新型冠状病毒信息简报

第 39 期（2020 年 4 月 26 日报）

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

联系人：蒋立春 jianglch@shanghaitech.edu.cn

内容介绍

分类	标题名称
疫情播报	1. 2020 年 4 月 25 日疫情
疾病检测	2. SARS-CoV-2 酶联免疫吸附法用于接触调查和血清学检测的方法验证
疾病病理	3. 中国西部地区儿童感染 SARS-CoV-2 的随访研究
疾病治疗	4. Mesoblast 公司的干细胞治疗在需要呼吸机的 COVID-19 病人中初现疗效
药物研发	5. 重组 ACE2-Ig 中和 SARS-CoV-2 刺突蛋白假病毒
基础研究	6. SARS-CoV-2 突变的基因组、地理和时间分布 7. 冠状病毒感染人细胞的转录调控图谱 8. 冠状病毒通过校对核糖核酸外切酶介导广泛的病毒重组
疾病模型	9. III 型干扰素在控制原代人肠上皮细胞中 SARS-CoV-2 感染、复制、传播的关键作用

1. 2020 年 4 月 25 日疫情

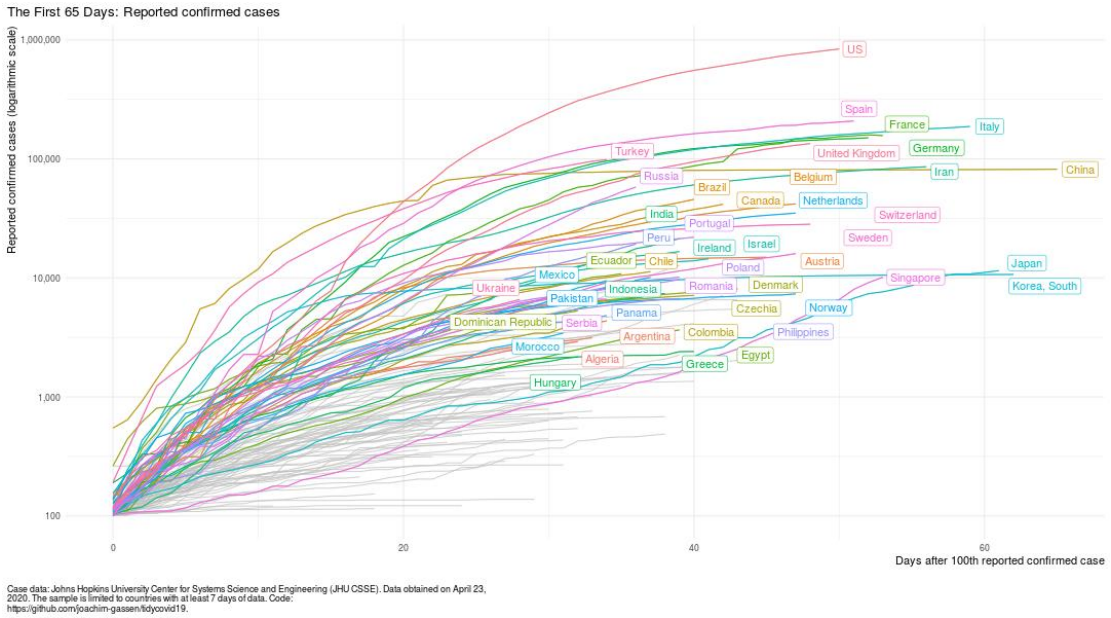
数据来源：WHO

发布时间：2020 年 4 月 25 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 4 月 25 日全球累计确诊新型冠状病毒病人 2719897 例，当日新增确诊 93716 例，累计死亡 187705 例，当日新增死亡 5767。

中国累计确诊 84325 例，累计死亡 4642 例，当日新增确诊 14 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 4 月 25 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（4 月 25 日，来源：http://2019ncov.chinacdc.cn/2019-nCoV/）

2. SARS-CoV-2 酶联免疫吸附法用于接触调查和血清学检测的方法验证

Validation of a SARS-CoV-2 spike ELISA for use in contact investigations and serosurveillance

来源：bioRxiv

发布时间：2020-04-24

链接：https://www.biorxiv.org/content/10.1101/2020.04.24.057323v1

第一作者：Brandi Freeman

通讯作者：Natalie J. Thornburg

通讯作者单位：佐治亚州亚特兰大疾病控制和预防中心

DOI 或 PUBMED ID：Preprint

编译者：孔娟

中文摘要：

研究者对基于 SARS-CoV-2 S 蛋白的 ELISA 相关参数进行了优化，并进行了大规模的验证，使检测的特异性和敏感性达到最大化。研究中包括 519 名对照血清，其中健康者血清（n=377）、疑似汉塔病毒患者（n=101）、艾滋病毒（n=21）、乙型肝炎病毒（n=10）或丙型肝炎病毒阳性（n=10）。COVID-19 患者（n=99）症状出现后第 10 天或之后采集的恢复期血清，同时收集来自常见冠状病毒（SARS-1 n=4、229E n=3、NL63 n=8、OC43 n=17 和 HKU1 n=14）感染患者的急性和恢复期成对血清对特异性进行检测。具体而言，研究者对抗原包被浓度，血清稀释比例及检测的二抗进行了优化，分别采用三种二抗：抗人广谱 Ig（anti-human pan Ig），抗人 IgG，抗人 IgM 进行检测。结果显示，0.15μg/ml 抗原包被浓度，血清稀释比例 1:100 的条件下抗人广谱 Ig 特异性为 99.3%（置信区间 98.32-99.88%），敏感性为 96%（置信区间 89.98-98.89%）优于其它两种抗体，抗人 IgG 敏感性 94.74%（置信区间 85.38-98.9%），抗人 IgM 敏感性 76.19%（置信区间 63.79-86.02%）。利用抗人广谱 Ig 检测结果显示 SARS-CoV-2 与 SARS1 和 MERS-CoV 存在交叉反应，但对其它常见病毒的交叉反应较弱。

Abstract

Since emergence of SARS-CoV-2 in late 2019, there has been a critical need to understand prevalence, transmission patterns, to calculate the burden of disease and case fatality rates. Molecular diagnostics, the gold standard for identifying viremic cases, are not ideal for determining true case counts and rates of asymptomatic infection. Serological detection of SARS-CoV-2 specific antibodies can contribute to filling these knowledge gaps. In this study, we describe optimization and validation of a SARS-CoV-2-specific-enzyme linked immunosorbent assay (ELISA) using the prefusion-stabilized form of the spike protein [1]. We performed receiver operator characteristic (ROC) analyses to define the specificities and sensitivities of the optimized assay and examined cross reactivity with immune sera from persons confirmed to have had infections with other coronaviruses. These assays will be used to perform contact investigations and to conduct large-scale, cross sectional surveillance to define disease burden in the population.

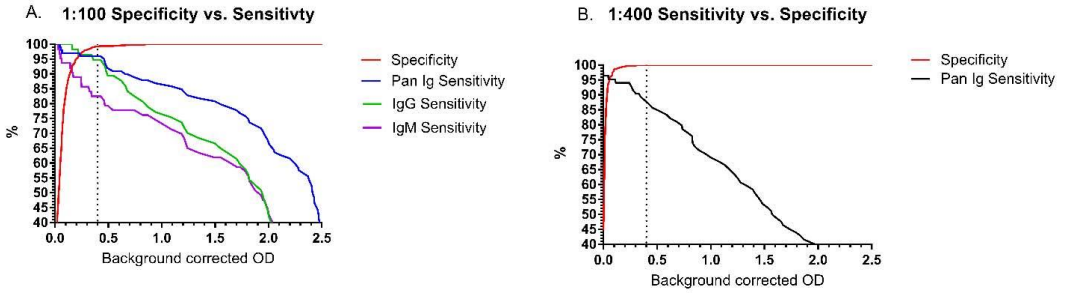


Figure 1. ROC analysis of 519 true negative and 99 true positive sera. A. Specificity was calculated at a 1:100 dilution using pan-Ig secondary (red). Sensitivities were calculated with sera diluted to 1:100 using anti-Pan Ig (blue), anti-IgG (green) or anti-IgM (purple) secondary antibodies. B. Results were analyzed with sera diluted to 1:400 using anti-Pan Ig secondary antibody.

3. 中国西部地区儿童感染 SARS-CoV-2 的随访研究

A follow-up study of children infected with SARS-CoV-2 from Western China

来源：medRxiv

发布时间：2020-04-24

链接：https://www.medrxiv.org/content/10.1101/2020.04.20.20073288v1

第一作者：Hongmei Xu1, Enmei Liu1

通讯作者：陈耀龙 2, Gary Wong3, 李秋 1

通讯作者单位：1 重庆医科大学附属儿童医院，2 兰州大学，3 香港中文大学

DOI 或 PUBMED ID：https://doi.org/10.1101/2020.04.20.20073288

编译者：宋张悦

中文摘要：

背景：为了阐明感染严重急性呼吸系统综合征冠状病毒 2 型(SARS-CoV-2)的儿童，包括无症状儿童的特点，以及核酸检测阳性的持续时间。

方法：本研究是一项多中心回顾性研究，选取 2020 年 1 月 24 日至 2 月 12 日在中国西部 4 个省（重庆市、贵州省、陕西省和四川省）的几家定点医院确诊的 32 例 SARS-CoV-2 感染的年龄小于 18 岁的患儿作为研究对象，随访至出院并隔离 14 天后。研究人员从每个定点医院的医院信息系统(HIS)和实验室信息系统(LIS)获取 COVID-19 确诊患儿的电子病历，进行数据统计分析。

结果：11 名儿童(34%)无症状，其中 6 名儿童 CT 扫描图像正常。年龄和性别与感染 SARS-CoV-2 患儿的临床症状或 CT 扫描结果无关。无症状感染儿童的白细胞和中性粒细胞浓度高于有临床症状或 CT 异常的儿童。CT 异常患者的 D-二聚体或总胆红素低于 CT 扫描正常但有临床症状者。所有患儿最终都康复了，没有人死亡，也没有人被送入儿童重症监护病房(PICU)。SARS-CoV-2 核酸检测阳性的平均持续时间为 15.4 (SD=7.2)天，无症状儿童和有症状或 CT 异常儿童的情况相似。研究人员们还发现淋巴细胞计数与核酸检测阳性的持续时间呈显著负相关。

结论：儿童感染 SARS-CoV-2 的临床特征、放射学和实验室检查结果以及临床结局与成人有本质的不同。无症状感染的儿童应与有症状的 SARS-CoV-2 感染患者隔离相同的时间。淋巴细胞数量与 SARS-CoV-2 阳性的持续时间呈负相关，这背后的临床意义及机制有待进一步研究。

Abstract:
Background: To clarify the characteristic and the duration of positive nucleic acid in children infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), including asymptomatic children. Methods: A total of 32 children confirmed with SARS-CoV-2 infection between January 24 and February 12, 2020 from four provinces in Western China were enrolled in this study and followed up until discharge and quarantine 14 days later. Results: Eleven children (34%) were asymptomatic, among whom six children had normal computed tomographic (CT) scan images. Age and gender were not associated with clinical symptoms or the results of CT scan in children infected with SARS-CoV-2. The concentrations of white blood cells and neutrophils were higher in children with asymptomatic infection than in children with clinical symptoms or CT abnormalities. Patients who presented with CT abnormalities had lower D-dimer or lower total bilirubin than those who had normal CT scan but clinical symptoms. All children recovered and no one died or was admitted to the pediatric intensive care unit (PICU). The mean duration of positive SARS-CoV-2 nucleic acid was 15.4 (SD=7.2) days and similar for both asymptomatic children and children with symptoms or CT abnormalities. We found a significant negative correlation between the lymphocyte count and the duration of positive nucleic acid test. Conclusions: Children with asymptomatic infection should be quarantined for the same duration as symptomatic patients infected with SARS-CoV-2. The clinical significance and mechanism behind the negative correlation between the number of lymphocytes and the duration of positive SARS-CoV-2 needs further study.

4. Mesoblast 公司的干细胞治疗在需要呼吸机的 COVID-19 病人中初现疗效

Mesoblast’s Stem Cell Therapy Shows 83% Survival in Ventilator-Dependent COVID-19 Patients

来源：biospace

发布时间：2020-04-25

链接：https://www.biospace.com/article/mesoblast-ltd-s-stem-cell-therapy-shows-83-percent-survival-in-covid-19-patients/

编译者：蒋立春

中文摘要：

经过 Mesoblast 公司的同种异体间充质干细胞候选物 Ryoncil (remestemcel-L)治疗后，12 个需要呼吸机的病人里面有 9 个（75%）在 10 天之内脱离了呼吸机。其中的 7 个病人已经出院。在使用间充质干细胞候选物 remestemcel-L 之前，所有病人都接受了其他实验性治疗。

同时期同在纽约的一个主要转诊医院的所有 445 个上呼吸机的 COVID-19 病人，只有 9%也就是 38 个病人脱离了呼吸机。纽约市的第二个主要转诊医院中上呼吸机的 COVID-19 病人的存活率也只有 12%。

基于这些,Mesoblast 公司认为他们的间充质干细胞候选物 remestemcel-L 可能在 COVID-19 引起的急性呼吸窘迫症中起到很强的抗炎作用，避免了细胞因子风暴的发生。该公司正在加紧完成一项在 COVID-19 急性呼吸窘迫症病人中开展的随机安慰剂对照的 Phase II/III 临床试验以充分证明 remestemcel-L 可以提高危重病人的生存率。

FDA 在 4 月 5 日批准了 remestemcel-L 用于 COVID-19 急性呼吸窘迫症的新药申请。

5. 重组 ACE2-Ig 中和 SARS-CoV-2 刺突蛋白假病毒

Neutralization of SARS-CoV-2 spike pseudotyped virus by recombinant ACE2-Ig
来源：NATURE COMMUNICATIONS
发布时间：2020-04-24
链接：https://www.nature.com/articles/s41467-020-16048-4
第一作者：雷长海
通讯作者：胡适
通讯作者单位：第二军医大学基础医学院生物物理系，Pharchoice Therapeutics, Inc
DOI 或 PUBMED ID：https://doi.org/10.1038/s41467-020-16048-4
编译者：刘焕珍
中文摘要：
针对 SARS-CoV-2，目前尚无特定的抗病毒治疗或疫苗可用。SARS-CoV-2 已被报道与 SARS-CoV 使用相同的受体进入细胞，这种受体是血管紧张素转化酶 2（ACE2）。在此报告中，作者通过将人 ACE2 的胞外域连接到人免疫球蛋白 IgG1 的 Fc 区来生成重组蛋白。在这项研究中还使用了具有低催化活性的 ACE2 突变体的融合蛋白。这两种融合蛋白对 SARS-CoV 和 SARS-CoV-2 的受体结合域具有高结合亲和力，并且在小鼠模型中表现出理想的药理特性。此外，这两种融合蛋白在体外可中和病毒（用 SARS-CoV 或 SARS-CoV-2 刺突蛋白假型化的病毒）。由于这些融合蛋白对冠状病毒表现出交叉反应性，因此它们在 SARS-CoV-2 的诊断，预防和治疗中具有潜在的应用。
Abstract:
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan, China, at the end of 2019, and there are currently no specific antiviral treatments or vaccines available. SARS-CoV-2 has been shown to use the same cell entry receptor as SARS-CoV, angiotensin-converting enzyme 2 (ACE2). In this report, we generate a recombinant protein by connecting the extracellular domain of human ACE2 to the Fc region of the human immunoglobulin IgG1. A fusion protein containing an ACE2 mutant with low catalytic activity is also used in this study. The fusion proteins are then characterized. Both fusion proteins have a high binding affinity for the receptor-binding domains of SARS-CoV and SARS-CoV-2 and exhibit desirable pharmacological properties in mice. Moreover, the fusion proteins neutralize virus pseudotyped with SARS-CoV or SARS-CoV-2 spike proteins in vitro. As these fusion proteins exhibit cross-reactivity against coronaviruses, they have potential applications in the diagnosis, prophylaxis, and treatment of SARS-CoV-2.

6. SARS-CoV-2 突变的基因组、地理和时间分布

Genomic, geographic and temporal distributions of SARS-CoV-2 mutations
来源：biorxiv
发布时间：2020-04-24
链接：https://www.biorxiv.org/content/10.1101/2020.04.22.055863v1
第一作者：Hsin-Chou Yang
通讯作者：Hsin-Chou Yang, James C. Liao

通讯作者单位：台湾中央研究院
DOI 或 PUBMED ID：
编译者：王玮
中文摘要：

COVID-19 大流行是近年来最重要的公共卫生问题。其病原体 SARS-CoV-2 自 2019 年 12 月首次出现以来，进化迅速。病毒基因组的突变对病毒株适应周围环境具有重要影响，并可能改变病毒的传播特性、疾病表现以及治疗和疫苗接种的效果。该研究利用 1932 个 SARS-CoV-2 基因组的全序列（基因组数据来自 GISAID, NCBI Genbank 和 CNCB），分析了 SARS-CoV-2 的流行时间、新突变和高频突变在基因组中、地理和时间上的分布，确定了 6 个系统发育簇，这 6 个系统发育簇在不同大陆上表现出地理偏好。6 个系统发育簇为：欧洲-1，大洋洲/亚洲，美洲，欧洲-2 ，亚洲-1，亚洲-2（图一）。该研究使用了在中国分离的菌株作为参考基因组，在所研究的 1932 株 SARS-CoV-2 病毒中分析发现欧洲和美洲每个样本的平均变异数远高于亚洲。14 个单核苷酸变异（SNV）形式的突变为这六个系统发育簇提供了直接的解释。连锁不平衡、单倍型结构、进化过程、突变的全球分布揭示了突变史的概貌。此外，该研究发现平均突变数与病死率呈正相关，并且这种相关性随着时间的推移而增强，这表明 SNVs 对疾病结局有重要作用。这项研究表明，SNV 可能成为病毒检测、临床治疗、药物设计和疫苗开发中的一个重要考虑因素，帮助检测研究靶标的变化，而持续的病毒分离和测序是抗击这一流行病的关键组成部分。

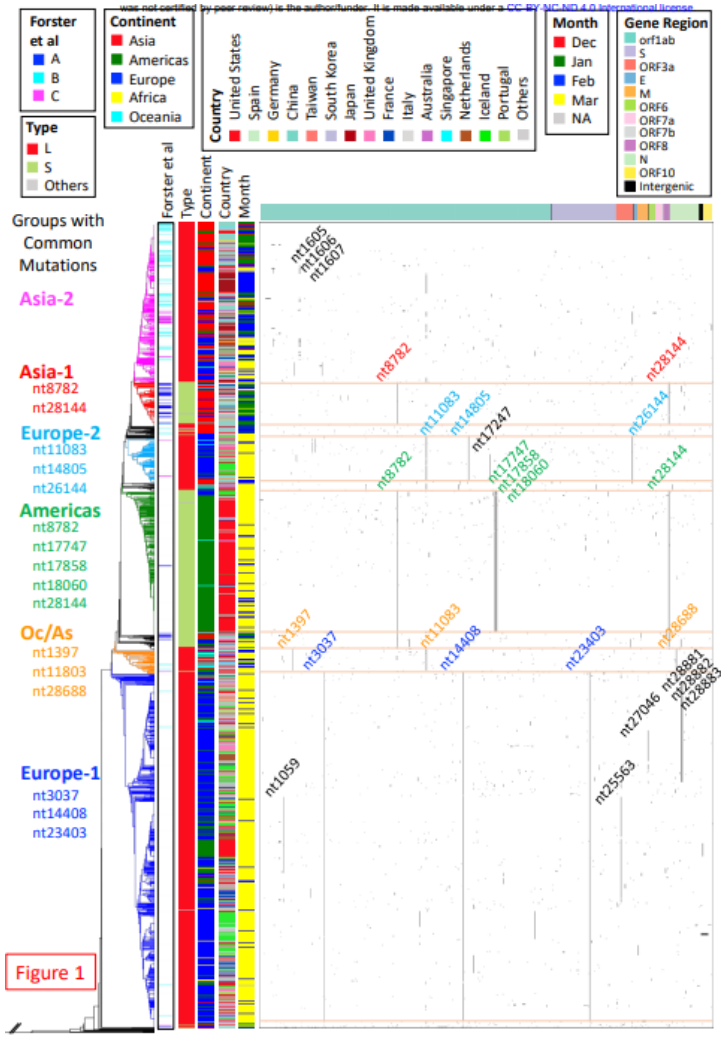


Figure 1

图一. Phylogenetic tree with mutation matrix map of 1,932 SARS-CoV-2 strains. On the right side is the mutation matrix map of 1,932 SARS-CoV-2 strains on 2,139 nucleotides. A black dot at position (i, j) indicates an occurrence of a mutation at nucleotide j (=1,…, 2139) for virus strain i (=1,…, 1932). All 2,139 nucleotides have at least one mutation among 1,932 virus strains. Nucleotides are listed by relative positions in the genome with color bands indicating their corresponding gene regions on the top panel. Five color panels to the left of the mutation matrix were utilized to identify auxiliary information for each virus strain - three strain types (A, B, C) defined by Forster et al. (3), two strain types (L, S) defined by Tang et al (2), and continent, country, with month of data collection. Virus strains were sorted by their relative positions in the phylogenetic tree displayed on the left. Six groups of virus strains were identified via their mutation patterns in the matrix and their relative positions in the phylogenetic tree, Europe-1 (in blue), Oceania/Asia (in orange), Americas (in green), Europe-2 (in cyan), Asia-1 (in red), and Asia-2 (in magenta). Common mutations for each of the six groups of virus strains are also labeled with corresponding colors in the mutation matrix.

Abstract:

The COVID-19 pandemic is the most significant public health issue in recent history. Its causal agent, SARS-CoV-2, has evolved rapidly since its first emergence in December 2019. Mutations in the viral genome have critical impacts on the adaptation of viral strains to the local environment, and may alter the characteristics of viral transmission, disease manifestation, and the efficacy of treatment and vaccination. Using the complete sequences of 1,932 SARS-CoV-2 genomes, we examined the genomic, geographic and temporal distributions of aged, new, and frequent mutations of SARS-CoV-2, and identified six phylogenetic clusters of the strains, which also exhibit a geographic preference in different continents. Mutations in the form of single nucleotide variations (SNVs) provide a direct interpretation for the six phylogenetic clusters. Linkage disequilibrium, haplotype structure, evolutionary process, global distribution of mutations unveiled a sketch of the mutational history. Additionally, we found a positive correlation between the average mutation count and case fatality, and this correlation had strengthened with time, suggesting an important role of SNVs on disease outcomes. This study suggests that SNVs may become an important consideration in virus detection, clinical treatment, drug design, and vaccine development to avoid target shifting, and that continued isolation and sequencing is a crucial component in the fight against this pandemic.

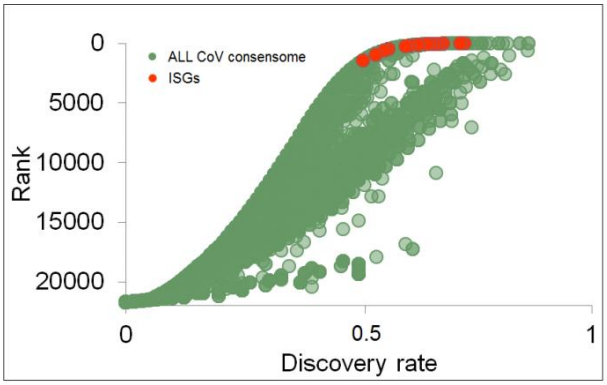
7. 冠状病毒感染人细胞的转录调控图谱

A transcriptional regulatory atlas of coronavirus infection of human cells

来源：medrxiv

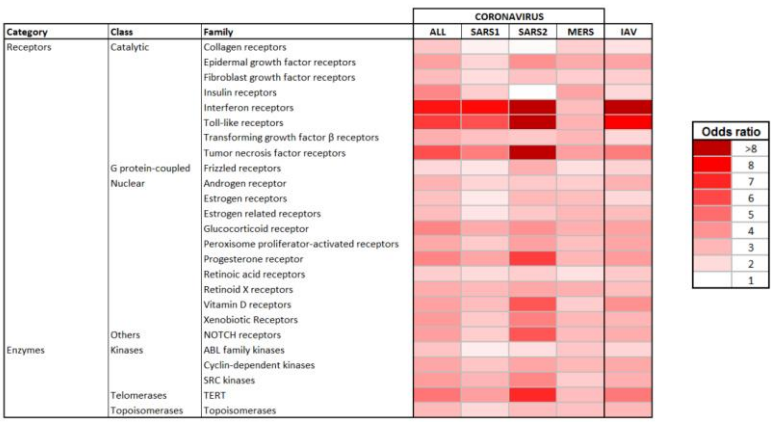
发布时间：2020-04-25

链接： https://www.biorxiv.org/content/10.1101/2020.04.24.059527v1.full.pdf
第一作者：Scott A Ochsner
通讯作者：Neil J McKenna
通讯作者单位： Baylor College of Medicine, USA
DOI: preprint
编译者：蒋立春
中文摘要：
鉴定出冠状病毒感染实验里宿主细胞最一致的基因转录变化可以帮助我们阐明人类细胞被冠状病毒感染后的细胞通路。作者收集了已经发表的 25 个独立的源于 111 个实验的冠状病毒感染的转录组数据集合，从 3 百万个数据点提取了一致的表达调控特征。研究者们把这个一致的表达调控特征叫做 consensomes，将基因按它们对 MERS, SARS-CoV-1 (SARS1)以及 SARS-CoV-2 (SARS2)的转录响应进行排序。
接着研究者们将在 consensomes 里面排列靠前的基因和 880 个整合于转录组数据和 ChIP-Seq 数据的细胞信号通路节点基因(原 SPP 项目数据*)进行比较，看有哪些基因是它们之间一致的基因。研究们发现在 consensomes 里面排名靠前的基因和细胞信号通路里面和冠状病毒感染相关的通路的基因有很好的一致性，这一定程度上证明研究者们分析病毒感染转录数据得到的 consensomes 的可靠性。作者们用一系列的使用例子进一步说明怎么使用冠状病毒 consensomes 来就宿主细胞对冠状病毒感染的细胞学机制提出假设。作者们通过一个叫做信号通路的项目（SPP）的网页版知识库来对外免费公开相关数据和分析方法（https://www.signalingpathways.org/index.jsf）。
为了验证作者采用的方法，作者也对甲流病毒感染的数据进行了整合分析，并和冠状病毒的数据进行了比较分析。



Interferon-stimulated genes (ISG)		ALL CoV CONSENSOME			
Symbol	Name	DR	CPV	Rank	%ile
IFIT1	interferon induced protein with tetratricopeptide repeats 1	0.73	1.5E-78	20	99
IFIT3	interferon induced protein with tetratricopeptide repeats 3	0.72	7.4E-77	25	99
IFI35	interferon induced protein 35	0.72	7.4E-77	28	99
ISG20	interferon stimulated exonuclease gene 20	0.68	2.8E-70	55	99
IFIT2	interferon induced protein with tetratricopeptide repeats 2	0.67	1.1E-68	62	99
ISG15	ISG15 ubiquitin like modifier	0.66	4.2E-67	69	99
IRF9	interferon regulatory factor 9	0.66	4.2E-67	71	99
IFIT5	interferon induced protein with tetratricopeptide repeats 5	0.65	1.5E-65	82	99
MX1	MX dynamin like GTPase 1	0.65	5.4E-64	89	99
IFIH1	interferon induced with helicase C domain 1	0.65	5.4E-64	91	99
IFITM3	interferon induced transmembrane protein 3	0.65	5.4E-64	94	99
IFITM1	interferon induced transmembrane protein 1	0.63	5.9E-61	135	99
IRF7	interferon regulatory factor 7	0.63	5.9E-61	138	99
IFITM2	interferon induced transmembrane protein 2	0.63	5.9E-61	140	99
OAS1	2'-5'-oligoadenylate synthetase 1	0.61	5.6E-58	197	99
IFI16	interferon gamma inducible protein 16	0.60	1.6E-56	259	98
DDX58	DExD/H-box helicase 58	0.56	7.8E-51	520	97
IFI27	interferon alpha inducible protein 27	0.55	1.9E-49	624	96
IFI30	IFI30 lysosomal thiol reductase	0.54	9.8E-47	954	95
IFI6	interferon alpha inducible protein 6	0.51	8.9E-43	1472	92

Figure 1. Ranking of ISGs in the human ALL CoV infection transcriptomic



			CORONAVIRUS				
Category	Class	Family	ALL	SARS1	SARS2	MERS	IAV
Receptors	Catalytic	Collagen receptors					
		Epidermal growth factor receptors					
		Fibroblast growth factor receptors					
		Insulin receptors					
		Interferon receptors					
		Toll-like receptors					
		Transforming growth factor β receptors					
		Tumor necrosis factor receptors					
	G protein-coupled	Frizzled receptors					
	Nuclear	Androgen receptor					
		Estrogen receptors					
		Estrogen related receptors					
		Glucocorticoid receptor					
		Peroxisome proliferator-activated receptors					
		Progesterone receptor					
		Retinoic acid receptors					
		Retinoid X receptors					
		Vitamin D receptors					
		Xenobiotic Receptors					
	Others	NOTCH receptors					
Enzymes	Kinases	ABL family kinases					
		Cyclin-dependent kinases					
		SRC kinases					
	Telomerases	TERT					
	Topoisomerases	Topoisomerases					

Figure 2 GeneOverlap analysis of CoV and IAV TC95s and SPP receptor or enzyme TC95s
注：
作者将转录组 95 分位 consensome 的叫做 TC95s。
SPP 项目：
信号通路项目是一个基于公共数据、进行过手工整理的转录组和 ChIP-Seq 数据集合的多组学的知识池。数据集合收集了遗传干预以及小分子处理的数据。该项目的目的是能给科学家提供一个可以不断产生研究设想或者对和细胞信号通路相关的实验发现进行验证的资源。

Abstract
Identifying transcriptional responses that are most consistently associated with experimental coronavirus (CoV) infection can help illuminate human cellular signaling pathways impacted by CoV infection. Here, we distilled over three million data points from publically archived CoV infection transcriptomic datasets into consensus regulatory signatures, or consensomes, that rank genes based on their transcriptional responsiveness to infection of human cells by MERS, SARS-CoV-1 (SARS1), SARS-CoV-2 (SARS2) subtypes. We computed overlap between genes with elevated rankings in the CoV consensomes against those from transcriptomic and ChIP-Seq consensomes for nearly 880 cellular signaling pathway nodes. Validating the CoV infection consensomes, we identified robust overlap between their highly ranked genes and high confidence targets of signaling pathway nodes with known roles in CoV infection. We then developed a series of use cases that illustrate the utility of the CoV consensomes for hypothesis generation around mechanistic aspects of the cellular response to CoV infection. We make the CoV infection consensomes and their universe of underlying data points freely accessible through the Signaling Pathways Project web knowledgebase.

8. 冠状病毒通过校对核糖核酸外切酶介导广泛的病毒重组

The coronavirus proofreading exoribonuclease mediates extensive viral recombination
来源：bioRxiv
发布时间：2020-04-25
链接：https://www.biorxiv.org/content/10.1101/2020.04.23.057786v1
第一作者：Jennifer Gribble
通讯作者：Andrew L. Routh[4,5], Mark R. Denison[1,2,3]
通讯作者单位：
1 Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA
2 Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN, USA
3 Vanderbilt Institute for Infection, Immunology, and Inflammation (VI4), Vanderbilt University Medical Center, Nashville, TN, USA
4 Department of Biochemistry and Molecular Biology, University of Texas -

Medical Branch, Galveston, TX, USA
5 Sealy Center for Structural Biology and Molecular Biophysics, University of Texas - Medical Branch, Galveston, TX, USA
DOI 或 PUBMED ID：
编译者：宋珂
中文摘要：
SARS-CoV-2（COVID-19）疫情的肆虐表明，冠状病毒（CoV）可以导致人畜共患病，而且是致命的人类疾病。在冠状病毒通常的复制过程中，RNA 重组对病毒亚基因组 mRNA（sgmRNA）的合成不可或缺，从而能产生功能未知的有缺陷的病毒基因组（DVG）。但是，冠状病毒重组的决定因素和模式目前仍不明确。本文中，作者发现不同的 β-CoVs：SARS-CoV-2，MERS-CoV 和鼠肝炎病毒（MHV）在培养过程中发生了广泛的 RNA 重组，产生了模式相似的重组接头，以及多样性的有缺陷的病毒基因组（DVGs）和 sgmRNA 分布。作者认为，在正常的 CoV 重组过程中，需要 CoV 校对非结构蛋白（nsp14）3 至 5 端的核糖核酸外切酶（nsp14-ExoN）。而且，在被感染的细胞和释放的病毒中，nsp14-ExoN 的基因失活会导致重组模式的改变和重组发生频率的显著降低。因此，nsp14-ExoN 高度保守，同时是 CoV 高准确性复制和重组的关键决定因素，可能是一个合适的抑制和减弱病毒的药物靶点。

Summary:
Coronaviruses (CoVs) emerge as zoonoses and cause severe disease in humans, demonstrated by the SARS-CoV-2 (COVID-19) pandemic. RNA recombination is required during normal CoV replication for subgenomic mRNA (sgmRNA) synthesis and generates defective viral genomes (DVGs) of unknown function. However, the determinants and patterns of CoV recombination are unknown. Here, we show that divergent β-CoVs SARS-CoV-2, MERS-CoV, and murine hepatitis virus (MHV) perform extensive RNA recombination in culture, generating similar patterns of recombination junctions and diverse populations of DVGs and sgmRNAs. We demonstrate that the CoV proofreading nonstructural protein (nsp14) 3-to-5 exoribonuclease (nsp14-ExoN) is required for normal CoV recombination and that its genetic inactivation causes significantly decreased frequency and altered patterns of recombination in both infected cells and released virions. Thus, nsp14-ExoN is a key determinant of both high fidelity CoV replication and recombination, and thereby represents a highly-conserved and vulnerable target for virus inhibition and attenuation.

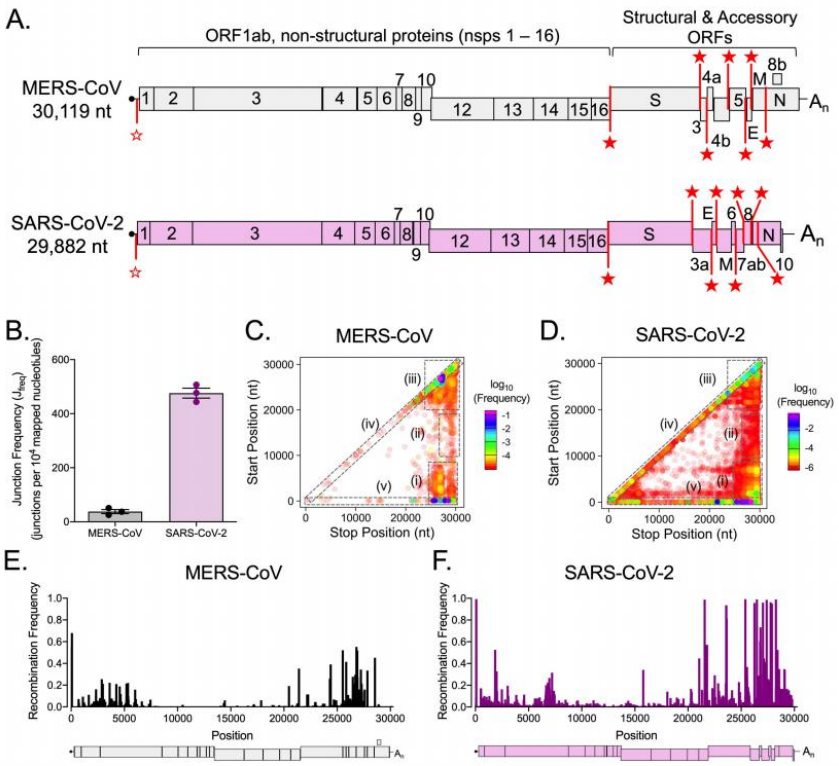


Figure 1. Genome-wide recombination generates populations of diverse RNA molecules in MERS-CoV and SARS-CoV-2. (A) Genome organization of MERS-CoV (violet) and SARS-CoV-2 (gray). Nonstructural (nsps 1 – 16) and structural (S, E, M, N) and accessory open reading frames (ORFs) are labelled. The common 5' leader transcription leader sequence (TRS-L) is denoted with an unfilled red star. Body TRSs are labelled with filled red stars. MERS-CoV total cell lysates (black) and SARS-CoV-2 infected cell monolayers (violet) were sequenced by RNA-seq. (B) Junction frequency (J_{freq}) was calculated by comparing the number of nucleotides in *ViReMa*-detected junctions to all mapped nucleotides. Error bars represent standard errors of the mean for three independent sequencing libraries (N = 3). Recombination junctions are mapped according to their genomic position (5' junction site, Start Position; 3' junction site, Stop Position) and colored according to their frequency in the population of all junctions in MERS-CoV (C) and SARS-CoV-2 (D). The highest frequency junctions are magenta and completely opaque. The lowest frequency junctions are red and the most transparent. Dashed boxes represent clusters of junctions: (i) 5' → 3'; (ii) mid-genome → 3' UTR; (iii) 3' → 3'; (iv) local deletions; (v) 5' UTR → rest of genome. Recombination frequency is quantified across the MERS-CoV (E) and SARS-CoV-2 (F) genomes. Recombination frequency is represented as a mean of three independent sequencing libraries (N = 3). See also Figure S1.

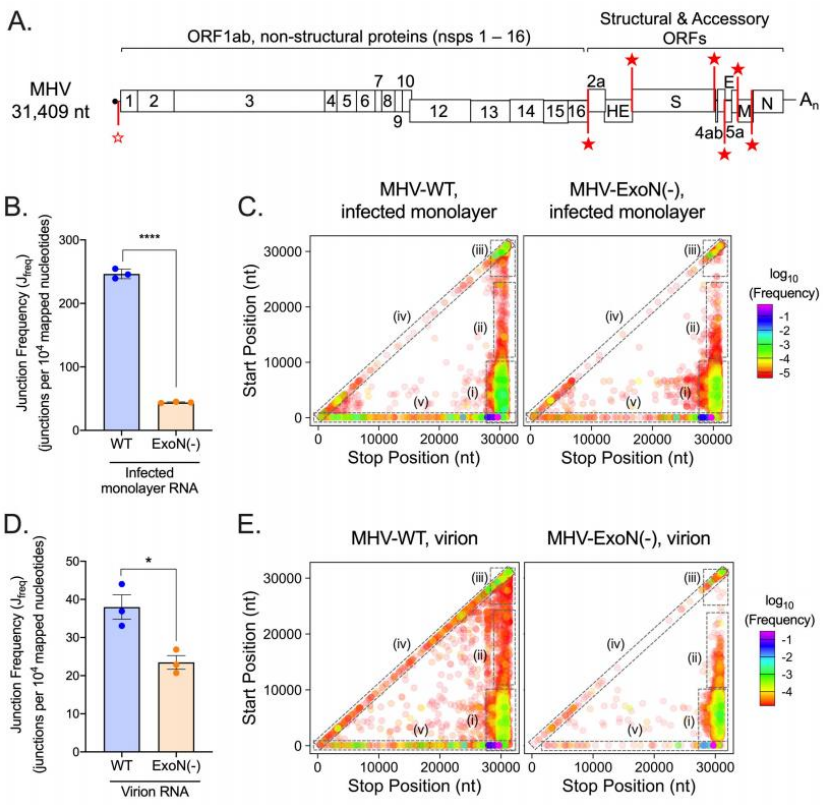


Figure 3. Loss of nsp14-ExoN activity decreases recombination frequency and alters recombination junction patterns across the genome. (A) Genome organization of MHV. Nonstructural (nsps 1 – 16) and structural (S, E, M, N) and accessory open reading frames (ORFs) are labelled. The common 5' leader transcription leader sequence (TRS-L) is denoted with an unfilled red star. Body TRSs are labelled with filled red stars. Infected monolayer and virion RNA from independent experiments were poly(A) selected, sequenced by RNA-seq, and aligned to the MHV genome using *ViReMa*. Junction frequency (J_{freq}) in infected monolayer RNA (B) and virion RNA (D) was calculated by dividing the number of sequenced nucleotides in all junctions by the total number of nucleotides sequenced in the library. Error bars represent standard error of the means (SEM) (N = 3). Statistical significance was determined by the unpaired student's t-test. *, $p < 0.05$. Unique forward (5' → 3') recombination junctions detected in infected monolayers (C) and virions (E) were mapped in MHV-WT and MHV-ExoN(-) according to their genomic position. Junctions are colored according to their frequency in the population (high frequency = magenta; low frequency = red). Clusters are marked by dashed boxes: (i) 5' → 3'; (ii) mid-genome → 3'; (iii) 3' → 3'; (iv) local deletions; (v) 5' UTR → rest of genome. See also Figure S2, Figure S3.

9. III 型干扰素在控制原代人肠上皮细胞中 SARS-CoV-2 感染、复制、传播的关键作用

Critical role of type III interferon in controlling SARS-CoV-2 infection, replication and spread in primary human intestinal epithelial cells

来源：biorxiv

发布时间：2020.04.24
文章链接：https://www.biorxiv.org/content/10.1101/2020.04.24.059667v1
第一作者：Megan L. Stanifer
通讯作者：Megan L. Stanifer, , Steeve Boulant

通讯作者单位：Megan L. Stanifer: Department of Infectious Diseases, Molecular Virology, Heidelberg University; Research Group “Cellular polarity and viral infection”, German Cancer Research Center (DKFZ); Steeve Boulant: Research Group “Cellular polarity and viral infection”, German Cancer Research Center (DKFZ); Department of Infectious Diseases, Virology, Heidelberg University;
10. 编译者：张怡
中文摘要：
SARS-CoV-2 是一个前所未有的全球健康问题，需要采取协调一致的全球方法来更好地了解该病毒，以便开发新的治疗方法来阻止 COVID-19 大流行，并更好地准备应对未来可能出现的新大流行病毒。虽然 SARS-CoV-2 主要作用于肺上皮细胞，引起呼吸道感染和病变，但越来越多的证据表明，肠上皮细胞也受到感染。然而，对 SARS-CoV-2 病毒引起的病理、传播和预后的肠期重要性仍然未知。文中作者利用结肠来源的细胞系和原代非转化结肠细胞质，首次全面分析了人肠上皮细胞中 SARS-CoV-2 的生命周期。作者的研究结果表明，人类肠上皮细胞完全支持 SARS-CoV-2 感染、复制和产生感染性全新的病毒颗粒。重要的是，他们发现肠上皮细胞是繁殖 SARS-CoV-2 的最佳培养模型。作者还发现，病毒感染引发了一种非常强大的内在免疫反应，有趣的是，与 I 型干扰素相比，III 型干扰素介导的反应在控制 SARS-CoV-2 复制和传播方面显著更有效。综上所述，文中数据表明，人类肠上皮细胞是 SARS-CoV-2 复制的一个位点，并提示 SARS-CoV-2 的肠相期可能通过增加患者病毒血症和加剧细胞因子反应，影响到 COVID-19 患者的病理观察。

Abstract
SARS-CoV-2 is an unprecedented worldwide health problem that requires concerted and global approaches to better understand the virus in order to develop novel therapeutic approaches to stop the COVID-19 pandemic and to better prepare against potential future emergence of novel pandemic viruses. Although SARS-CoV-2 primarily targets cells of the lung epithelium causing respiratory infection and pathologies, there is growing evidence that the intestinal epithelium is also infected. However, the importance of the enteric phase of SARS-CoV-2 for virus-induced pathologies, spreading and prognosis remains unknown. Here, using both colon-derived cell lines and primary non-transformed colon organoids, we engage in the first comprehensive analysis of SARS-CoV-2 lifecycle in human intestinal epithelial cells. Our results demonstrate that human intestinal epithelial cells fully support SARS-CoV-2 infection, replication and production of infectious de-novo virus particles. Importantly, we identified intestinal epithelial cells as the best culture model to propagate SARS-CoV-2. We found that viral infection elicited an extremely robust intrinsic immune response where, interestingly, type III interferon mediated response was significantly more efficient at controlling SARS-CoV-2 replication and spread compared to type I interferon. Taken together, our data demonstrate that human intestinal epithelial cells are a productive site of SARS-CoV-2 replication and suggest that the

enteric phase of SARS-CoV-2 may participate in the pathologies observed in COVID-19 patients by contributing in increasing patient viremia and by fueling an exacerbated cytokine response.