新型冠状病毒信息简报

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上海科技大学免疫化学研究所

生物医学大数据平台和高通量筛选平台领衔编译制作

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1. 2020 年 5 月 8 日疫情

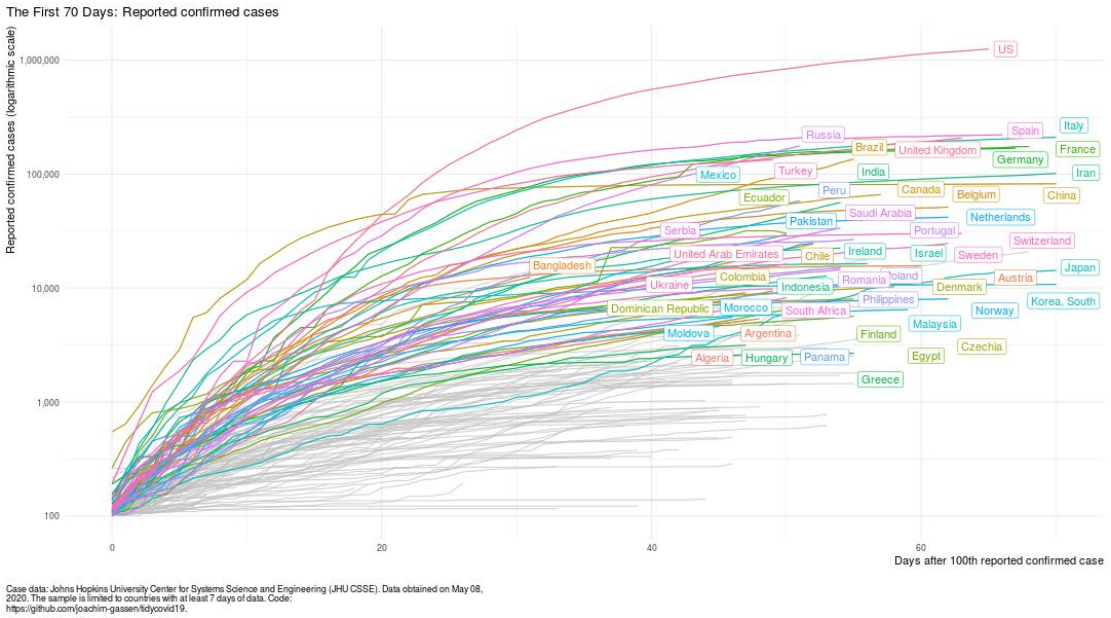
数据来源：WHO

发布时间：2020 年 5 月 8 日北京时间下午 4 点

链接：https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports

根据 WHO 提供的数据，2020 年 5 月 8 日全球累计确诊新型冠状病毒病人 3759967 例，当日新增确诊 87729 例，累计死亡 259474 例，当日新增死亡 5429。

中国累计确诊 84415 例，累计死亡 4643 例，当日新增确诊 6 例，新增死亡 0 例。



重点国家确诊数量曲线（https://jgassen.shinyapps.io/tidycovid19/，数据截止 5 月 8 日北京时间下午 4 点）

全国新型冠状病毒肺炎新增确诊病例分布图（5 月 8 日，来源：http://2019ncov.chinacdc.cn/2019-nCoV/）

2. 美国 FDA 首次批准基于 CRISPR 技术的 COVID-19 检测方法

First CRISPR test for the coronavirus approved in the United States

来源：Nature news
发布时间：2020-05-08
链接：https://www.nature.com/articles/d41586-020-01402-9
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DOI： 10.1038/d41586-020-01402-9
编译者：孔娟
中文摘要：

5 月 6 日，美国 FDA 首次批准了利用 CRISPR 技术进行新型冠状病毒检测的紧急使用。该诊断试剂盒是由 Sherlock Biosciences 公司开发，它的工作原理是通过对 CRISPR 系统进行编程，以检测鼻、口、喉拭子或肺部液体中的 SARS-CoV-2 基因物质片段。如果发现病毒的遗传物质，CRISPR 酶会产生荧光，该测试约一小时就能得到结果。纽约罗切斯特大学的生物化学家米切尔·奥康奈尔表示广泛使用 FDA 批准的新试剂盒有助于检测能力的进一步提高。但同时提醒与标准测试相比，该测试在医院等现实条件下的表现如何，还有待观察。

这套新的诊断工具是基于麻省理工学院和哈佛大学剑桥分校 CRISPR 先驱张峰开发的一种方法，它被允许在得到认证的实验室中检测 SARS-CoV-2。张峰等研究人员在 2017 年首次描述了这种检测方法的基础，并表明它可以检测出低水平的寨卡病毒和登革热病毒。其他实验室也在开发基于 CRISPR 基因编辑技术的 SARS-CoV-2 诊断实验。上个月，旧金山的研究人员公布了一项分析的细节，该分析可以在大约 40 分钟内出结果，该方法是一个基于 CRISPR-Cas12 的对呼吸道拭子抽提的 RNA 进行分析的侧相层析方法(详见 4 月 17 日简报)。阿根廷和加利福尼亚的科学家在预印本中也报道了类似的方法。Sherlock Bioscience 公司目前正在开发一种无需在实验室进行处理、可以在家使用的简便测试，但这种测试需要额外的验证和 FDA 的授权。自 4 月初以来，该机构已经发布了 60 多项 SARS-CoV-2 诊断检测的紧急使用许可，但这些测试均未许可在家中使用。

Abstract:

The US drug regulator has granted its first emergency-use approval for a new coronavirus test that takes advantage of the gene-editing technology CRISPR on 6 May. It will be used to test for SARS-CoV-2 in laboratories that are certified to provide clinical-test results.

The CRISPR-based diagnostic kit has been developed by Sherlock Biosciences. It works by programming the CRISPR machinery to detect a snippet of SARS-CoV-2 genetic material in a nose, mouth or throat swab, or in fluid from the lungs. If the virus’s genetic material is found, a CRISPR enzyme generates a fluorescent glow. The test can return results in about an hour, according to the company.

The new diagnostic kit is based on an approach developed by CRISPR pioneer Feng Zhang at the Broad Institute of MIT and Harvard in Cambridge, Massachusetts. Researchers led by Zhang first described the basis of the test approach in 2017, and showed that it could detect low levels of Zika and Dengue virus. Other labs are also developing SARS-CoV-2 diagnostic tests based on CRISPR gene-editing technology. Last month, researchers in San Francisco, California, published details of an assay that could return results in about 40 minutes. A similar

approach has been reported in a preprint by scientists in Argentina and in California. The chief executive of Sherlock Bioscience, Rahul Dhanda, says that the company is now working to create a single cartridge that would not need to be processed in a laboratory and could be used at home. Since early April, the agency has issued emergency-use authorizations for more than 60 SARS-CoV-2 diagnostic tests. None of these tests have received clearance to be used and processed entirely at home.

3. 使用 SHERLOCK 诊断对 COVID-19 进行护理点测试

Point-of-care testing for COVID-19 using SHERLOCK diagnostics

来源：medRxiv
发布时间：2020-05-08
链接：https://www.medrxiv.org/content/10.1101/2020.05.04.20091231v1
第一作者：Julia Joung
通讯作者：张锋
通讯作者单位：MIT
DOI 或 PUBMED ID：https://doi.org/10.1101/2020.05.04.20091231
编译者：张丽双

中文摘要：

最近爆发的导致 COVID-19 的新型冠状病毒 SARS-CoV-2 可通过 RT-qPCR 进行诊断，但由于缺乏足够的试剂和设备，疾病检测速度减慢，并阻碍了减缓病毒传播的努力。基于等温扩增和 CRISPR 介导的检测相结合的替代方法，如 SHERLOCK（特异性高灵敏度酶报告系统脱锁）技术，降低了对 RT-qPCR 设备的依赖性，但先前报道的方法需要多个流体处理步骤，使其较难部署在临床实验室之外。文中研究者们开发了一种简化的测试化学方法，称为 STOP（一锅化的 SHERLOCK 检测），用于在一小时内检测 SARS-CoV-2，适用于护理点使用。这种简化的检测，STOPCovid，提供了与基于 RT-qPCR 的 SARS-CoV-2 检测相当的灵敏度，并且每个反应可检测唾液或鼻咽拭子中低至 100 个拷贝起始病毒基因组。使用免疫层析读数，测试在 70 分钟内返回结果，使用荧光读数，测试在 40 分钟内返回结果。此外，研究者们使用 COVID-19 患者的鼻咽拭子对 STOPCovid 进行了验证，能够在 3 个重复中正确诊断 12 例阳性和 5 例阴性患者。预计 STOPCovid 的实施将大大有助于“测试-跟踪-隔离”工作，特别是在低资源环境下，这将对长期的公共卫生安全和有效的社会重新开放至关重要。

Abstract:

The recent outbreak of the novel coronavirus SARS-CoV-2, which causes COVID-19, can be diagnosed using RT-qPCR, but inadequate access to reagents and equipment has slowed disease detection and impeded efforts to mitigate viral spread. Alternative approaches based on combinations of isothermal amplification and CRISPR-mediated detection, such as the SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing) technique, offer reduced dependence on RT-qPCR equipment, but previously reported methods required multiple fluid handling steps, complicating their deployment outside clinical labs. Here we developed a simple test chemistry called STOP (SHERLOCK Testing in One Pot) for detecting SARS-CoV-2 in one hour that is suitable for point-of-care use. This simplified test, STOPCovid, provides sensitivity comparable to RT-qPCR-based SARS-CoV-2 tests and has a limit of detection of 100 copies of viral genome input in saliva or

nasopharyngeal swabs per reaction. Using lateral flow readout, the test returns result in 70 minutes, and using fluorescence readout, the test returns result in 40 minutes. Moreover, we validated STOPCovid using nasopharyngeal swabs from COVID-19 patients and were able to correctly diagnose 12 positive and 5 negative patients out of 3 replicates. We envision that implementation of STOPCovid will significantly aid "test-trace-isolate" efforts, especially in low-resource settings, which will be critical for long-term public health safety and effective reopening of the society.

4. 5 分钟 RNA 制备方法用于 COVID-19 RT-qPCR 检测

A 5-min RNA preparation method for COVID-19 detection with RT-qPCR

来源： medRxiv

发布时间： 2020-05-08

链接： https://www.medrxiv.org/content/10.1101/2020.05.07.20055947v1

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通讯作者： 张锋

通讯作者单位： MIT

DOI 或 PUBMED ID： Preprint

编译者： 宋张悦

中文摘要：

RNA 提取已成为 COVID-19 检测的瓶颈，部分原因是试剂短缺。研究人员在本文中提出了一个快速的操作流程，避免了 RNA 提取的需要，这与基于 RT qPCR 的检测方法兼容。该方法可以在 5 分钟内完成无过柱的一步法 RNA 制备，而且该反应可直接用于 CDC COVID-19 RT-qPCR 检测方案，从而提高了检测通量，缓解了供应链问题。

操作流程：

1. 用快速提取 DNA 溶液（Quick ExtractTM DNA Extraction Solution，货号：QE09050，供应商：Lucigen）1:1 稀释保存在病毒转运介质或人体标本对照(HSC)中的鼻咽拭子。例如，在一个新的 PCR 管中，将 20μl 拭子样本与 20μl 的快速提取溶液混合。

2. 将拭子和快速提取混合物 95° C 孵育 5 分钟，冰上冷却后进行下一步骤。

3. 使用步骤(2)的反应产物进行 qRT-PCR。在 qRT-PCR 反应体系中加入 10%的步骤(2)产物的量。例如，一个反应总体系是 50μl，使用 5μl 步骤(2)的反应产物。

方法开发和初步验证如下 Figure 1 所示。

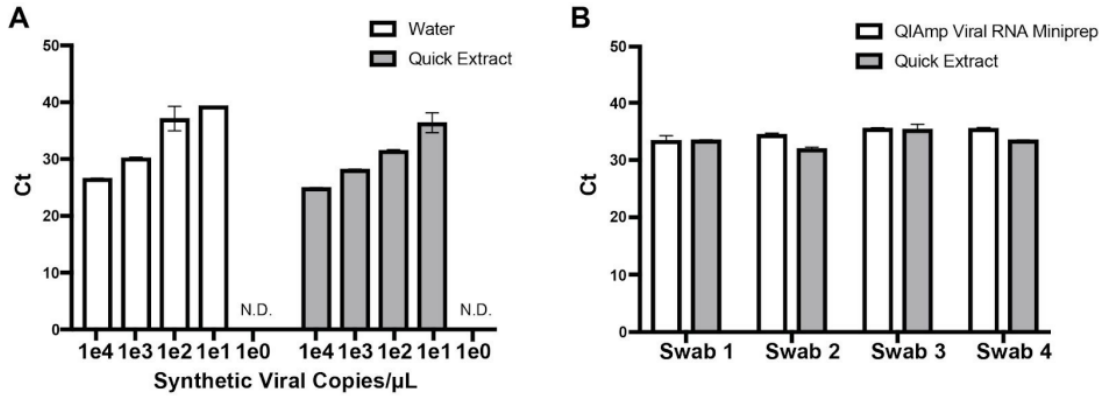

Figure 1. A) RT-qPCR of synthetic SARS-CoV-2 RNA control (Twist Synthetic SARS-CoV-2 RNA Control 1, SKU:102019) diluted in water or 50:50 ddH_2O:Quick Extract

mixture. Final sample volume was 1 µL in a 10 µL reaction. B) RT-qPCR of diluted COVID-19 positive nasopharyngeal swabs treated at 95° C for 5 minutes in Quick Extract or purified using QIAmp Viral RNA Miniprep.

Abstract:
RNA extraction has become a bottleneck for detection of COVID-19, in part because of reagent shortages. We present here a rapid protocol that circumvents the need for RNA extraction that is compatible with RT-qPCR-based detection methods.
Protocol
1. Dilute nasopharyngeal swab stored in Viral Transport Medium or Human Specimen Control (HSC) 1:1 with Quick Extract™ DNA Extraction Solution. For example, in a fresh PCR tube, mix 20 µl of swab sample with 20 µl of Quick Extract.
2. Incubate swab and Quick Extract mix at 95° C for 5 minutes. Allow reaction to cool on ice before proceeding.
3. Use reaction from step (2) for qRT-PCR. Use an amount from step (2) that corresponds to 10 % of the total qRT-PCR reaction volume. For example, for a reaction with total volume of 50 µl, use 5 µl of the reaction from step (2).

5. HLA A*02:01 的 COVID-19 患者共有对 SARS-COV-2 刺突蛋白的抗原特异性 CD8+ T 细胞应答

Shared Antigen-specific CD8+ T cell Responses Against the SARS-COV-2 Spike Protein in HLA A*02:01 COVID-19 Participants

来源： medRxiv
发布时间： 2020-05-08
链接： https://www.medrxiv.org/content/10.1101/2020.05.04.20085779v1
第一作者： William Chour
通讯作者： Jason D. Goldman, James R. Heath
通讯作者单位： 美国西雅图瑞典医学中心瑞典研究和创新中心，华盛顿大学医学系传染病科，系统生物学研究所
DOI 或 PUBMED ID： Preprint
编译者： 雷颖
中文摘要：
文中报告了来自 SARS-CoV-2 病毒刺突蛋白的抗原，当由 MHC I 展示时，可导致 COVID-19 患者的细胞毒性 CD8+ T 细胞抗病毒反应。作者提出了一种方法，将 SARS-CoV-2 刺突蛋白转化为肽抗原-主要组织相容性复合物（pMHCs）的文库，成为包含肽抗原、MHC HLA 等位基因和 β-2 微球蛋白亚基的单链三聚体。该文库用于检测四个 COVID-19 研究参与者中的病毒特异性 T 细胞群体在感染初期的两个时间点上的进化，其中两位有同一个 HLA 等位基因，另两位有另一个 HLA 等位基因相同。HLA 匹配的参与者表现出相似的病毒特异性 T 细胞群体，但这些群体的时间轨迹非常不同。这一策略可用于跟踪感染过程中这些病毒特异性 T 细胞群体，从而深入了解在不同 COVID-19 患者中观察到的 SARS-CoV-2 免疫系统轨迹。

Abstract
We report here on antigens from the SARS-CoV-2 virus spike protein, that when presented by Class I MHC, can lead to cytotoxic CD8+ T cell anti-viral responses in COVID-19 patients. We present a method in which the SARS-CoV-2

spike protein is converted into a library of peptide antigen-Major Histocompatibility Complexes (pMHCs) as single chain trimers that contain the peptide antigen, the MHC HLA allele subunit, and the β-2 microglobulin subunit. This library is used to detect the evolution of virus-specific T cell populations in four COVID-19 study participants two of which share one HLA allele, and the other two a second HLA allele, at two time points over the initial course of infection. HLA-matched participants exhibit similar virusspecific T cell populations, but very different time-trajectories of those populations. This strategy can be used to track those virus-specific T cell populations over the course of an infection, thus providing deep insight into the SARS-CoV-2 immune system trajectories observed in different COVID-19 patients.

6. 在 COVID-19 患者中快速产生的中和抗体反应

Rapid generation of neutralizing antibody responses in COVID-19 patients

来源： medRxiv

发布时间： 2020-05-08

链接： https://www.medrxiv.org/content/10.1101/2020.05.03.20084442v1

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通讯作者： Mehul S. Suthar, Jens Wrammert

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DOI 或 PUBMED ID： Preprint

编译者： 张鹏伟

中文摘要：

SARS-CoV-2 目前正在造成毁灭性的大流行，因此迫切需要了解急性感染过程中体液免疫反应的动力学、特异性和中和潜能。文中作者报道了 44 例 COVID-19 患者中，对刺突蛋白的受体结合域（RBD）的抗体反应和病毒中和活性的动态。PCR 确诊 6 天后，所有患者均检测到 RBD 特异性 IgG 反应。用临床分离的 SARS-CoV-2，在 PCR 确诊 6 天后所有患者均检测到中和抗体滴度。RBD 特异性 IgG 结合滴度的高低与病毒中和作用密切相关。在临床环境中，对 RBD 特异性 IgG 滴度动态的初步分析，在一个更大的 PCR 确诊患者队列中得到证实（n=231）。这些发现对于了解 SARS-CoV-2 的保护性免疫、利用免疫血浆作为治疗手段以及开发急需的疫苗具有重要意义。

Abstract:

SARS-CoV-2 is currently causing a devastating pandemic and there is a pressing need to understand the dynamics, specificity, and neutralizing potency of the humoral immune response during acute infection. Herein, we report the dynamics of antibody responses to the receptor-binding domain (RBD) of the spike protein and virus neutralization activity in 44 COVID-19 patients. RBD-specific IgG responses were detectable in all patients 6 days after PCR confirmation. Using a clinical isolate of SARS-CoV-2, neutralizing antibody titers were also detectable in all patients 6 days after PCR confirmation. The magnitude of RBD-specific IgG binding titers correlated strongly with viral neutralization. In a clinical setting, the initial analysis of the dynamics of RBD-specific IgG

titers was corroborated in a larger cohort of PCR-confirmed patients (n=231). These findings have important implications for our understanding of protective immunity against SARS-CoV-2, the use of immune plasma as a therapy, and the development of much-needed vaccines.

7. 基于展示在病毒样颗粒上的受体结合域的 COVID-19 疫苗的开发

Development of a COVID-19 vaccine based on the receptor binding domain displayed on virus-like particles

来源： bioRxiv

发布时间： 2020-05-07

链接： https://www.biorxiv.org/content/10.1101/2020.05.06.079830v1

第一作者： Lisha Zha

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DOI 或 PUBMED ID： https://doi.org/10.1101/2020.05.06.079830

编译者： 刘焕珍

中文摘要：

COVID-19 的严重程度低于 SARS 和 MERS，但是具有更强的传播力，因为它在症状出现之前就已经传播，或者可能由无症状的人传播。因此，快速开发针对 COVID-19 的保护性疫苗至关重要。文中作者证明与 SARS 同源的刺突蛋白的重组表达受体结合域（RBD）可以与病毒受体 ACE2 结合。在免疫优化过的来自黄瓜花叶病毒的类病毒颗粒上高度反复表达 RBD，产生了一种候选疫苗（RBD-$CuMV_{TT}$），该疫苗能在小鼠体内诱导高水平的特异性抗体，这种抗体能够阻断刺突蛋白与 ACE2 的结合，并在体外有效中和 SARS-CoV-2 病毒。

Abstract:

The disease COVID-19 is less severe than SARS and MERS, however, the new coronavirus spreads more strongly, as it sheds long before onset of symptoms or may be transmitted by people without symptoms. Rapid development of a protective vaccine against COVID-19 is therefore of paramount importance. Here we demonstrate that recombinantly expressed receptor binding domain (RBD) of the spike protein homologous to SARS binds to ACE2, the viral receptor. Higly repetitive display of RBD on immunologically optimized virus-like particles derived from cucumber mosaic virus resulted in a vaccine candidate (RBD-$CuMV_{TT}$) that induced high levels of specific antibodies in mice which were able to block binding of spike protein to ACE2 and potently neutralized the SARS-CoV-2 virus in vitro.

8. 针对 COVID-19 疫情快速开展的老药新用研究

Rapid repurposing of drugs for COVID-19

来源： Science

发布时间： 2020-05-08

链接： https://science.sciencemag.org/content/early/2020/05/07/science.abb9332

第一作者： R. Kiplin Guy

通讯作者： R. Kiplin Guy

通讯作者单位：College of Pharmacy，University of Kentucky，Lexington，KY，USA.
DOI 或 PUBMED ID：10.1126/science.abb9332
编译者：宋珂
中文摘要：

由一种新型冠状病毒引起呼吸道疾病急需对现有药物进行老药新用研究

世界卫生组织宣布，由 SARS-CoV-2 病毒引起的 COVID-19 已经构成大流行。针对 COVID-19 患者，当前尚无直接治疗方案。利用现有的人体药理学和毒理学详细信息，对已经批准的用于其他病症的药物进行老药新用研究，快速开展临床试验和监管审查，有助于尽快发现直接治疗方法。

在 COVID-19 之前，只有 SARS-CoV-1 和 MERS-CoV 造成了严重的病症。因此，与其他病毒引起的疾病（如流感）相比，针对冠状病毒而开展的药物研发工作并不多。鉴于 COVID-19 的快速传播和相对较高的死亡率，迫切需要开发针对冠状病毒的特异性药物。

冠状病毒的生命周期涉及许多可成为潜在靶点的过程。包括：内吞进入宿主细胞[涉及血管紧张素转化酶 2（ACE2）和跨膜蛋白丝氨酸酶 2（TMPRSS2）]，RNA 复制和转录[涉及解旋酶和 RNA 依赖 RNA 聚合酶（RdRp）]，病毒蛋白（涉及胰凝乳蛋白酶和木瓜蛋白酶）的翻译和蛋白水解过程,病毒组装以及通过外泌体系统释放新病毒等。除了病毒自身编码的靶点外，许多宿主靶点对于病毒复制和疾病进程也很关键。

SARS-CoV-2 的细胞受体是 ACE2。目前正在开发的重组人 ACE2 蛋白（rhACE2 或 APN01），主要用于治疗急性肺损伤和肺动脉高压。在临床 1 期试验中，已在健康的志愿者身上验证了其耐受性。rhACE2 可以显著降低病毒侵入人体类器官细胞的速度。这一结果为开展使用 APN01 阻断病毒侵入 COVID-19 患者的临床试验提供了有力支持。病毒进入宿主细胞的过程需要 Spike 蛋白进行酶解，这需要借助 TMPRSS2 进行。在日本，TMPRSS2 抑制剂 camostat 已获准用于治疗慢性胰腺炎和术后胃反流。总体具有良好的耐受性，但也有零星报道指出，存在严重的副作用。Camostat 和相关药物 nafamostat 均会阻止 SARS-CoV-2 在有 TMPRSS2 表达的人类细胞中复制。在小鼠模型中已经发现，camostat 可以阻止 SARS-CoV-2 的感染。在荷兰和德国，已启动了这些药物用于 COVID-19 的临床试验。

在脱膜前，冠状病毒利用内溶酶体途径侵入细胞。氯喹（CQ）和羟氯喹（HCQ）是抗疟疾药物，会对细胞核内体功能产生影响，阻止自噬体与溶酶体融合。在细胞模型中，两种药物均可以抑制 SARS-CoV-2 复制。阿奇霉素（AZ）是被广泛使用的广谱抗生素，它还可以阻止人体细胞中自噬体的清除。在体外实验中，AZ 还可以阻止 Zika 病毒和流感病毒在人体细胞中的复制。

然而，HCQ 和 AZ 均有潜在的心脏毒性（QT 延长，并造成致命的心律失常）。HCQ 还可能对眼睛产生不良影响。

尽管针对 HIV 和其他病毒开发了一些针对病毒蛋白酶的抑制剂，但均对病毒蛋白酶具有较高的特异性。已有临床实验证明，HIV 蛋白酶抑制剂洛匹那韦和利托那韦的组合对 COVID-19 患者无效，如同之前针对 SARS-CoV-1 感染的情况一样。

理论上,解旋酶是一个有潜力的靶标,但 SARS-CoV-2 的解旋酶与其他病毒的解旋酶不同。而且，目前尚无证据表明，单纯性疱疹病毒解旋酶抑制剂阿美那韦或普瑞替韦对冠状病毒有效。

RdRp 同时执行病毒 RNA 的复制和转录功能，是阻断病毒生命周期的明确靶标。许多广谱 RdRp 抑制剂已被批准或正在进行临床试验，包括瑞德西韦和法匹拉韦。瑞德西韦最初被开发用于治疗引起埃博拉和马尔堡疾病的黄病毒，并在过去的两次埃博拉疫情中，证明了其安全性。瑞德西韦在动物模型中对 SARS-CoV-1 和 MERS-CoV 均表现出活性。法匹拉韦是为应对流感而开发，并于 2014 年在日本获批，专门用于预防新型流感的爆发。瑞德西韦和法匹拉

韦在体外实验中，均对人体细胞中的 SARS-CoV-2 表现出抗病毒活性。瑞德西韦已迅速进入 COVID-19 的多项临床试验。试验发布的早期非正式数据表明，瑞德西韦是有效的。

针对这些潜在治疗药物进行的临床试验中，都存在一个关键的问题：在疫情流行高峰期间，针对个体患者给出治疗方案，与涉及少量样本的临床研究中，快速开展精心设计的随机临床试验，并证明方案的安全有效性，需要在两者对立的需求之间的寻找平衡点。

Abstract:

The emergence of a new coronaviral respiratory disease calls for repurposing existing drugs

9. SARS-CoV-2 的细胞进入机制

Cell entry mechanisms of SARS-CoV-2

来源： PNAS

发布时间： 2020.05.06

文章链接： https://www.pnas.org/content/early/2020/05/05/2003138117

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DOI： https://doi.org/10.1073/pnas.2003138117

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中文摘要：

了解 SARS-CoV-2 是如何进入人体细胞的是遏制其传播的一项重要任务。病毒表面刺突蛋白介导 SARS-CoV-2 进入细胞。SARS-CoV-2 刺突蛋白通过其受体结合域(RBD)与其受体人 ACE2（hACE2）结合，并被人类蛋白酶蛋白水解激活。作者使用生化方法和假病毒侵入试验，研究了受体结合和蛋白酶活化的 SARS-CoV-2 刺突蛋白。他们的发现确定了 SARS-CoV-2 进入细胞的关键机制。首先，SARS-CoV-2 RBD 比 SARS-CoV RBD 具有更高的 hACE2 结合亲和力，更利于它进入细胞。第二，矛盾的是，完整的 SARS-CoV-2 刺突蛋白与 hACE2 的结合亲和力，比 SARS-CoV 刺突蛋白的结合力要低，这表明尽管 SARS-CoV-2 RBD 结合力更强，但却比 SARS-CoV RBD 暴露得更少。第三，与 SARS-CoV 不同的是，SARS-CoV-2 进入细胞会被前体蛋白转化酶弗林蛋白酶预先激活，减少其对靶细胞蛋白酶的依赖。RBD 与 hACE2 的高亲和力、弗林蛋白酶对刺突蛋白的预激活和隐藏在刺突蛋白中的 RBD 可能使 SARS-CoV-2 逃避免疫监视，有效地进入细胞。这些特征可能有助于病毒的广泛传播。成功的干预策略必须同时针对 SARS-CoV-2 的效力和它的隐蔽性。

Abstract

Understanding how SARS-CoV-2 enters human cells is a high priority for deciphering its mystery and curbing its spread. A virus surface spike protein mediates SARS-CoV-2 entry into cells. To fulfill its function, SARS-CoV-2 spike binds to its receptor human ACE2 (hACE2) through its receptor-binding domain (RBD) and is proteolytically activated by human proteases. Here we investigated receptor binding and protease activation of SARS-CoV-2 spike using biochemical and pseudovirus entry assays. Our findings have identified key cell entry mechanisms of SARS-CoV-2. First, SARS-CoV-2 RBD has higher hACE2 binding affinity than SARS-CoV RBD, supporting efficient cell entry. Second, paradoxically, the hACE2 binding affinity of the entire SARS-CoV-2 spike is comparable to or lower than that of SARS-CoV spike, suggesting that SARS-CoV-2

RBD, albeit more potent, is less exposed than SARS-CoV RBD. Third, unlike SARS-CoV, cell entry of SARS-CoV-2 is preactivated by proprotein convertase furin, reducing its dependence on target cell proteases for entry. The high hACE2 binding affinity of the RBD, furin preactivation of the spike, and hidden RBD in the spike potentially allow SARS-CoV-2 to maintain efficient cell entry while evading immune surveillance. These features may contribute to the wide spread of the virus. Successful intervention strategies must target both the potency of SARS-CoV-2 and its evasiveness.

10. SARS-CoV-2 在人的呼吸道和结膜中的细胞偏好性，复制能力以及引起的固有免疫反应：一项采用离体以及体外培养体系的研究

Tropism, replication competence, and innate immune responses of the coronavirus SARS-CoV-2 in human respiratory tract and conjunctiva: an analysis in ex-vivo and in-vitro cultures

来源：Lancet

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链接：https://www.thelancet.com/journals/lanres/article/PIIS2213-2600(20)30193-4/fulltext

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中文摘要：

我们对 SARS-CoV-2 的传染性以及致病机制仍然知之甚少。该研究比较了 SARS-CoV-2 和其他冠状病毒以及流感病毒在呼吸道、结膜里的的组织和细胞偏好性以及所引起的固有免疫反应。

研究者们在离体培养的人支气管（n=5）和肺(n=4)中比较了从病人分离的 SARS-CoV-2，引起中东呼吸症 MERS-CoV，以及 2009 大流行的流感 H1N1pdm 的组织细胞偏好性以及复制能力。研究者们用离体培养的人结膜(n=3）以及体外培养的人结肠癌细胞系来评估了肺外感染。在肺泡上皮细胞以及巨噬细胞里面研究了固有免疫和 ACE2 的表达。在体外研究体系中用高致病性的禽流感 H5N1 以及空白对照感染做为对照。

SARS-CoV-2 感染支气管中有纤毛的、分泌粘液细胞以及棒状细胞，以及 1 型肺细胞，结膜的黏膜。在支气管里，SARS-CoV-2 的复制效率和 MERES-CoV 相似，比 SARS-CoV 高，但是低于 H1N1pdm.在肺部，SARS-CoV-2 的复制效率和 SARS-CoV 以及 H1N1pdm 相似，但是低于 MERS-CoV.在结膜，SARS-CoV-2 的复制效率高于 SARS-CoV。SARS-CoV2 诱导炎性细胞因子的能力弱于 H5N1, H1N1pdm, 以及 MERS-CoV。

结膜上皮以及气管看上去像是 SARS-CoV-2 的潜在的感染路径。SARS-CoV 和 SARS-CoV-2 在肺部上皮细胞里复制效率相似，而 SASR-CoV-2 在支气管里的复制能力强于 SARS-CoV.这些发现对了解 SARS-CoV-2 的传染以及病理学以及和其他冠状病毒的差异提供了重要线索。

Abstract:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in December 2019, causing a respiratory disease (coronavirus disease 2019, COVID-19) of varying severity in Wuhan, China, and subsequently leading to a pandemic. The transmissibility and pathogenesis of SARS-CoV-2 remain poorly

understood. We evaluate its tissue and cellular tropism in human respiratory tract, conjunctiva, and innate immune responses in comparison with other coronavirus and influenza virus to provide insights into COVID-19 pathogenesis. We isolated SARS-CoV-2 from a patient with confirmed COVID-19, and compared virus tropism and replication competence with SARS-CoV, Middle East respiratory syndrome-associated coronavirus (MERS-CoV), and 2009 pandemic influenza H1N1 (H1N1pdm) in ex-vivo cultures of human bronchus (n=5) and lung (n=4). We assessed extrapulmonary infection using ex-vivo cultures of human conjunctiva (n=3) and in-vitro cultures of human colorectal adenocarcinoma cell lines. Innate immune responses and angiotensin-converting enzyme 2 expression were investigated in human alveolar epithelial cells and macrophages. In-vitro studies included the highly pathogenic avian influenza H5N1 virus (H5N1) and mock-infected cells as controls.
SARS-CoV-2 infected ciliated, mucus-secreting, and club cells of bronchial epithelium, type 1 pneumocytes in the lung, and the conjunctival mucosa. In the bronchus, SARS-CoV-2 replication competence was similar to MERS-CoV, and higher than SARS-CoV, but lower than H1N1pdm. In the lung, SARS-CoV-2 replication was similar to SARS-CoV and H1N1pdm, but was lower than MERS-CoV. In conjunctiva, SARS-CoV-2 replication was greater than SARS-CoV. SARS-CoV-2 was a less potent inducer of proinflammatory cytokines than H5N1, H1N1pdm, or MERS-CoV.
The conjunctival epithelium and conducting airways appear to be potential portals of infection for SARS-CoV-2. Both SARS-CoV and SARS-CoV-2 replicated similarly in the alveolar epithelium; SARS-CoV-2 replicated more extensively in the bronchus than SARS-CoV. These findings provide important insights into the transmissibility and pathogenesis of SARS-CoV-2 infection and differences with other respiratory pathogens.

11. SARS-CoV-2 感染人支气管上皮细胞的单细胞时间序列分析

Single-cell longitudinal analysis of SARS-CoV-2 infection in human bronchial epithelial cells

来源： biorxiv

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中文摘要：

SARS-CoV-2 是 COVID-19 的病原体，目前已造成 300 多万人感染，20 万人死亡。目前还没有用于治疗或预防 COVID-19 的批准药物或疫苗。加强对 SARS-CoV-2 感染及发病机制的认识，是治疗学发展的关键。为了揭示 SARS-CoV-2 的病毒复制、细胞倾向性和宿主病毒相互作用，该研究对实验感染的人支气管上皮细胞（HBECs）进行了一段时间的气液界面培养，并进行了单细胞 RNA 测序。单细胞 RNA 测序揭示了新的多聚腺苷酸病毒转录物，并强调纤毛细胞是

感染的主要目标，并通过电子显微镜证实了这一点。在感染过程中，SARS-CoV-2 的细胞向性扩展到其他上皮细胞类型，包括基底细胞和棒状细胞。感染诱导细胞内 I 型和 III 型 IFNs 和 IL6 的表达，而不是 IL1 的表达。这导致干扰素刺激基因在感染细胞和旁观者细胞中的表达。该文章对 HBECs 中的 SARS-CoV-2 感染进行了深入分析，并提供了与感染相关的基因、细胞类型和细胞状态变化的详细特征。

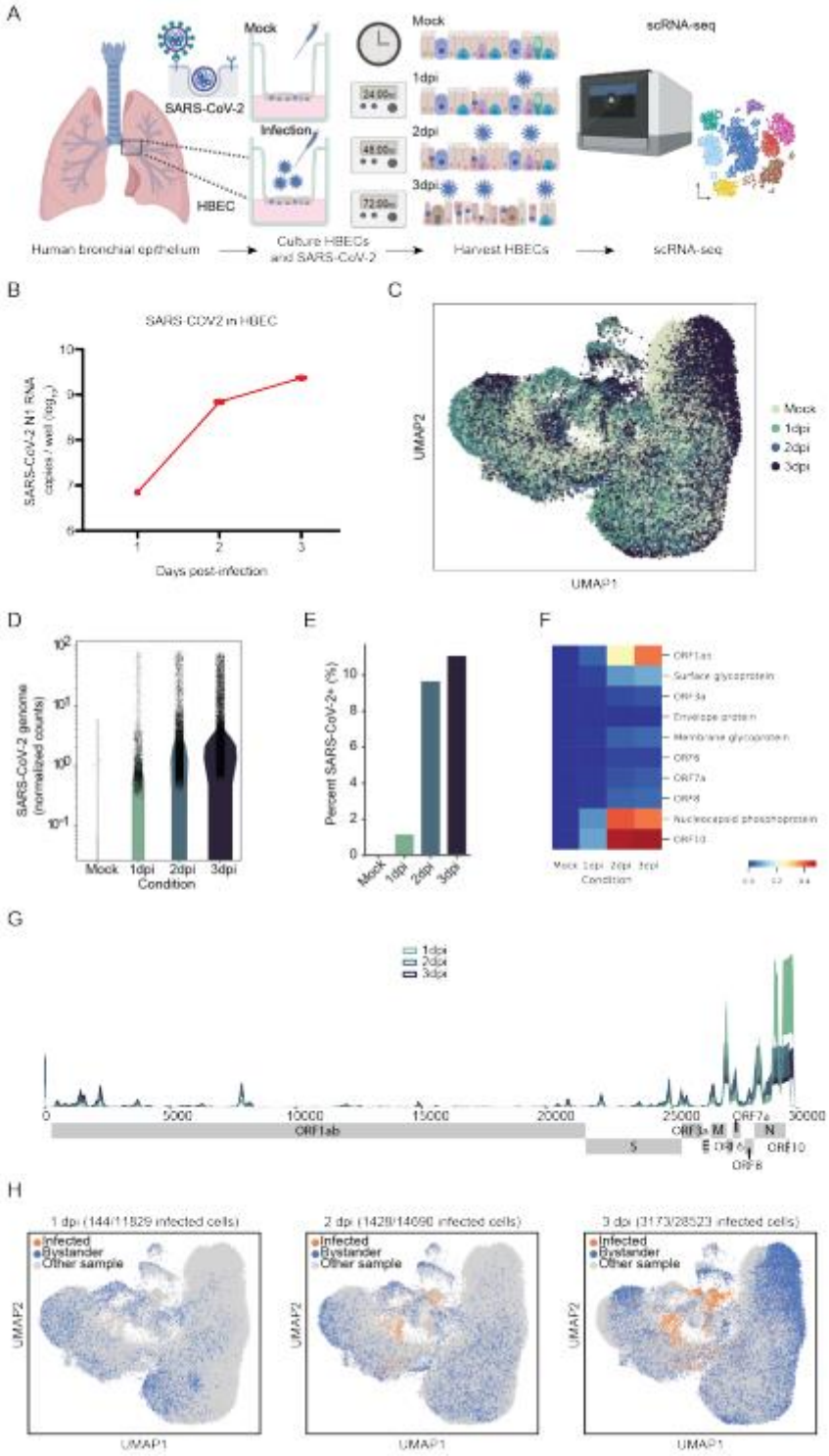

Figure 1: scRNA-seq reveals SARS-CoV-2 infection of HBECs. A. Schematic of the experiment. Human bronchial epithelial cells (HBECs) were cultured and infected

or not (mock) with SARS-CoV-2. Infected cultures were collected for scRNA-seq at 1, 2 and 3 days post infection (dpi). B. RT-qPCR in cultured HBEC to detect viral transcripts at each dpi (copies/well). C. UMAP visualization of the scRNA-seq gene counts after batch correction. Each point represents a cell, colored by sample. D. Normalized counts of viral counts in each condition. For each cell, viral counts were determined by aligning reads to a single, genome-wide reference. E. Percent of cells infected by SARS-CoV-2, based on a viral genes count threshold (see Materials and Methods) F. Normalized heatmap of the viral Open Reading Frame (ORF) counts in each condition. Reads were aligned to each 10 SARS-CoV-2 ORFs. G. Coverage plot of viral reads aligned to SARS-CoV-2 genome. The sequencing depth was computed for each genomic position for each condition. As infection progresses, coverage becomes more dispersed on the genome. H. UMAP visualizations of infected and bystander cells in each condition after batch correction. Bystander cells are defined as cells that remained uninfected in infected HBEC samples.

Abstract:
SARS-CoV-2, the causative agent of COVID-19, has resulted in more than 3,000,000 infections and 200,000 deaths. There are currently no approved drugs or vaccines for the treatment or prevention of COVID-19. Enhanced understanding of SARS-CoV-2 infection and pathogenesis is critical for the development of therapeutics. To reveal insight into viral replication, cell tropism, and host-viral interactions of SARS-CoV-2 we performed single-cell RNA sequencing of experimentally infected human bronchial epithelial cells (HBECs) in air-liquid interface cultures over a time-course. This revealed novel polyadenylated viral transcripts and highlighted ciliated cells as the major target of infection, which we confirmed by electron microscopy. Over the course of infection, cell tropism of SARS-CoV-2 expands to other epithelial cell types including basal and club cells. Infection induces cell intrinsic expression of type I and type III IFNs and IL6 but not IL1. This results in expression of interferon stimulated genes in both infected and bystander cells. Here, we have conducted an in depth analysis of SARS-CoV-2 infection in HBECs and provide a detailed characterization of genes, cell types, and cell state changes associated with the infection.

12. 通过混合测序以及单细胞测序分析感染SARS-CoV-2的人细胞系的基因表达谱鉴定出新药物靶点

Bulk and single-cell gene expression profiling of SARS-CoV-2 infected human cell lines identifies molecular targets for therapeutic intervention

来源：biorxiv

发布时间：2020-05-05

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中文摘要

作者们用混合测序以及单细胞测序对 SARS-CoV 和 SARS-CoV-2 感染 3 种细胞系（ H1299,Calu-3 为肺癌细胞，Caco-2 为结肠癌细胞 ）的基因表达谱进行了研究。数据显示和免疫以及炎症相关的 microRNA miRNA-155 在细胞感染病毒后都有很强的表达。
在易感的 Calu-3 细胞系中，SARS-CoV-2 相比 SARS-CoV 激发的干扰素反应高约 2 倍，诱导的诸如 CXCL10 和 IL6 细胞因子的水平也更高。单细胞测序结果显示典型的干扰素激活的基因比如 IFIT2 以及 OAS2 都被广泛地发生了诱导，而 beta 和 labmda 干扰素只在一部分被感染的细胞中表达。转录反应的时序分析可以发现干扰素调控因子的活性比 NF-κB 早。作者鉴定出热休克蛋白 HSP90 可能和感染相关。用化学分析 17-AAG 抑制 HSP90 的伴侣分子活性会降低病毒的复制，以及 TNF 和 IL1B mRNA 的水平。作者认为 HSP90 可能是治疗 SARS-CoV-2 感染的潜在药靶。

Abstract

The coronavirus disease 2019 (COVID-19) pandemic, caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is an ongoing global health threat with more than two million infected people since its emergence in late 2019. Detailed knowledge of the molecular biology of the infection is indispensable for understanding of the viral replication, host responses, and disease progression. We provide gene expression profiles of SARS-CoV and SARS-CoV-2 infections in three human cell lines (H1299, Caco-2 and Calu-3 cells), using bulk and single-cell transcriptomics. Small RNA profiling showed strong expression of the immunity and inflammation-associated microRNA miRNA-155 upon infection with both viruses. SARS-CoV-2 elicited approximately two-fold higher stimulation of the interferon response compared to SARS-CoV in the permissive human epithelial cell line Calu-3, and induction of cytokines such as CXCL10 or IL6. Single cell RNA sequencing data showed that canonical interferon stimulated genes such as IFIT2 or OAS2 were broadly induced, whereas interferon beta (IFNB1) and lambda (IFNL1-4) were expressed only in a subset of infected cells. In addition, temporal resolution of transcriptional responses suggested interferon regulatory factors (IRFs) activities precede that of nuclear factor-κB (NF-κB).Lastly, we identified heat shock protein 90 (HSP90) as a protein relevant for the infection. Inhibition of the HSP90 charperone activity by Tanespimycin/17-N-allylamino-17-demethoxygeldanamycin (17-AAG) resulted in a reduction of viral replication, and of TNF and IL1B mRNA levels. In summary, our study established in vitro cell culture models to study SARS-CoV-2 infection and identified HSP90 protein as potential drug target for therapeutic intervention of SARS-CoV-2 infection.

13. COVID-19 患者的外周血转录的时间序列分析捕捉疾病进展并揭示潜在的生物标志物

Longitudinal peripheral blood transcriptional analysis of COVID-19 patients captures disease progression and reveals potential biomarkers

来源：medRxiv

发布时间：2020-05-08
链接：https://www.medrxiv.org/content/10.1101/2020.05.05.20091355v1
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中文摘要：
COVID-19 是由 SARS-CoV-2 引起的，它在大多数患者中是一种急性自溶性疾病，但也有部分患者会发展成重症甚至死亡。为了研究疾病进展过程中宿主的反应和识别潜在的生物标记物，研究人员对 4 例 COVID-19 患者从症状开始到恢复的 4 个不同时间点采集的外周血单核细胞（PBMCs）进行了转录组时间序列分析。研究发现不同 COVID-19 疾病阶段的 PBMCs 具有独特的转录组特征。SARS-CoV-2 感染可引起机体先天免疫功能失调，尤其是 I 型干扰素应答，以及炎性细胞因子和脂质介质释放紊乱，低密度中性粒细胞异常增多，可能引起组织损伤。细胞死亡、衰竭和迁移途径的激活可能导致淋巴细胞减少和适应性免疫功能紊乱。COVID-19 引起的缺氧可加重凝血障碍。基于本研究的分析，作者提出了一套用于监测疾病进展和预测严重性风险的潜在生物标志物。

Abstract:
COVID-19, caused by SARS-CoV-2, is an acute self-resolving disease in most of the patients, but some patients can develop a severe illness or even death. To characterize the host responses and identify potential biomarkers during disease progression, we performed a longitudinal transcriptome analysis for peripheral blood mononuclear cells (PBMCs) collected from 4 COVID-19 patients at 4 different time points from symptom onset to recovery. We found that PBMCs at different COVID-19 disease stages exhibited unique transcriptome characteristics. SARS-CoV-2 infection dysregulated innate immunity especially type I interferon response as well as the disturbed release of inflammatory cytokines and lipid mediators, and an aberrant increase of low-density neutrophils may cause tissue damage. Activation of cell death, exhaustion and migratory pathways may lead to the reduction of lymphocytes and dysfunction of adaptive immunity. COVID-19 induced hypoxia may exacerbate disorders in blood coagulation. Based on our analysis, we proposed a set of potential biomarkers for monitoring disease progression and predicting the risk of severity.

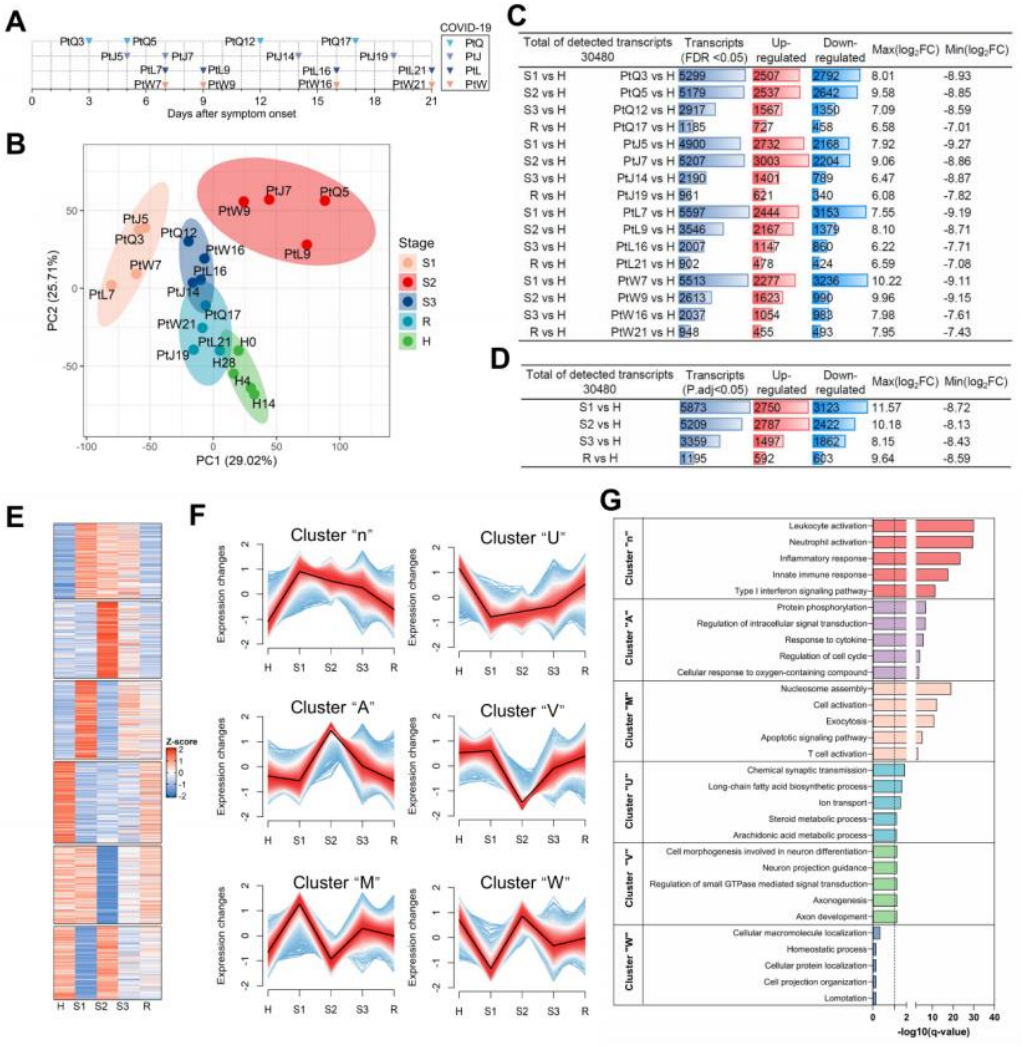

Fig. 1. Global transcriptional analysis across COVID-19 patients and health donor samples. **(A)** Time points of blood sample collection from 4 COVID-19 patients. **(B)** Principal component analysis in COVID-19 patients and health donor samples, depicting the variation in the global gene expression profiles across different stages (S1, S2, S3, and R) and healthy control (H). Principal components 1 (PC1) and 2 (PC2), which represent the greatest variation in gene expression, are shown. **(C)** Paired comparison between S1 and R, S2 and R, S3 and R for each COVID-19 patient. The numbers of up-regulated and down-regulated gene are listed. **(D)** Grouped comparison between S1 and R, S2 and R, S3 and R. Samples from different patients in the same stage were combined and compared with samples from the convalescent stage. The numbers of up-regulated and down-regulated gene are listed. **(E)** All DEGs were grouped into six clusters according to their expression pattern. Heatmap showing the relative expression of individual transcript at different disease stages. **(F)** The relative expression changes of each cluster are shown. Each line in the plots presents a unique DEG and the black line indicates the median. **(G)** Gene ontology (Go) analysis for each cluster. Top 5 Go terms enriched in each cluster are shown. The dotted line indicates the threshold value (q-value = 0.05) of significantly enriched.

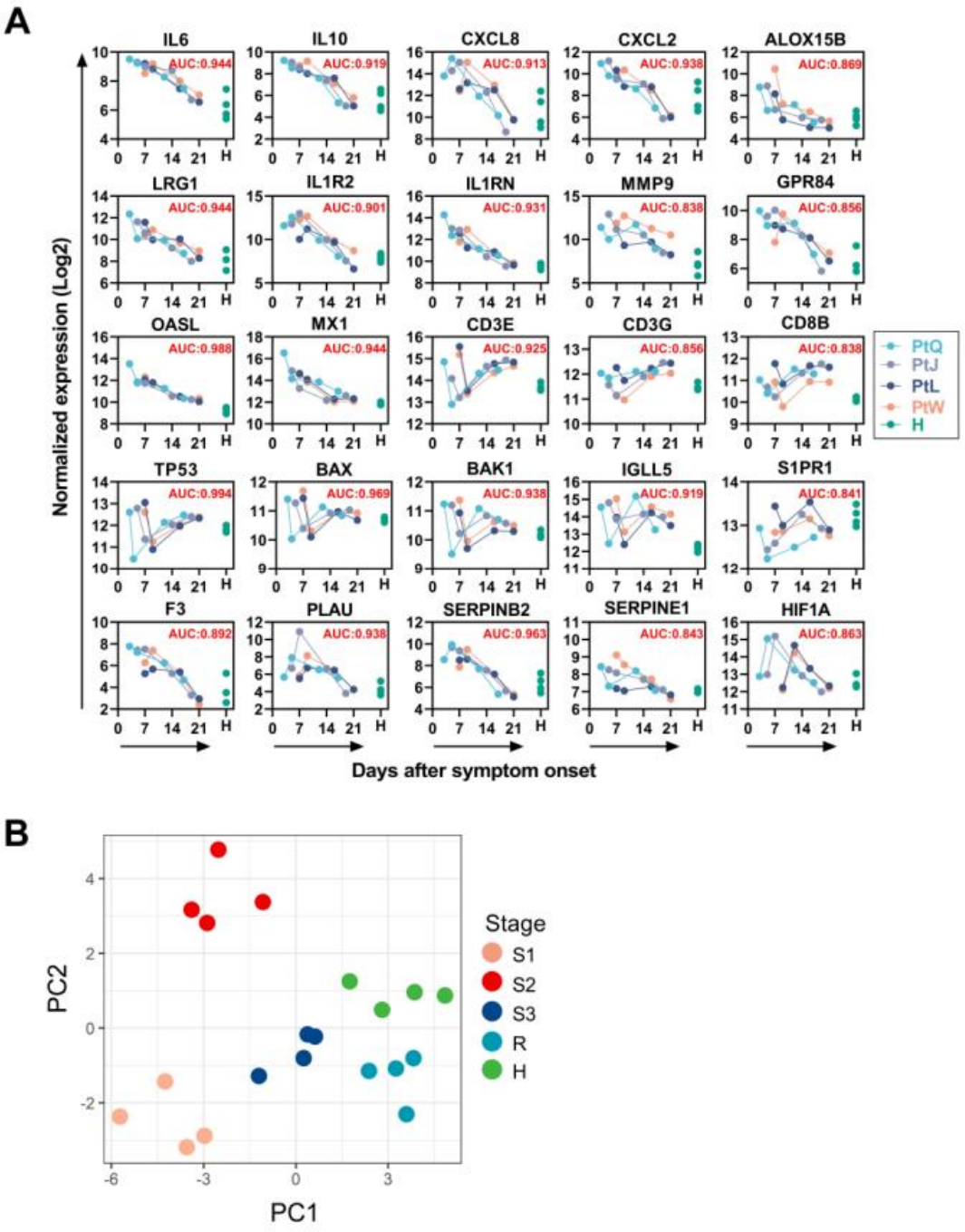

Fig. S7. Potential biomarkers that predict outcomes of COVID-19. **(A)** Normalized log2 expression and AUC values of 25 candidate biomarkers for monitoring disease progression. X axis denotes the days from onset on which the corresponding sample was collected. Y axis denotes log2 normalized gene expression. AUC values of individual candidate biomarker are labeled on the top left corner of each panel. **(B)** Sample scores from probabilistic principal components analysis using the 25 candidate biomarkers shown in Fig. S7A.