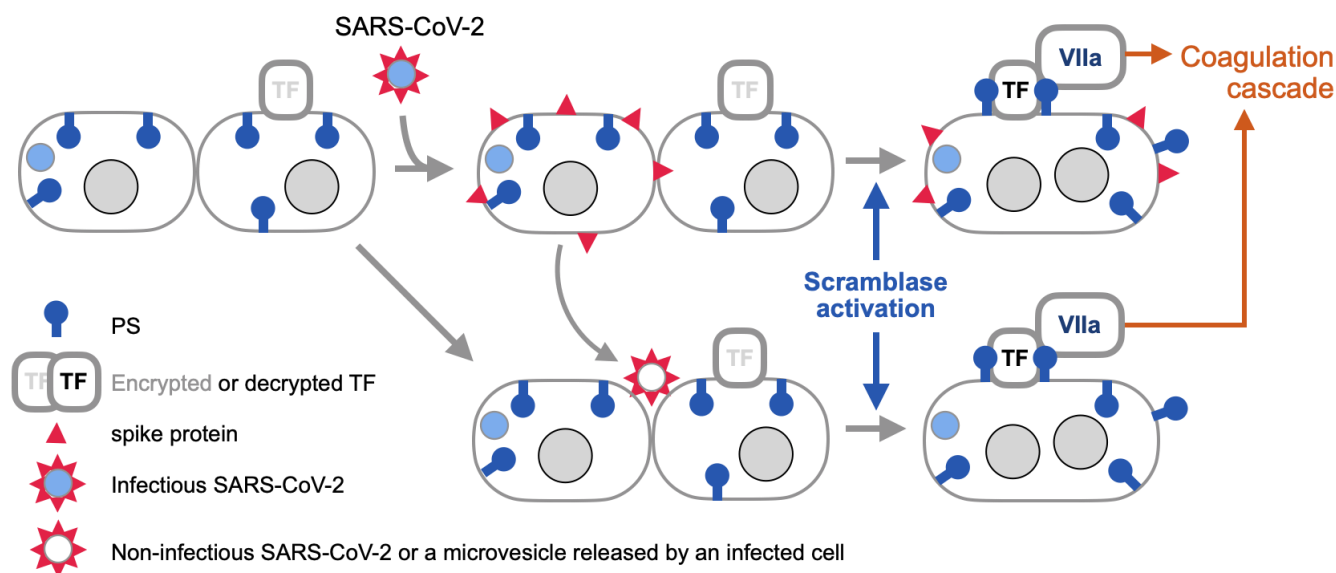
Diamond, 2016), the patch exposed by sloughing of a syncytium made of more than several cells might be large enough to produce a thrombus.

Cell fusion and vaccines. The hypothesis that cell fusion induced by spike can trigger blood coagulation might also explain the rare cases of thrombosis following the injection of vaccines that use viral vectors or mRNA delivery vesicles to express SARS-CoV-2 spike in human cells. A current explanation for this phenomenon (Kowarz *et al.,* 2021) states that expressing spike using adenoviral vectors, such as used in some of the vaccines, produces a soluble fragment of the protein that induces thrombosis through signaling pathways mediated by ACE2, a receptor of spike.

I would like to suggest that since spike transduced into cultured cells by viral vectors fuses cells even in undetectable amounts (Theuerkauf *et al.,* 2021), it is possible the amounts produced in cells by the vehicles used in the vaccines would be sufficient to fuse some cells in humans. The extent of this fu-

sion would depend on the level of spike expression, which is determined by the gene delivery vehicle used, the cell type of the cells transduced in the body, and whether these cells release extracellular vesicles that can fuse cells at sites distant to the site of injection (Rosell *et al.*, 2021). Whether the resulting syncytia, if they arise, would cause any problems would depend on the number of syncytia, their size, and their access to blood. Should the hypothesis linking cell fusion to thrombosis be proven correct, or out of an abundance of caution, a solution would be to use spike mutants that are deficient in cell fusion or (and) in inducing scramblases.

Cell fusion and SARS-CoV-2 strains. While spike can fuse viruses to cells and cells to each other, the underlying mechanisms of these two activities are not identical. For example, sera from convalescent COVID-19 patients neutralize fusion of the virus to cells but fail to prevent the fusion of cells (Theuerkauf *et al.*, 2021). Likewise, modifying the spike of SARS-CoV, which causes severe

Figure 2. Syncytia induced by SARS-CoV-2 spike as a platform for triggering blood coagulation cascade. SARS-CoV-2 is covered by an envelope, which is fused to the cell membrane by spike once this protein binds one of its receptors an is activated by a membrane protease (both are not shown for simplicity). The infected cell produces viral components, including spike. Now, spike can fuse the membrane of the host cell with the membrane of an adjacent cell if that cell also has a spike receptor. Braga and colleagues (Braga et al., 2021) found that spike-induced cell fusion is associated with activation of TMEM16F, a scramblases that externalizes PS. This commentary proposes that PS externalized by spike enables the formation of the extrinsic tenase (Fig. 1), the key trigger of blood coagulation cascade during viral infections.

SARS-CoV-2 spike can also fuse cells if the virus is not infectious, or even if spike is incorporated into membrane vesicles (Theuerkauf et al., 2021), like extracellular vesicles released by infected cells. This mechanism is known as fusion from without, as the viral particle or a vesicle provides a bridge between the membranes. Syncytia produced by this mechanism would have no trace of SARS-CoV-2.

Note that TF is encrypted, that is unable to activate FVIIa, until it is de-encrypted by externalized PS (reviewed in (Grover and Mackman, 2018)).

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acute respiratory syndrome, to enable maturation of this protein by furin, a protease that also processes SARS-CoV-2 spike, increased the ability of SARS-CoV to fuse cells manyfold with little effect on virus-cell fusion (Follis, York and Nunberg, 2006). Finally, a single mutation in a porcine coronavirus spike enables this protein to cause cell-cell fusion at barely detectably amounts without affecting the ability of the virus to infect (Wanitchang *et al.*, 2019). These observations mean that some strains of SARS-CoV-2 can be equally infective but differ in how well they fuse cells and thus in the consequences of this fusion.

Inflammation and fibrosis. Besides mediating blood clotting, the coagulation cascade is interwoven with signaling pathways regulating inflammation, fibrosis and some other conditions associated with COVID-19 (Sriram and Insel, 2021). Therefore, if syncytia produced by spike trigger blood coagulation cascade, this activity would contribute to COVID-19 beyond inducing thrombosis.

Overall, the outlined possibilities suggest that syncytia produced by spike might be another wrench thrown into the gears of the human body by SARS-CoV-2. Yet, as we are about to discuss, the potential ability of the syncytia to trigger the blood coagulation cascade might be only one of many abnormalities associated with these cells.

Syncytia and other products of cell fusion are heterogeneous abnormal cell types with emergent properties.

In the lungs of COVID-19 patients, SARS-CoV-2 infects, and thus can fuse, ciliated cells in the airway, alveolar type 2 pneumocytes, and epithelial progenitors among others (Buchrieser *et al.*, 2020). What are the properties of, say, syncytia that united pneumocytes with an epithelial progenitor? What happens if a leukocyte or another cell happens to join in? Because spike receptors ACE2 (Zhou *et al.*, 2020), CD147 (Wang *et al.*, 2020), and Neuropilin-1 (Cantuti-Castelvetri *et al.*, 2020) are present in a variety of cell types, the diversity of cells SARS-CoV-2 can produce might be substantial.

Moreover, SARS-CoV-2 may be not the only cause of syncytia in COVID-19. For example, HERV-W ENV, the fusogen of an endogenous retrovirus, was detected in the leukocytes of COVID-19 patients at concentrations that exceeded that in

the cells from healthy donors by orders of magnitude (Balestrieri *et al.*, 2021). HERV-W ENV is also known as syncytin-1 because it mediates the fusion of trophoblasts into syncytiotrophoblast and can fuse other cells in culture if expressed ectopically (Blond *et al.*, 2000). Should expression of syncytin-1 in leukocytes enable them to fuse with each other and other cells, the assortment of cell types in COVID-19 patients would increase even further.

Syncytia made by exogenous viruses are abnormal by definition because cell fusion in the body is normally restricted to a handful of physiological processes, such as fertilization, myogenesis, and the formation of osteoclasts, the cell that remodel bones (Brukman *et al.*, 2019; Petrany and Millay, 2019).

What is known about the mechanisms of physiological fusion – which is much less that one would expect given its function in the body – gives the impression that these mergers are planned and rehearsed down to detail to ensure that only the right cells fuse in a right time and at a right place and that, with the exception of fertilization and stem cell fusion, the resulting syncytia do not attempt to proliferate.

These sophisticated mechanisms, however, are overridden by many infectious viruses, including SARS CoV-2, that fuse cells randomly as long as the cells carry a cognate receptor (Hernández and Podbilewicz, 2017; Brukman *et al.*, 2019). This randomness means that cell fusion induced by infectious viruses is a violent event that forcefully unites two or more finely tuned and specialized systems that just happened to be next to each other but may be quite different in their functions, gene expression patterns, cell cycle stage, age, activation status, and other features. All these features have to be reconciled in the syncytia and hybrids if they are to stay alive (Lazebnik, 2014).

What are the properties of abnormal syncytia? The properties and the fates of non-physiological syncytia in the body remain practically unknown. However, observations made in experimental systems and by studying physiological syncytia provide some clues. One of them is that syncytia can become abnormal not only by combining distinct features of parental cells that are not found together in normal cell types, but also by having emergent properties that appear as a result of rec-

onciling two underlying gene expression patterns (Koulakov and Lazebnik, 2012; Lazebnik, 2014).

For example, fusion of human bronchial epithelial cells to human multipotent stromal cells resulted in cells that appeared epithelial but failed to function properly because the two ion channel required to maintain bronchial and alveolar fluid balance were impaired due to changes in gene expression. One lacked a subunit, the other was improperly expressed (Curril *et al.*, 2010). Likewise, bone marrow derived cells fused to hepatocytes in a mouse model of chronic liver damage yielded cells that differed in their gene expression patterns from both parental types and, unexpectedly, expressed cytokines and genes involved in neurotransmission and in the TGF-β pathway (Quintana-Bustamante *et al.*, 2012). In an extreme case illustrating an enigmatic phenomenon called extinction, fusion of hepatoma cells to fibroblasts silenced hundreds of genes specific to either parental cell type, thus producing dedifferentiated cells (Bulla *et al.*, 2010). Even fusion of cells belonging to the same cell type can produce syncytia with new properties, as it happens with osteoclasts, which resorb bone better than their mononuclear precursors (Yagi *et al.*, 2005).

Given the outlined examples, it is not unreasonable to envision that some syncytia created by SARS CoV-2, associated viral infections, or induced endogenous viruses can produce cytokines or other signaling factors capable of deregulating tissue homeostasis either locally or even systemically, as it happens in COVID-19 during cytokine storm (Sriram and Insel, 2021). These cells might also become sanctuaries for the virus as it has been described for HIV (Symeonides *et al.*, 2015; Bracq *et al.*, 2018), or by evading immune surveillance, perhaps by fusing to immune cells.

What could abnormal syncytia do if they proliferate? While the published reports on COVID-19 discuss large syncytia, as these cells are the most noticeable products of cell fusion due to their size and numerous nuclei (thus often called multinucleated giant cells), they are not the only outcome of cell fusion. Cell fusion can produce binuclear or trinuclear cells, which are often more abundant in experimental systems than large syncytia but could go unnoticed in human tissues or not attributed to cell fusion because distinguishing them reliably from binuclear cells produced by failed mitosis is difficult or impossible with available tools (Gast *et al.*, 2018). A syncytium, especially if it has

only two or three nuclei, can also enter mitosis and produce mononuclear daughter cells, an outcome that might contribute to the pool of dysmorphic cells in the lung, another hallmark of COVID-19 (Bussani *et al.*, 2020). Multipolar mitoses that are common in these mitoses are prone to produce aneuploid cells with chromosomal aberrations, adding another abnormal feature to the offspring of cell fusion (Duelli *ets al.*, 2007), a phenomenon that may be particular relevant to patients with neoplastic lesions.

Fusion of proliferating cells introduces additional abnormalities by combining cells that are at different phases of the cell cycle. Because nuclei united in one cell can progress through the cell cycle independently, one nucleus can force the others to enter mitosis prematurely, causing fragmentation of their chromatin in a phenomenon known as chromatin pulverization (reviewed in (Stevens *et al.*, 2010)). The resulting fragments can incorporate into the chromatin of the hybrid (Pantelias *et al.*, 2019). The common features of chromosomal aberrations in cell hybrids and cancer cells (Ogle *et al.*, 2004; Duelli *et al.*, 2007; Delespaul *et al.*, 2019; Lartigue *et al.*, 2020) along with the shared clonal heterogeneity of cancers and neoplastic cell hybrids, which enables their evolution (Miroshnychenko *et al.*, 2021) has provided support for a long standing model that cell fusion is involved in cancer development, progression, recurrence, dormancy, and acquired drug resistance (reviewed in: (Duelli and Lazebnik, 2003; Parris, 2013; Shabo *et al.*, 2020)). This model has been supported by recent reports of cell hybrids in human cancers (Gast *et al.*, 2018; Laberge *et al.*, 2019) and by multiple observations in animal models (reviewed in (Duelli and Lazebnik, 2003; Pawelek and Chakraborty, 2008; Noubissi and Ogle, 2016)). Whether any neoplastic hybrids found in humans are made by viral fusogens, as it has been suggested (Duelli *et al.*, 2005; Parris, 2005; Duelli and Lazebnik, 2007), is yet to be learned.

This incomplete list of options that cell fusion can uniquely use to produce diverse abnormal cells suggests that drugs that target this largely unexplored biological process, including those identified by Braga and colleagues (Braga *et al.*, 2021), might also be useful in conditions other than COVID-19. If true, discovering otherwise neglected syncytia in COVID-19 patients may help to dissect and control cell fusion and its consequences in

health and disease. After all, we tend to see only what we expect to see.

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REFERENCES

Antoniak, S. and Mackman, N. (2014) 'Multiple roles of the coagulation protease cascade during virus infection', *Blood*, 123(17), pp. 2605–2613. doi: 10.1182/blood-2013-09-526277.

Balestrieri, E., Minutolo, A., Petrone, V., Fanelli, M., Iannetta, M., Malagnino, V., Zordan, M., Vitale, P., Charvet, B., Horvat, B., Bernardini, S., Garaci, E., di Francesco, P., Sinibaldi Vallebona, P., Sarmati, L., Grelli, S., Andreoni, M., Perron, H. and Matteucci, C. (2021) 'Evidence of the pathogenic HERV-W envelope expression in T lymphocytes in association with the respiratory outcome of COVID-19 patients', *EBioMedicine*, 66, p. 103341. doi: 10.1016/j.ebiom.2021.103341.

Blond, J. L., Lavillette, D., Cheynet, V., Bouton, O., Oriol, G., Chapel-Fernandes, S., Mandrand, B., Mallet, F. and Cosset, F. L. (2000) 'An envelope glycoprotein of the human endogenous retrovirus HERV-W is expressed in the human placenta and fuses cells expressing the type D mammalian retrovirus receptor', *Journal of Virology*, 74(7), pp. 3321–3329. doi: 10.1128/jvi.74.7.3321-3329.2000.

Bom, V. J. and Bertina, R. M. (1990) 'The contributions of Ca2+, phospholipids and tissue-factor apoprotein to the activation of human blood-coagulation factor X by activated factor VII', *The Biochemical Journal*, 265(2), pp. 327–336. doi: 10.1042/bj2650327.

Bracq, L., Xie, M., Benichou, S. and Bouchet, J. (2018) 'Mechanisms for Cell-to-Cell Transmission of HIV-1', *Frontiers in Immunology*, 9, p. 260. doi: 10.3389/fimmu.2018.00260.

Braga, L., Ali, H., Secco, I., Chiavacci, E., Neves, G., Goldhill, D., Penn, R., Jimenez-Guardeño, J. M., Ortega-Prieto, A. M., Bussani, R., Cannatà, A., Rizzari, G., Collesi, C., Schneider, E., Arosio, D., Shah, A. M., Barclay, W. S., Malim, M. H., Burrone, J. and Giacca, M. (2021) 'Drugs that inhibit TMEM16 proteins block SARS-CoV-2 spike-induced syncytia', *Nature*. doi: 10.1038/s41586-021-03491-6.

Brukman, N. G., Uygur, B., Podbilewicz, B. and Chernomordik, L. V. (2019) 'How cells fuse', *Journal of Cell Biology*, 218(5), pp. 1436–1451. doi: 10.1083/jcb.201901017.

Buchrieser, J., Dufloo, J., Hubert, M., Monel, B., Planas, D., Rajah, M. M., Planchais, C., Porrot, F., Guivel-Benhassine, F., Van der Werf, S., Casartelli, N., Mouquet, H., Bruel, T. and Schwartz, O. (2020) 'Syncytia formation by SARS-CoV-2-infected cells', *The EMBO journal*, 39(23), p. e106267. doi: 10.15252/embj.2020106267.

Bulla, G. A., Luong, Q., Shrestha, S., Reeb, S. and Hickman, S. (2010) 'Genome-wide analysis of hepatic gene silencing in mammalian cell hybrids', *Genomics*, 96(6), pp. 323–332. doi: 10.1016/j.ygeno.2010.08.006.

Bussani, R., Schneider, E., Zentilin, L., Collesi, C., Ali, H., Braga, L., Volpe, M. C., Colliva, A., Zanconati, F., Berlot, G., Silvestri, F., Zacchigna, S. and Giacca, M. (2020) 'Persistence of viral RNA, pneumocyte syncytia and thrombosis are hallmarks of advanced COVID-19 pathology', *EBioMedicine*, 61, p. 103104. doi: 10.1016/j.ebiom.2020.103104.

Cañas, C. A., Cañas, F., Bautista-Vargas, M. and Bonilla-Abadía, F. (2021) 'Role of Tissue Factor in the Pathogenesis of COVID-19 and the Possible Ways to Inhibit It', *Clinical and Applied Thrombosis/Hemostasis*, 27, p. 107602962110039. doi: 10.1177/10760296211003983.

Cantuti-Castelvetri, L., Ojha, R., Pedro, L. D., Djannatian, M., Franz, J., Kuivanen, S., van der Meer, F., Kallio, K., Kaya, T., Anastasina, M., Smura, T., Levanov, L., Szirovicza, L., Tobi, A., Kallio-Kokko, H., Österlund, P., Joensuu, M., Meunier, F. A., Butcher, S. J., Winkler, M. S., Mollenhauer, B., Helenius, A., Gokce, O., Teesalu, T., Hepojoki, J., Vapalahti, O., Stadelmann, C.,

Balistreri, G. and Simons, M. (2020) 'Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity', *Science (New York, N.Y.)*, 370(6518), pp. 856–860. doi: 10.1126/science.abd2985.

Curril, I. M., Koide, M., Yang, C. H., Segal, A., Wellman, G. C. and Spees, J. L. (2010) 'Incomplete reprogramming after fusion of human multipotent stromal cells and bronchial epithelial cells', *FASEB journal: official publication of the Federation of American Societies for Experimental Biology*, 24(12), pp. 4856–4864. doi: 10.1096/fj.09-152991.

Delespaul, L., Merle, C., Lesluyes, T., Lagarde, P., Le Guellec, S., Pérot, G., Baud, J., Carlotti, M., Danet, C., Fèvre, M., Rousseau, B., Durrieu, S., Teichmann, M., Coindre, J.-M., Lartigue, L. and Chibon, F. (2019) 'Fusion-mediated chromosomal instability promotes aneuploidy patterns that resemble human tumors', *Oncogene*, 38(33), pp. 6083–6094. doi: 10.1038/s41388-019-0859-6.

Duelli, D. and Lazebnik, Y. (2003) 'Cell fusion: a hidden enemy?', *Cancer Cell*, 3(5), pp. 445–448. doi: 10.1016/s1535-6108(03)00114-4

Duelli, D. and Lazebnik, Y. (2007) 'Cell-to-cell fusion as a link between viruses and cancer', *Nature Reviews. Cancer*, 7(12), pp. 968–976. doi: 10.1038/nrc2272.

Duelli, D. M., Hearn, S., Myers, M. P. and Lazebnik, Y. (2005) 'A primate virus generates transformed human cells by fusion', *The Journal of Cell Biology*, 171(3), pp. 493–503. doi: 10.1083/jcb.200507069.

Duelli, D. M., Padilla-Nash, H. M., Berman, D., Murphy, K. M., Ried, T. and Lazebnik, Y. (2007) 'A virus causes cancer by inducing massive chromosomal instability through cell fusion', *Current biology: CB*, 17(5), pp. 431–437. doi: 10.1016/j.cub.2007.01.049.

Follis, K. E., York, J. and Nunberg, J. H. (2006) 'Furin cleavage of the SARS coronavirus spike glycoprotein enhances cell-cell fusion but does not affect virion entry', *Virology*, 350(2), pp. 358–369. doi: 10.1016/j.virol.2006.02.003.

Gallaher, W. R. and Bratt, M. A. (1972) 'Temperature-dependent inhibition of fusion from without', *Journal of Virology*, 10(1), pp. 159–161. doi: 10.1128/JVI.10.1.159-161.1972.

Gast, C. E., Silk, A. D., Zarour, L., Riegler, L., Burkhart, J. G., Gustafson, K. T., Parappilly, M. S., Roh-Johnson, M., Goodman, J. R., Olson, B., Schmidt, M., Swain, J. R., Davies, P. S., Shasthri, V., Iizuka, S., Flynn, P., Watson, S., Korkola, J., Courtneidge, S. A., Fischer, J. M., Jaboin, J., Billingsley, K. G., Lopez, C. D., Burchard, J., Gray, J., Coussens, L. M., Sheppard, B. C. and Wong, M. H. (2018) 'Cell fusion potentiates tumor heterogeneity and reveals circulating hybrid cells that correlate with stage and survival', *Science Advances*, 4(9), p. eaat7828. doi: 10.1126/sciadv.aat7828.

Grover, S. P. and Mackman, N. (2018) 'Tissue Factor: An Essential Mediator of Hemostasis and Trigger of Thrombosis', *Arteriosclerosis, Thrombosis, and Vascular Biology*, 38(4), pp. 709–725. doi: 10.1161/ATVBAHA.117.309846.

Hanff, T. C., Mohareb, A. M., Giri, J., Cohen, J. B. and Chirinos, J. A. (2020) 'Thrombosis in COVID-19', *American Journal of Hematology*, 95(12), pp. 1578–1589. doi: 10.1002/ajh.25982.

Hernández, J. M. and Podbilewicz, B. (2017) 'The hallmarks of cell-cell fusion', *Development*, 144(24), pp. 4481–4495. doi: 10.1242/dev.155523.

Koulakov, A. A. and Lazebnik, Y. (2012) 'The problem of colliding networks and its relation to cell fusion and cancer', *Biophys J*, 103(9), pp. 2011–20. doi: 10.1016/j.bpj.2012.08.062.

Kowarz, E., Krutzke, L., Reis, J., Bracharz, S., Kochanek, S. and Marschalek, R. (2021) *"Vaccine-Induced Covid-19 Mimicry" Syndrome:Splice reactions within the SARS-CoV-2 Spike open reading frame result in Spike protein variants that may cause thromboembolic events in patients immunized with vector-based vaccines*. preprint. In Review. doi: 10.21203/rs.3.rs-558954/v1.

Laberge, G., Duvall, E., Haedicke, K. and Pawelek, J. (2019) 'Leukocyte–Cancer Cell Fusion—Genesis of a Deadly Journey', *Cells*, 8(2), p. 170. doi: 10.3390/cells8020170.

Lartigue, L., Merle, C., Lagarde, P., Delespaul, L., Lesluyes, T., Le Guellec, S., Pérot, G., Leroy, L.,

Coindre, J.-M. and Chibon, F. (2020) 'Genome remodeling upon mesenchymal tumor cell fusion contributes to tumor progression and metastatic spread', *Oncogene*, 39(21), pp. 4198–4211. doi: 10.1038/s41388-020-1276-6.

Lazebnik, Y. (2014) 'The shock of being united and symphiliosis', *Cell Cycle*, 13(15), pp. 2323–2329. doi: 10.4161/cc.29704.

Mackman, N., Antoniak, S., Wolberg, A. S., Kasthuri, R. and Key, N. S. (2020) 'Coagulation Abnormalities and Thrombosis in Patients Infected With SARS-CoV-2 and Other Pandemic Viruses', *Arteriosclerosis, Thrombosis, and Vascular Biology*, 40(9), pp. 2033–2044. doi: 10.1161/ATVBAHA.120.314514.

Miroshnychenko, D., Baratchart, E., Ferrall-Fairbanks, M. C., Velde, R. V., Laurie, M. A., Bui, M. M., Tan, A. C., Altrock, P. M., Basanta, D. and Marusyk, A. (2021) 'Spontaneous cell fusions as a mechanism of parasexual recombination in tumour cell populations', *Nature Ecology & Evolution*, 5(3), pp. 379–391. doi: 10.1038/s41559-020-01367-y.

Noubissi, F. and Ogle, B. (2016) 'Cancer Cell Fusion: Mechanisms Slowly Unravel', *International Journal of Molecular Sciences*, 17(9), p. 1587. doi: 10.3390/ijms17091587.

Ogle, B. M., Butters, K. A., Plummer, T. B., Ring, K. R., Knudsen, B. E., Litzow, M. R., Cascalho, M. and Platt, J. L. (2004) 'Spontaneous fusion of cells between species yields transdifferentiation and retroviral transfer in vivo', *FASEB journal: official publication of the Federation of American Societies for Experimental Biology*, 18(3), pp. 548–550. doi: 10.1096/fj.03-0962fje.

Pantelias, Karachristou, Georgakilas, and Terzoudi (2019) 'Interphase Cytogenetic Analysis of Micronucleated and Multinucleated Cells Supports the Premature Chromosome Condensation Hypothesis as the Mechanistic Origin of Chromothripsis', *Cancers*, 11(8), p. 1123. doi: 10.3390/cancers11081123.

Parris, G. E. (2005) 'The role of viruses in cell fusion and its importance to evolution, invasion and metastasis of cancer clones', *Medical Hypotheses*, 64(5), pp. 1011–1014. doi: 10.1016/j.mehy.2004.11.012.

Parris, G. E. (2013) 'Historical perspective of cell-cell fusion in cancer initiation and progression', *Critical Reviews in Oncogenesis*, 18(1–2), pp. 1–18. doi: 10.1615/critrevoncog.v18.i1-2.20.

Pawelek, J. M. and Chakraborty, A. K. (2008) 'Fusion of tumour cells with bone marrow-derived cells: a unifying explanation for metastasis', *Nature Reviews. Cancer*, 8(5), pp. 377–386. doi: 10.1038/nrc2371.

Petrany, M. J. and Millay, D. P. (2019) 'Cell Fusion: Merging Membranes and Making Muscle', *Trends in Cell Biology*, 29(12), pp. 964–973. doi: 10.1016/j.tcb.2019.09.002.

Quintana-Bustamante, O., Grueso, E., Garcia-Escudero, R., Arza, E., Alvarez-Barrientos, A., Fabregat, I., Garcia-Bravo, M., Meza, N. W. and Segovia, J. C. (2012) 'Cell Fusion Reprogramming Leads to a Specific Hepatic Expression Pattern during Mouse Bone Marrow Derived Hepatocyte Formation In Vivo', *PLoS ONE*. Edited by A. Wutz, 7(3), p. e33945. doi: 10.1371/journal.pone.0033945.

Rosell, A., Havervall, S., von Meijenfeldt, F., Hisada, Y., Aguilera, K., Grover, S. P., Lisman, T., Mackman, N. and Thålin, C. (2021) 'Patients With COVID-19 Have Elevated Levels of Circulating Extracellular Vesicle Tissue Factor Activity That Is Associated With Severity and Mortality-Brief Report', *Arteriosclerosis, Thrombosis, and Vascular Biology*, 41(2), pp. 878–882. doi: 10.1161/ATVBAHA.120.315547.

Scudieri, P., Caci, E., Venturini, A., Sondo, E., Pianigiani, G., Marchetti, C., Ravazzolo, R., Pagani, F. and Galietta, L. J. V. (2015) 'Ion channel and lipid scramblase activity associated with expression of TMEM16F/ANO6 isoforms: TMEM16F variants', *The Journal of Physiology*, 593(17), pp. 3829–3848. doi: 10.1113/JP270691.

Shabo, I., Svanvik, J., Lindström, A., Lechertier, T., Trabulo, S., Hulit, J., Sparey, T. and Pawelek, J. (2020) 'Roles of cell fusion, hybridization and polyploid cell formation in cancer metastasis', *World Journal of Clinical Oncology*, 11(3), pp. 121–135. doi: 10.5306/wjco.v11.i3.121.

Smith, S. A. and Morrissey, J. H. (2019) 'Interactions Between Platelets and the Coagulation Sys-

tem', in Michelson, A. D. (ed.) *Platelets (Fourth Edition)*. Academic Press, pp. 393–400. doi: 10.1016/B978-0-12-813456-6.00021-7.

Smith, S. A., Travers, R. J. and Morrissey, J. H. (2015) 'How it all starts: Initiation of the clotting cascade', *Critical Reviews in Biochemistry and Molecular Biology*, 50(4), pp. 326–336. doi: 10.3109/10409238.2015.1050550.

Sriram, K. and Insel, P. A. (2021) 'Inflammation and thrombosis in COVID-19 pathophysiology: proteinase-activated and purinergic receptors as drivers and candidate therapeutic targets', *Physiological Reviews*, 101(2), pp. 545–567. doi: 10.1152/physrev.00035.2020.

Stevens, J. B., Abdallah, B. Y., Regan, S. M., Liu, G., Bremer, S. W., Ye, C. J. and Heng, H. H. (2010) 'Comparison of mitotic cell death by chromosome fragmentation to premature chromosome condensation', *Molecular Cytogenetics*, 3, p. 20. doi: 10.1186/1755-8166-3-20.

Suzuki, J., Umeda, M., Sims, P. J. and Nagata, S. (2010) 'Calcium-dependent phospholipid scrambling by TMEM16F', *Nature*, 468(7325), pp. 834–838. doi: 10.1038/nature09583.

Symeonides, M., Murooka, T., Bellfy, L., Roy, N., Mempel, T. and Thali, M. (2015) 'HIV-1-Induced Small T Cell Syncytia Can Transfer Virus Particles to Target Cells through Transient Contacts', *Viruses*, 7(12), pp. 6590–6603. doi: 10.3390/v7122959.

Theuerkauf, S. A., Michels, A., Riechert, V., Maier, T. J., Flory, E., Cichutek, K. and Buchholz, C. J. (2021) 'Quantitative assays reveal cell fusion at minimal levels of SARS-CoV-2 spike protein and fusion from without', *iScience*, 24(3), p. 102170. doi: 10.1016/j.isci.2021.102170.

Wang, K., Chen, W., Zhang, Z., Deng, Y., Lian, J.-Q., Du, P., Wei, D., Zhang, Y., Sun, X.-X., Gong, L., Yang, X., He, L., Zhang, L., Yang, Z., Geng, J.-J., Chen, R., Zhang, H., Wang, B., Zhu, Y.-M., Nan, G., Jiang, J.-L., Li, L., Wu, J., Lin, P., Huang, W., Xie, L., Zheng, Z.-H., Zhang, K., Miao, J.-L., Cui, H.-Y., Huang, M., Zhang, J., Fu, L., Yang, X.-M., Zhao, Z., Sun, S., Gu, H., Wang, Z., Wang, C.-F., Lu, Y., Liu, Y.-Y., Wang, Q.-Y., Bian, H., Zhu, P. and Chen, Z.-N. (2020) 'CD147-spike protein is a novel route for SARS-CoV-2 infection to host

cells', *Signal Transduction and Targeted Therapy*, 5(1), p. 283. doi: 10.1038/s41392-020-00426-x.

Wanitchang, A., Saenboonrueng, J., Kaewborisuth, C., Srisutthisamphan, K. and Jongkaewwattana, A. (2019) 'A Single V672F Substitution in the Spike Protein of Field-Isolated PEDV Promotes Cell⁻Cell Fusion and Replication in VeroE6 Cells', *Viruses*, 11(3). doi: 10.3390/v11030282.

Yagi, M., Miyamoto, T., Sawatani, Y., Iwamoto, K., Hosogane, N., Fujita, N., Morita, K., Ninomiya, K., Suzuki, T., Miyamoto, K., Oike, Y., Takeya, M., Toyama, Y. and Suda, T. (2005) 'DC-STAMP is essential for cell-cell fusion in osteoclasts and foreign body giant cells', *The Journal of Experimental Medicine*, 202(3), pp. 345–351. doi: 10.1084/jem.20050645.

Zhang, Y., Le, T., Grabau, R., Mohseni, Z., Kim, H., Natale, D. R., Feng, L., Pan, H. and Yang, H. (2020) 'TMEM16F phospholipid scramblase mediates trophoblast fusion and placental development', *Science Advances*, 6(19), p. eaba0310. doi: 10.1126/sciadv.aba0310.

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., Chen, H.-D., Chen, J., Luo, Y., Guo, H., Jiang, R.-D., Liu, M.-Q., Chen, Y., Shen, X.-R., Wang, X., Zheng, X.-S., Zhao, K., Chen, Q.-J., Deng, F., Liu, L.-L., Yan, B., Zhan, F.-X., Wang, Y.-Y., Xiao, G.-F. and Shi, Z.-L. (2020) 'A pneumonia outbreak associated with a new coronavirus of probable bat origin', *Nature*, 579(7798), pp. 270–273. doi: 10.1038/s41586-020-2012-7.

Zhu, S., Tomaiuolo, M. and Diamond, S. L. (2016) 'Minimum wound size for clotting: flowing blood coagulates on a single collagen fiber presenting tissue factor and von Willebrand factor', *Integrative biology : quantitative biosciences from nano to macro*, 8(8), pp. 813–820. doi: 10.1039/c6ib00077k.

Zwaal, R. F., Comfurius, P. and Bevers, E. M. (1998) 'Lipid-protein interactions in blood coagulation', *Biochimica Et Biophysica Acta*, 1376(3), pp. 433–453. doi: 10.1016/s0304-4157(98)00018-5.