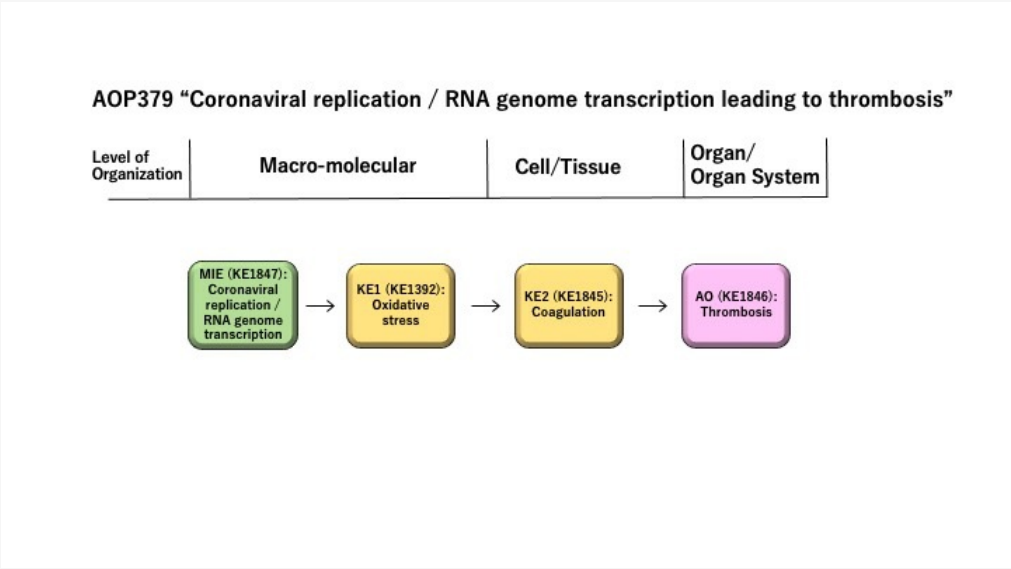
AOP ID and Title:

AOP 379: Coronaviral replication / RNA genome transcription leading to thrombosis
Short Title: SARS-CoV2 to thrombosis

Graphical Representation



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Status

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Abstract

Coronavirus disease-19 (COVID-19) is circulating all over the world. To understand and find a way of the COVID-19 treatment, the therapeutic mechanism of COVID-19 is focused on in this Editorial. The pathogenesis of COVID-19 includes molecular networks such as the binding of the membrane proteins, signaling pathways, and RNA replication. The mechanism of infection and targets of the therapeutics are explored and summarized. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is a new type of coronavirus causing COVID-19, infects the cells via the binding of the membrane proteins of human cells and is internalized by the cells. The viral genome is replicated by RNA-dependent RNA polymerase (RdRp), followed by the packaging and releasing of the viral particles. These steps can be the main targets for the therapeutics of COVID-19. This AOP379 "Coronaviral replication / RNA genome transcription leading to thrombosis" consists of the molecular initiating event (MIE) as "Coronaviral replication / RNA genome transcription" (KE1847), key events (KEs) as "Oxidative stress" (KE1: KE1392) and "Coagulation" (KE2: KE1845), and adverse outcome (AO) as "thrombosis" (KE1846).

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
	MIE	1847	Coronaviral replication / RNA genome transcription	RNA genome transcription
	KE	1392	Oxidative Stress	Oxidative Stress
	KE	1845	Coagulation	Coagulation

Sequence	Type	Event ID	Title	Short name
	AO	1846	Thrombosis	Thrombosis

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Coronaviral replication / RNA genome transcription	adjacent	Oxidative Stress	Moderate	
Oxidative Stress	adjacent	Coagulation	Moderate	
Coagulation	adjacent	Thrombosis	High	

Stressors

Name	Evidence
Sars-CoV-2	High

Overall Assessment of the AOP

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI

Sex Applicability

Sex	Evidence
Unspecific	High

References

Tanabe S. The Therapeutic Mechanism of COVID-19. J Clin Med Res. 2020;2(5):1-3. DOI: https://doi.org/10.37191/Mapsci-2582-4333-2(5)-048

Appendix 1

List of MIEs in this AOP

Event: 1847: Coronaviral replication / RNA genome transcription

Short Name: RNA genome transcription

Key Event Component

Process	Object	Action
viral RNA genome replication		increased

AOPs Including This Key Event

AOP ID and Name	Event Type

AOP ID and Name	Event Type
Aop:379 - Coronaviral replication / RNA genome transcription leading to thrombosis	MolecularInitiatingEvent

Biological Context

Level of Biological Organization

Molecular

Domain of Applicability

Homo sapiens

Key Event Description

Coronavirus is a class of viruses which have single-stranded positive-sense RNA genomes in their envelopes [Cui J, et al. Nature Reviews Microbiology. 2019;17(3):181-192.]. Infected virus particles release their genomic RNA inside the human cells, followed by RNA translation or genomic RNA replication by RNA-dependent RNA polymerase (RdRp). RNA viral genome is transcribed into messenger RNA by viral RdRps [Ahlquist, P. Science 2002, 296, 1270, Florindo HF, Nature Nanotechnology. 2020:15(8):630-45.]. Viral RdRps act in combination with other viral and host factors involved in selecting template RNAs, elongating RNA synthesis, differentiating genomic RNA replication from mRNA transcription, modifying product RNAs with 5' caps or 3' polyadenylate [Ahlquist, P. Science 2002, 296, 1270]. Positive-sense (messenger-sense) RNA viruses replicate their genomes through negative-strand RNA intermediates [Schwartz, Michael et al. Molecular Cell. 2002;9(3):505-514]. Upon virus entry into host cells, genomic RNA serves as mRNA for the first open reading frame (ORF1), being thus translated into viral replicase polyproteins [Florindo HF, Nature Nanotechnology. 2020:15(8):630-45]. The cleaved-polyproteins assemble on double-membrane vesicles, where the RNA genome replication and subgenomic RNA transcription occur [Florindo HF, Nature Nanotechnology. 2020:15(8):630-45, Schwartz, Michael et al. Molecular Cell. 2002;9(3):505-514]. The RdRp complex uses the genome as a template to generate negative-sense subgenome and genome-length RNAs, which are in turn used as templates for synthesis of positive-sense full-length progeny genomes and subgenomic mRNAs [Hartenian E, et al. J Biol Chem. 2020;295(37):12910-12934].

How it is Measured or Detected

The mRNA transcripts are detected by the real-time reverse transcription-PCR (RT-PCR) assay. Several methods targeting the mRNA transcripts have been developed, which includes the RT-PCR assays targeting RdRp/helicase (Hel), spike (S) and nucleocapsid (N) genes of SARS-CoV-2 [Chan, Jasper Fuk-Woo et al. J Clin Microbiol. 2020:58(5)e00310-20]. RT-PCR assays detecting SARS-CoV-2 RNA in saliva include quantitative RT-PCR (RT-qPCR), direct RT-qPCR, reverse transcription-loop-mediated isothermal amplification (RT-LAMP) [Nagura-Ikeda M, Imai K, Tabata S, et al. J Clin Microbiol. 2020;58(9):e01438-20]. The viral mRNAs are reverse-transcribed with RT, followed by the amplification by PCR.

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List of Key Events in the AOP

Event: 1392: Oxidative Stress

Short Name: Oxidative Stress

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:220 - Cyp2E1 Activation Leading to Liver Cancer	KeyEvent
Aop:17 - Binding of electrophilic chemicals to SH(thiol)-group of proteins and /or to seleno-proteins involved in protection against oxidative stress during brain development leads to impairment of learning and memory	KeyEvent
Aop:284 - Binding of electrophilic chemicals to SH(thiol)-group of proteins and /or to seleno-proteins involved in protection against oxidative stress leads to chronic kidney disease	KeyEvent
Aop:379 - Coronaviral replication / RNA genome transcription leading to thrombosis	KeyEvent

Stressors

Name
Acetaminophen
Chloroform
furan

Biological Context

Level of Biological Organization
Molecular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rodents	rodents	High	NCBI
Homo sapiens	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Oxidative stress is produced in, and can occur in, any species from bacteria through to humans.

Key Event Description

Oxidative stress is defined as an imbalance in the production of reactive oxygen species (ROS) and antioxidant defenses. High levels of oxidizing free radicals can be very damaging to cells and molecules within the cell. As a result, the cell has important defense mechanisms to protect itself from ROS. For example, Nrf2 is a transcription factor and master regulator of the oxidative stress response. During periods of oxidative stress, Nrf2-dependent changes in gene expression are important in regaining cellular homeostasis (Nguyen, et al. 2009) and can be used as indicators of the presence of oxidative stress in the cell.

In addition to the directly damaging actions of ROS, cellular oxidative stress also changes cellular activities on a molecular level. Redox sensitive proteins have altered physiology in the presence and absence of ROS, which is caused by the oxidation of sulfhydryls to disulfides (2SH àSS) on neighboring amino acids (Antelmann and Helmann 2011). Importantly Keap1, the negative regulator of Nrf2, is regulated in this manner (Itoh, et al. 2010).

The brain possesses several key physiological features, such as high O2 utilization, high polyunsaturated fatty acids content, presence of autooxidable neurotransmitters, and low antioxidant defenses as compared to other organs, that make it highly susceptible to oxidative stress (Halliwell, 2006; Emerit and al., 2004; Frauenberger et al., 2016).

How it is Measured or Detected

Oxidative Stress. Direct measurement of ROS is difficult because ROS are unstable. The presence of ROS can be assayed indirectly by measurement of cellular antioxidants, or by ROS-dependent cellular damage:

- Detection of ROS by chemiluminescence (https://www.sciencedirect.com/science/article/abs/pii/S0165993606001683)
- Glutathione (GSH) depletion. GSH can be measured by assaying the ratio of reduced to oxidized glutathione (GSH:GSSG) using a commercially available kit (e.g., http://www.abcam.com/gshgssg-ratio-detection-assay-kit-fluorometric-green-ab138881.html).
- TBARS. Oxidative damage to lipids can be measured by assaying for lipid peroxidation using TBARS (thiobarbituric acid reactive substances) using a commercially available kit.
- 8-oxo-dG. Oxidative damage to nucleic acids can be assayed by measuring 8-oxo-dG adducts (for which there are a number of ELISA based commercially available kits),or HPLC, described in Chepelev et al. (Chepelev, et al. 2015).

Molecular Biology: Nrf2. Nrf2's transcriptional activity is controlled post-translationally by oxidation of Keap1. Assay for Nrf2 activity include:
- Immunohistochemistry for increases in Nrf2 protein levels and translocation into the nucleus;
- Western blot for increased Nrf2 protein levels;
- Western blot of cytoplasmic and nuclear fractions to observe translocation of Nrf2 protein from the cytoplasm to the nucleus;
- qPCR of Nrf2 target genes (e.g., Nqo1, Hmox-1, Gcl, Gst, Prx, TrxR, Srxn), or by commercially available pathway-based qPCR array (e.g., oxidative stress array from SABiosciences)
- Whole transcriptome profiling by microarray or RNA-seq followed by pathway analysis (in IPA, DAVID, metacore, etc.) for enrichment of the Nrf2 oxidative stress response pathway (e.g., Jackson et al. 2014).

References

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Nguyen, T., Nioi, P., Pickett, C.B., 2009. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J. Biol. Chem. 284, 13291-13295.

Event: 1845: Coagulation

Short Name: Coagulation

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:379 - Coronaviral replication / RNA genome transcription leading to thrombosis	KeyEvent

Stressors

Name
Sars-CoV-2

Biological Context

Level of Biological Organization

Cellular

Cell term

Cell term

blood cell

Organ term

Organ term

blood plasma

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Sex Applicability

Sex	Evidence
Unspecific	Moderate

Homo sapiens

Key Event Description

Coagulation is a process that responds to injury by the rapid formation of a clot. Activation of coagulation factor proteins are involved in coagulation. In the extrinsic pathway, platelets, upon the contact with collagen in the injured blood vessel wall, release thromboxane A2 (TXA2) and adenosine 2 phosphates (ADP), leading to the clot formation. Extravascular tissue factor (TF) binds to plasma factor VIIa (FVIIa) and promotes the activation of FXa. Activated FXa assembles with cofactors FVa and FVIIIa on the surface of aggregated platelets, which lead to generation of thrombin (FIIa). Thrombin catalyzes the production of fibrin (FG) which creates a clot.
The binding of prekallikrein and high-molecular weight kininogen activate FXIIa in the intrinsic pathway.
Many regulators are involved in coagulation system. Plasmin is one of the modulators required for dissolution of the fibrin clot. Plasmin is activated by tissue plasminogen activator (tPA) and urokinase plasminogen activation (uPA). SERPINs inhibit thrombin, plasmin and tPA. For example, SERPINE1 or plasminogen activator inhibitor-1 (PAI-1) inhibits tPA/uPA and results in hypofibrinolysis [Bernard I,et al. Viruses. 2021; 13(1):29.]. In addition, SERPING1 inhibits FXII, and thus down-regulation of SERPING1 lifts suppression of FXII of the intrinsic coagulation cascade [Garvin et al. eLife 2020;9:e59177]. Protein C, protein S and thrombomodulin degrade FVa and FVIIIa. [Ref. IPA, Coagulation System, version60467501, release date: 2020-11-19]

How it is Measured or Detected

Coagulation and inflammatory parameters are measured in COVID-19 patients [Di Nisio et al. 2021]. Coagulation parameters include platelet count, prothrombin time, activated partial thromboplastin time, D-dimer, fibrinogen, antithrombin III [Di Nisio et al. 2021]. These parameters are measured in the blood.

In vitro systems

Whole human blood model for testing the activation of coagulation and complement system, as well as clot formation [Ekstrand-Hammarström, B. et al. Biomaterials 2015, 51, 58-68, Ekdahl, K.N., et al. Nanomedicine: Nanotechnology, Biology and Medicine 2018, 14, 735-744, Ekdahl, K.N., et al. Science and Technology of Advanced Materials, 20:1, 688-698,]

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List of Adverse Outcomes in this AOP

Event: 1846: Thrombosis

Short Name: Thrombosis

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:379 - Coronaviral replication / RNA genome transcription leading to thrombosis	AdverseOutcome

Biological Context

Level of Biological Organization

Organ

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 2291: RNA genome transcription leads to Oxidative Stress

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Coronaviral replication / RNA genome transcription leading to thrombosis	adjacent	Moderate	

Relationship: 2289: Oxidative Stress leads to Coagulation

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Coronaviral replication / RNA genome transcription leading to thrombosis	adjacent	Moderate	

Relationship: 2290: Coagulation leads to Thrombosis

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Coronaviral replication / RNA genome transcription leading to thrombosis	adjacent	High	

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Key Event Relationship Description

Many regulators are involved in coagulation system. Plasmin is one of the modulators required for dissolution of the fibrin clot. Plasmin is activated by tissue plasminogen activator (tPA) and urokinase plasminogen activation (uPA). SERPINs inhibit thrombin, plasmin and tPA. For example, SERPINE1 or plasminogen activator inhibitor-1 (PAI-1) inhibits tPA/uPA and results in hypofibrinolysis [Bernard I,et al. Viruses. 2021; 13(1):29.]. In addition, SERPING1 inhibits FXII, and thus down-regulation of SERPING1 lifts suppression of FXII of the intrinsic coagulation cascade [Garvin et al. eLife 2020;9:e59177]. Protein C, protein S and thrombomodulin degrade FVa and FVIIIa. [Ref. IPA, Coagulation System, version60467501, release date: 2020-11-19]

Quantitative Understanding of the Linkage

Known Feedforward/Feedback loops influencing this KER

Decreased fibrinolysis is involved in coagulation system. Coagulopathy may also be involved in this KER.

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