Culture of SARS-CoV-2 in a panel of laboratory cell lines

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Abstract

Purpose

The emergence of COVID-19 disease due to SARS-CoV-2 at the end of 2019 was rapidly associated with the isolation of the strain from co-culture onto VERO cells. These isolations quickly made it possible to carry out the first tests for antiviral agents' susceptibility and drug repurposing. However, it seems important to make an inventory of all the cells that can support the growth of this virus, with the aim of producing it in large quantities, to test new antiviral molecules on cells closer to human lung cells, to better understand its cell cycle, to start developing vaccines based on attenuated strains.

Methods

In the present work, we tested a strain of SARS-CoV-2 locally isolated on a panel of 30 cell lines present in our laboratory and commonly used for the isolation of human pathogenic microorganism. After inoculation, cells were observed for cytopathic effects and quantitative real time polymerase reaction was used to measure the virus replication on the cells.

Results

We were able to obtain growth on 7 cell lines, 6 simian and 1 human, Caco-2. The cytopathogenic effects are variable, ranging from lysis of the cell monolayer in 48-72 hours to no cytopathic effect in spite of intense multiplication, as in Caco-2 cells.

Conclusion

In this paper, we explored the species specificity and tissue tropism of SARS-CoV-2 in vitro on a panel of cells available in our laboratory and identified human and animal cell lines susceptible to support SARS-CoV-2 replication.

Keywords

SARS-Cov2; Covid-19; coronavirus; culture; cell lines

Introduction

The current outbreak of the novel Severe Acute Respiratory Syndrome (2019-nCov then Covid-19) due to SARS-Cov-2 started in Wuhan, China in late December 2019 and has spread to many other countries [1–4]. To date, more than 84,000 cases and more than 4,600 deaths have been reported across China due to SARS-Cov2, mostly in the region of Hubei (WHO, [5]). SARS-Cov-2 has disseminated in 188 countries, with currently more than 4,100,000 confirmed cases and 287,000 deaths around the world.

Coronaviruses are enveloped, positive single-stranded large RNA viruses that infect also a wide range of animals. The first description of coronavirus was made in 1966 by Tirell and Bynoe, who cultivated the viruses from patients with colds [6]. They were named coronavirus because of their morphology, spherical virions with a core shell and surface resembling to a solar crown, in Latin corona. Coronaviruses are divided into 4 subfamilies alpha, beta, delta and gamma-coronaviruses. The first two originate from mammals, in particular bats, while the other two come from pigs and birds. The genome size of coronaviruses ranges from approximately 27 to 34 kilobases. Severe disease and fatalities are caused essentially by beta-coronaviruses, whereas alpha-coronaviruses cause asymptomatic or mildly symptomatic infections. SARS-CoV and Middle East Respiratory Syndrome coronavirus (MERS-CoV) belong to the beta-coronavirus cluster [7], as well as the SARS-CoV-2 [8].

In this crisis situation, isolation of causative virus is indispensable for developing and evaluating diagnostic tools and therapeutics assays. The first isolation of SARS-CoV-2 was performed on human airway epithelial cells in China [8]. Subsequently, like SARS-CoV and MERS Cov [9,10], SARS-CoV-2 was isolated on Vero cells, which are kidney epithelial cells extracted from African green monkey [11–13]. In this paper, we investigated the susceptibility of a number of cells lines available in our laboratory collection to SARS-CoV-2. These cells were derived from a variety of species and tissues routinely used for the culture of micro-organisms. After inoculation with SARS-CoV-2, cells were observed for cytopathic effects and quantitative real time polymerase reaction was used to measure ongoing replication on the cells growing the virus.

Materials and Methods

Virus routine propagation

SARS-CoV-2-IHUMI2 strain was isolated from human nasopharyngeal swab as previously described [14] as used for all tests. The 4-passage strain was grown in VERO E6 before subculture in different cell lines in Minimum Essential Medium culture medium with 4% fetal calf serum and 1% glutamine, without antibiotics at 37°C under 5% CO_2. After 48h of incubation, supernatant was used to determine TCID50 and inoculation of cell lines.

Multiple cell lines assays

The cell lines tested are listed in Table 1. These cells are either routinely or occasionally used for microorganisms isolation or for various diverse research projects in our laboratory. Cell lines to be tested were inoculated in 96-wells microplates at $2*10^5$ cells/ml into their specific growth medium (Table 1), without antibiotics and incubated to reach sub-confluence. At this stage, cells were infected with SARS-CoV-2 at 10^{-1} dilution of VERO E6 supernatant. Each day, cells were observed for SARS-CoV-2 specific cytopathic effects (CPE) for 7 days. On day 0 and day 7 after infection, supernatants were collected for subsequent quantification using RT-PCR targeting E-gene as previously described [15]. For cells for which a CPE effect was observed or a growth detected by RT-PCR, the experiment was repeated at dilution 10^{-4} dilution to observe possible differences in permissivity of cells with respect to the virus. All experiments involving SARS-CoV-2 cultures were carried out in a Biosafety level 3 laboratory and conducted under appropriate conditions.

Results

Table 1 presents the panel of 34 cell lines present in the laboratory and tested for their susceptibility to the SARS-Cov-2 virus. Among these cell lines, 7 are able to support SARS-CoV-2 multiplication and are presented in Table 2. For these seven cell lines that supported growth of the virus, the Δ Ct between day 0 and day 7 at dilution 10^{-1} varied between 4.65 and 6.48, as shown in Table 2. Besides VERO E6 in which the virus was isolated and propagated, 4 African green monkey kidney cell lines supported replication of SARS-CoV-2 (VERO 81, VERO SLAM, MA104 and BGM cells) and produced CPE 48h after SARS-CoV-2 infection. All produced evident CPEs. One human cell line supported virus replication, an epithelial line from colorectal adenocarcinoma cell line (Caco-2). Caco-2 cell line showed only discrete modification as compared to control but no real CPE. The morphological changes observed in the different cell lines are shown in Figure 1. LLC-MK2, a rhesus macaque epithelial kidney cell line did not produce evident CPE. For these seven cell lines that supported growth of the virus, the Δ Ct between day 0 and day 7 at dilution 10^{-4} varied between 11.3 and 17.26 as shown in Table 1. Viral multiplication was not associated with the intensity of CPE.

Twenty-seven other cell lines, derived from various species like insect, human, rodent, bovine, dog, sheep and bat cell lines, did not present any morphological changes or CPE and no difference of Δ Ct was observed.

Discussion

In the context of the SARS-CoV-2 epidemic, it was first important to develop rapid methods to isolate the virus. This was done easily using the common Vero E6 cell line, a highly virus permissive interferon deficient cell line [17]. In order to produce the virus in large quantities for vaccine research, to identify potential antiviral compounds, to understand intracellular trafficking and to develop innovative therapeutic approach, it is important to have other cell line, especially from human origin. In this paper, we explored the species specificity and tissue tropism of SARS-CoV-2 *in vitro* on a panel of cells available in our microbiology laboratory and identified human and animal cell lines susceptible to support SARS-CoV-2 multiplication.

Previous published reports showed that several monkey kidney cell lines are susceptible to SARS-CoV-2, specifically classical VERO cells, VERO E6 cells, VERO h/SLAM cells [8,11–13,18–21]. In this paper, we showed that all kidney cells derived from two species of monkey (African green monkey and rhesus macaque) support the growth of SARS-CoV-2, and all these cells, except for LLC-MK2 cell lines, presented CPE at 48h post-infection. Unsurprisingly, MA104, BGM and LLC-MK2 already tested for SARS-CoV with very early CPE [22] and not previously tested with SARS -CoV-2, supported its growth.

In our first tests, HEP-2, an endothelial cell line suspected to be derived from laryngeal epidermoid carcinoma but in fact a clone derived from HELA cells [23], was first identified as susceptible to SARS-CoV-2 infection. SARS-CoV2 infection on our HEP-2 cells induced CPE after 120h of infection with high virus multiplication. This result was unexpected, asprevious studies on SARS CoV showed that this virus did not infect HEP-2 cell lines, with no observable CPE or virus multiplication [22]. Based on PCR amplification of HPV18, a virus that chronically infects HEP-2 and HELA cells, we could detect that our HEP-2 cell line was misidentified. A new batch of cells ordered to the ATCC allowed to confirm that HEP-2 cells do not support growth of SARS-CoV-2;

A unique human cell line, Caco-2, epithelial cells from colorectal adenocarcinoma, was susceptible to SARS-CoV-2 with medium virus multiplication, but no specific CPE. Instead of CPE, we observed that the cell layer appears to be mottled more rapidly than in the control. This effect is rather seen in ageing uninfected Caco-2. Previous studies showed that SARS CoV and SARS-CoV-2 can infect Caco-2 cell lines [24,25]. For SARS CoV infections, CPE appeared on Caco-2 cell line 48h post-infection [25], whereas, as observed, no obvious cell damage was found for SARS CoV-2 infections [24]. This capability of SARS-CoV-2 to infect Caco-2 cells, could explain why patients infected with the virus present commonly gastrointestinal symptoms [26]. Moreover, SARS-CoV-2 RNA was detected in stools of patients infected with the virus, raising the question of viral gastrointestinal infection and fecal-oral transmission routes [27,28]. However, to our knowledge, the virus could not be isolated from stools of infected patients.

We showed that 8 other human cells lines were not susceptible to SARS-CoV-2 (HEP-2, HT-29, HELA, HCT-8, ECV-304, HL-60, MRC5 and THP1 cell lines). In a recent paper of Chu et al.2020 [24], SARS-CoV-2 was inoculated on 9 human cell lines. They showed that SARS-CoV-2 replicates also on Calu3 (Lung adenocarcinoma), Huh7 (Hepatocellular carcinoma), U251 (Glioblastoma) and 293T (Embryonic kidney) cell lines, whereas no growth was observed on A549 (Lung adenocarcinoma), HFL (Embryonic lung fibroblasts) and RD (Rhabdomyosarcoma) cell lines. These data are consistent with the results observed in our study.

In this latter study, they evaluated the cell tropism profile of SARS-CoV-2 in non-human and non-primate cells originating from different animal species and showed that SARS-CoV-2 replicate in cat (Feline kidney CRFK cells), rabbit (RK-13 Rabbit kidney cells) and pig cells (PK-15 Porcine kidney cells). In our study, we evaluated the susceptibility of SARS-CoV-2 in 19 animal cell lines. SARS-CoV-2 did not infect insect cells (Aa23, C6/36, S2, ISE6 and IPL-LD-65Y cells), rodent cells (BHK-21, McCoy, L929, P388 D1 and RAW 264.7 cells), bovine cells (BA886), bats cells (R05T, R06E, TB1 Lu cells), frog cells (XTC-2), dog cells (DH-2, MDCK cells) and sheep cells (OA3.Ts, MDOK cells).

Cellular entry of coronaviruses depends on the binding of the spike (S) protein to a specific cellular receptor and subsequent S protein priming by cellular proteases. Similarly to SARS-CoV [29,30], SARS-CoV-2 seems to employ ACE2 (angiotensin-converting enzyme 2) as a receptor for cellular entry, and priming to be performed by the serine protease TMPRSS2 [19,31,32]. This likely explains the specific permissivity of animal and kidney cell lines to the virus. ACE2 is expressed in various human tissues, such as heart, kidney and testes, in addition to the lungs [33], indicating that SARS-CoV-2 may infect other tissues aside from the lungs. Moreover, Zhou et al. demonstrated that overexpressing ACE2 from different species in HeLa cells with human ACE2, pig ACE2, civet ACE2 (but not mouse ACE2) allowed SARS-CoV-2 infection and replication [19]. Hoffmann et al. reported similar findings for human and bat ACE-2 [34]. Additionally, Hoffmann et al. showed that treating Vero-E6 cells, a monkey kidney cell line known to permit SARS-CoV replication, with an Anti-ACE-2 Antibody blocked the entry of VSV pseudotypes expressing the SARS-CoV-2 S protein [34]. A recent study conducted by Wang et al. reported that the existence of the novel SARS-CoV-2 (CD147-SP) route in host cells [35]. All these data suggest that SARS-CoV-2 is able to infect different tissues in human, but is also able to infect animals, and these information are concomitant with the variety of cell line that SARS-CoV-2 is able to infect.

Declarations

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Conflicts of Interest

The authors declare no conflict of interest.

Ethics approval

Not applicable

Consent to participate

Not applicable

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author onreasonable request.

Code availability

Not applicable

Authors’ Contributions

Wurtz Nathalie: Writing – original draft preparation, Analysis of results

Penant Gwilherm: Methodology, Investigation, Writing – original draft preparation

Duclos Nathalie and Priscilla Jardot: Methodology, Investigation

Bernard La Scola: Conceptualization, Supervision, writing

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Table 1 Cell lines tested for their susceptibility to SARS-CoV-2

Cell lines	**Species of origin**	**Cell types**	**References**	**Culture medium**	**Culture conditions**
Aa23	*Aedes albopictus*	Epithelial larva cells	ATCC® CCL-125™	L15 Leibovitz + 10% FBS + 8% tryptose phosphate	28°C
C6/36	*Aedes albopictus*	Larva cells	ATCC® CRL-1660™	L15 Leibovitz + 10% FBS + 8% tryptose phosphate	28°C
S2	*Drosophila melanogaster*	Embryo cells	Thermo R69007	Schneider medium + 10% FBS	28°C
ISE6	*Ixodes scapularis*	Embryo cells	ATCC® CRL-11974	L15B + 10% FBS	28°C
IPL-LD-65Y	*Lymantria dispar*	Larvae cells	ACC 181 (DSMZ)	TC-100 + 101% FBS	28°C
BGM	*Cercopithecus aethiops*	Epithelial kidney cells	ECACC 90092601	MEM + 10% FBS	37°C 5% CO_2
Vero/hSLAM	*Cercopithecus*	Epithelial kidney cells	04091501-1VL	MEM + 5% FBS +	37°C 5% CO_2

	aethiops			0,4 mg/ml geneticin	
MA104	*Cercopithecus aethiops*	Epithelial kidney cells	ATCC® CRL-2378.1™	MEM + 10% FBS	37°C 5% CO_2
VERO	*Cercopithecus aethiops*	Epithelial kidneycells	ATCC® CCL-81™	MEM + 4% FBS	37°C 5% CO_2
VERO C1008	*Cercopithecus aethiops*	Epithelial kidney cells	ATCC® CRL-1586™	MEM + 10% FBS	37°C 5% CO_2
LLC-MK2	*Macaca mulatta*	Epithelial kidney cells	ATCC® CCL-7™	M199 + 1% FBS	37°C 5% CO_2
HT-29	*Homo sapiens*	Epithelial cells from colorectal adenocarcinoma	ATCC® HTB-38™	DMEM/F12 + 10% FBS	37°C 5% CO_2
Caco-2	*Homo sapiens*	Epithelial cells from Colorectal adenocarcinoma	ATCC® HTB-37™	DMEM + 10% FBS + 1%AA	37°C 5% CO_2
HELA	*Homo sapiens*	Epithelial cervix cells from adenocarcinoma	ATCC® CCL-2™	MEM + 10% FBS	37°C 5% CO_2
HCT-8	*Homo sapiens*	Epithelial colon cells from ileocecal colorectal adenocarcinoma	ATCC® CCL-244™	RPMI + 10% FBS	37°C 5% CO_2
HEP-2	*Homo sapiens*	HeLa derived cell line from Laryngeal epidermoid carcinoma	ATCC® CCL-23™	MEM + 5% FBS + 1% AA	37°C 5% CO_2

ECV304	*Homo sapiens*	Endothelial cells from human cord / urinary bladder carcinoma	ATCC® CRL-1998™	RPMI + 10% FBS	37°C 5% CO_2
HL-60	*Homo sapiens*	Promyeloblast cells from Human peripherical blood from acute promyelocytic leukemia	ATCC® CCL-240™	RPMI + 10% FBS	37°C 5% CO_2
MRC5	*Homo sapiens*	Fibroblast cells from lung	ATCC® CCL-171™	MEM + 10% FBS	37°C 5% CO_2
THP1	*Homo sapiens*	Monocytes from peripheral blood from acute monolytic leukemia	ATCC® TIB-202™	RPMI + 10% FBS	37°C 5% CO_2
BHK21	*Mesocricetus auratus*	Fibroblast kidney cells	ATCC® CCL-10™	MEM + 4% FBS	37°C 5% CO_2
McCoy	*Mus musculus*	Fibroblast cells	ATCC® CRL-1696™	MEM + 4% FBS	37°C 5% CO_2
L929	*Mus musculus*	Fibroblast cells from subcutaneous areolar and adipose	ATCC® CCL1™	MEM + 4% FBS	37°C 5% CO_2
P388 D1	*Mus musculus*	Macrophage cells from lymphoma	ATCC® CCL-46™	MEM + 10% FBS	37°C 5% CO_2
RAW 264.7	*Mus musculus*	Macrophage from Abelson murine leukemia virus-induced tumor	ATCC® TIB-71™	MEM + 10% FBS + AA	37°C 5% CO_2
BA 886	*Bos taurus*	Endothelial cells from bovine aorta	[16]	DMEM/F12 + 10% FBS	37°C 5% CO_2

MDCK	*Canis familiaris*	Epithelial cells froms kidney	ATCC® CCL-34™	MEM + 10% FBS	37°C 5% CO_2
DH82	*Canis familiaris*	Macrophage cells from malignant histiocytosis	ATCC® CRL-10389™	MEM + 10% FBS	37°C 5% CO_2
OA3.Ts	*Ovis aries*	Epithelial testis cells	ATCC® CRL-6546™	DMEM + 10% FBS	37°C 5% CO_2
MDOK	*Ovis aries*	Epithelial kidney cells	ATCC® CRL-1633™	MEM + 10% FBS + 1% AA + 1% pyruvate	37°C 5% CO_2
R05T	*Rousettus aegyptiacus*	Fetus cells	Bei resources NR-49169	DMEM/F12 + 10% FBS	37°C 5% CO_2
R06E	*Rousettus aegyptiacus*	Fetus cells	Bei resources NR-49168	DMEM/F12 + 10% FBS	37°C 5% CO_2
TB1 Lu	*Tadarida brasiliensis*	Epithelial lung cells	ATCC® CCL-88™	DMEM + 10% FBS	37°C 5% CO_2
XTC-2	*Xenopus laevis*	Tadpole cells	CellBank Riken® RCB0771	L15 Leibovitz + 5% FBS + 8% tryptose phosphate	28°C

FBS: fetal bovine serum

AA: non essential amino-acids

Table 2 Tested cell lines permissive to SARS-CoV-2

Cell lines	**CPE**	**Δ Ct day 0 - day 7 Dil. 10^{-1}**	**Δ Ct day 0 - day 7 Dil. 10^{-4}**
BGM	48H	5,17	11,3
Vero/hSLAM	48H	6,48	15,66
MA104	48H	5,6	16,17
VERO	48H	5,25	14,92
VERO C1008	48H	5,1	12,9
LLC-MK2	NO modifications	4,65	15,07
Caco-2	NO modifications	6,28	17,26

Dil.: SARS-Cov-2 virus dilution

Figure 1. Morphological changes observed in the different cell lines

a non infected VERO cells (X10) **b** SARS-Cov-2 infected VERO cells at 48h post-infection (X10) **c** non infected C1008 VERO cells (X10) **d** SARS-Cov-2 infected C1008 VERO cells at 48h post-infection (X10) **e** non infected VERO/hSLAM cells (X10) **f** SARS-Cov-2 infected VERO/hSLAM cells at 48h post-infection (X10) **g** non infected MA104 cells (X10) **h** SARS-Cov-2 infected MA104 cells at 48h post-infection (X10) **i** non infected BGM cells (X10) **j** SARS-Cov-2 infected BGM cells at 48h post-infection (X10).