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Clinically relevant cell culture models and their significance in isolation, pathogenesis, vaccine development, repurposing and screening of new drugs for SARS-CoV-2: a systematic review

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ABSTRACT

Background: In-Vitro/Cellular evidence is the backbone and vital proof of concept during the development of novel therapeutics as well as drugs repurposing against COVID-19. Choosing an ideal in-vitro model is vital as the virus entry is through ACE2, CD147, and TMPRSS2 dependant and very specific. In this regard, this is the first systematic review addressing the importance of specific cell lines used as potential in-vitro models in the isolation, pathogenesis, and therapeutics for SARS–COV-2.

Methods: We searched 17 literature databases with appropriate keywords, and identified 1173 non-duplicate studies. In the present study, 71 articles are included after a careful, thorough screening of the titles and their abstracts for possible inclusion using predefined inclusion/exclusion criteria (PRISMA Guidelines).

Results: In the current study, we compiled cell culture-based studies for SARS-CoV-2 and found the best compatible In-Vitro models for SARS-CoV-2 (Vero, VeroE6, HEK293 as well as its variants, Huh-7, Calu-3 2B4, and Caco2). Among other essential cell lines used include LLC-MK2, MDCKII, BHK-21, HepG2, A549, T cell leukemia (MT-2), stems cells based cell line DYR0100 for differentiation assays, and embryo-specific NIH3T3 cell line for vaccine production.

Conclusion: The Present study provides a detailed summary of all the drugs/compounds screened for drug repurposing and discovery purpose using the in-vitro models for SARS-CoV-2 along with isolation, pathogenesis and vaccine production. This study also suggests that after careful evaluation of all the cell line based studies, Kidney cells (VeroE6, HEK293 along with their clones), liver Huh-7 cells, respiratory Calu-3 cells, and intestinal Caco-2 are the most widely used in-vitro models for SARS-CoV-2.

Abbreviations: SARS-CoV-2, Severe Acute Respiratory Syndrome-Coronavirus-2; 2019-nCoV, 2019-novel Corona virus; ACE-2, Angiotensin-Converting Enzyme-2; TMPRSS2, Transmembrane Protease Serine 2.

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1. Introduction

1.1. Overview of Corona viruses and the current outbreak of COVID-19

Corona viruses (CoVs) have a long history and are the part of family Coronaviridae (subfamily Coronavirinae). They spread infection especially through the respiratory tract’s involvement (both lower and upper respiratory tract) with the symptoms of common cold, pneumonia, bronchiolitis, rhinitis, pharyngitis, sinusitis, diarrhea (Chang et al., 2016; Paules et al., 2020). Till date, a total of seven human Corona virus strains have been identified, [229E, NL63, OC43, HKU1, severe acute respiratory syndrome-Corona virus (SARS-CoV), middle east respiratory syndrome-corona virus (MERS-CoV), and severe acute respiratory syndrome - corona virus 2 (SARS-CoV-2)] or 2019 Novel coronavirus (2019-nCoV) (Paules et al., 2020). Though CoVs were known to cause milder symptoms, the outbreak of these three strains were deadly with high mortality and shown very adaptive potential as per environmental conditions and classified as “emerging viruses.” COVID-19 was initially named “2019-nCoV.” But later on, it was changed to “SARS-CoV-2” due to high similarity with severe acute respiratory syndrome corona virus (SARS-CoV) as per the recommendation of the Corona virus Study Group (CSG) of the International Committee on Taxonomy of Viruses (ICTV) (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses (ICTV), 2020). Corona viruses (CoVs) are single-stranded positively sense RNA viruses of the family Coronaviridae (subfamily Coronavirinae). Their genome is the largest among all RNA viruses, with a size range between 26.2 and 31.7 kb. They are either pleomorphic or spherical with a diameter of 80–120 nm and distinguished by bears club-shaped projections of glycoproteins on their surface. CoVs genome consists of six to ten open reading frames (ORFs) (Belouzard et al., 2012), and their genetic material is highly susceptible to frequent recombination process, resulting in new strains with alteration in their virulence (Hilgenfeld, 2014). The most important structural proteins of SARS–CoV-2 are spike (S) protein, which is a trimeric, membrane (M) protein, envelope (E) protein, and the nucleocapsid (N) protein, and all of them are potential drug development targets. Some viruses, such as beta–CoVs, also have hemagglutinin esterase (HE) glycoprotein (Belouzard et al., 2012). The RNA genome of CoV has seven conserved genes (ORF1a, ORF1b, S, ORF3, E, M, and N) arranged in 5′ to 3′: ORF1a/b alone covers two-third part of its RNA genome. It is responsible for the production of two viral replicase polyproteins which are PP1a and PP1ab. Further processing gives rise to sixteen mature nonstructural proteins (NSPs) and play a crucial role in various viral functions, including the formation of the replicative transcription complex (McBride et al., 2014). The rest of the virus’s genomic part encodes the mRNA, which produces its other essential structural proteins, including spike, envelope, membrane, and nucleocapsid (McBride et al., 2014). HE is another essential envelop-associated protein that is expressed by specific CoV strains (Structure, 2016). The entire RNA genome of corona virus is packed with nucleocapsid protein under covered with envelope (Guo et al., 2008).

1.2. Mechanism of SARS-CoV-2 entry into target cells and the importance of cell culture models

When SARS-CoV-2 enters into the body, the primary target cells are the enterocytes and pneumocytes (Guo et al., 2008; Gu and Korteweg, 2007; Hoffmann et al., 2020; Chan et al., 2020a; Corman et al., 2019; Iwata-Yoshikawa et al., 2019; Kleine-Weber et al., 2019). In contrast, other target cells include kidney tubular epithelial cells, cerebral neuronal cells, and immune cells (Guo et al., 2008; Gu and Korteweg, 2007). SARS-CoV-2 entry and infection into the host cells take place through host cell factors, angiotensin-converting enzyme-2 (ACE2), and subsequent proteolytic cleavage on the Spike protein by transmembrane protease serine 2 (TMPRSS2), which is responsible for membrane fusion (Hoffmann et al., 2020). In the case of ACE-2, after recognizing the receptor, the virus genome and its nucleocapsid are released in the cytoplasm of the target cells. Two viral polyproteins (pp1a and pp1b) encoded by ORF1a and ORF1b genes are further processed by proteases into 16 polyproteins and play a crucial role in the formation of the replication transcription complex (teVelthuis et al., 2012). This takes command over host translational machinery for the production of their own proteins (Stobart et al., 2013). All NSPs have their specific functions required starting from their entry into the target cell to suppression of target gene expression to replicate and translate their genome and necessary proteins and specific functions of NSPs and their cellular production (teVelthuis et al., 2012; Stobart et al., 2013; Wang et al., 2016; Egloff et al., 2004; Hu et al., 2017; Bouvet et al., 2014; Narayanan et al., 2000; de Wit et al., 2016; Nieto-Torres et al., 2011; Prajapati et al., 2020). Similarly, a recent study suggested that entry and viral spread among other organs such as lungs of the infected host also depend on serine protease TMPRSS-2 activity in 5′ protein priming of SARS-CoV-2 (Hoffmann et al., 2020). It is suggested that SARS-CoV-2 infection further leads to a surge of pro-inflammatory cytokines and chemokines, and causes severe pulmonary tissue damage (Ding et al., 2004), deteriorating lung function and cause lung failure (Du et al., 2009). Both host-directed therapies and virus-directed therapies are under investigation with variable success. The most important among the virus directed therapies, are Lopinavir/Ritonavir combination, hydroxychloroquine, chloroquine, remdesivir as we discussed in our previous studies (Prajapati et al., 2020; Sarma et al., 2020a, b; Sarma et al., 2021). Owing to the ease of the developmental process, repurposed drugs are taking the lead role in the fight against the covid with preliminary evidence generated in in-vitro, leading to further preclinical and clinical trials. In-vitro experimental model systems are required to confirm the safety, efficacy, standardization and validation of new potential drug in combinatorial doses of existing anti-viral drugs for COVID-19. Being the cornerstone of the preliminary evidences generation/preliminary proof of concept studies/repurposing of existing drugs/evaluation of new chemical entities, in-vitro studies are taking a lead role in the evidence generation process against SARS-CoV-2. The importance of detailed knowledge of the in-vitro systems would help the best system for optimal drug evaluation. Towards this effort, it is the first systematic review addressing the utility of different cell culture systems as potential in-vitro models for repurposing and drugs development, vaccine development, isolation and pathogenesis of SARS-CoV-2.

2. Methods

2.1. Objective

Elucidation of potential In-Vitro models to combat and understand the pathogenesis of SARS-CoV-2

2.2. Criteria for including studies in this review

In the systematic review, we included in-vitro studies (both primary culture and cell line experiments), which involved viral culture of SARS-CoV-2 for any purpose. Only original studies providing details of the culture process were included. On the other hand, review articles and other study designs were excluded.

2.3. Search strategy

A total of 17 literature databases (PubMed, Embase, Wiley Online Library, OVID, SCOPUS, Google Scholar, Epistemonomics, CINAHL, Web of Science, TRIP, Cochrane CENTRAL, Science Direct, Virtual Health Library, CNKI and journals including Nature, Mediterranean-infection.com/pre-prints-ihuand SSRN preprints) were searched using the keywords COVID-19, 2019-nCoV, novel corona virus, SARS-CoV-2, in-vitro, cell lines, cell culture and culture techniques. We included all search studies 19th April 2020, without any language restriction. We also
searched references of all the included articles for identification of articles with possible inclusion.

2.4. Selection of studies

After searching databases and removing duplicates, two authors (HK and SK) independently screened the titles/abstracts using predefined inclusion/exclusion criteria. For relevant articles, full-texts were obtained for further evaluation. In case of any discrepancy, BM was consulted, and the issue was resolved.

2.5. Data extraction

Data extraction was done separately by two authors (HK and SK). In articles published in a language other than English, Google translate was used to identify relevant data.

3. Results and discussion

3.1. Details of included studies

After searching a total of 17 literature databases, we identified 1173 relevant articles, which were screened for title and abstract. Following which 272 articles were selected for full-text screening and finally a total of 71 articles were selected for the systematic review. For the review process, various cell lines have been used for SARS-CoV-2 isolation, pathogenesis, and therapeutic purpose. The details of the selection process and PRISMA chart are included (Fig. 1). For studying the pathogenesis and evaluation of vaccines and therapeutics against SARS-CoV-2, suitable, rapid, and safe experimental models are a current necessity that could combat the present clinical disease (Gretebeck and Subbarao, 2015; Yong et al., 2019). Various animal models are utilized for SARS-CoV studies, such as golden Syrian hamsters, rabbits, guinea pigs, mice, ferrets, and non-human primates like rhesus macaques, marmosets, and cats (Lai et al., 2013; Martina et al., 2003; Lamirande et al., 2008; Roberts et al., 2008; Falzarano et al., 2014; Du et al., 2016; Enjuanes et al., 2016). The virus specificity to the ACE-2 receptor and TMPRSS-2 (both SARS-CoV-2 and SARS-CoV) was found to be a significant hindrance in developing the animal models for SARS-CoV-2. In this regard, based on the literature available regarding SARS-CoV-2 cellular binding and viral spread in the infected host, we tried to compile the available compatible in-vitro experimental model systems. Although there are some studies where the viral load of SARS-CoV was either not detected or identified very low, all cells are not susceptible to these viruses (Chan et al., 2013). In the present study, we compiled various human and other primates based cell lines used since the outbreak of SARS-CoVs and the compiled list of these cell lines is given in Table 1.

3.2. In-vitro model platforms for SARS-CoV-2

Cell culture models can replicate the different properties and functions of the various organs specific cells in in-vitro conditions and are the key to successfully translating research findings into real-world medical applications. Based on the receptor-specific cellular infection of SARS-CoV-2, the selection of a suitable experimental model becomes essential for further studies. SARS-CoV-2 and SARS-CoV represent highly similar genetic makeup with a similarity of approximately 70%. Both SARS-CoV and SARS-CoV-2 share a similar entrance system in the target cells as all the target cells with ACE-2, CD147, and TMPRSS-2 are susceptible to SARS-CoV-2 infection. In a recent study, the expression of

![Fig. 1. Prisma flow chart of the included studies.](image-url)
Table 1
Showing the list of cell lines, their origin, availability and purpose of use for SARS-CoV-2.

| Organ/ Tissue | Experimental Models: Cell Lines | Origin/ Details of culture model | Drug/vaccine Evaluated with reference | Availability | Remark |
|--------------|--------------------------------|---------------------------------|--------------------------------------|--------------|--------|
| Vero E6      | Cercopithecus aethiops, Kidney | Kidney                          | Chloroquine (Capobianchi et al., 2020) | Pathogenesis and Transmission (Hoffmann et al., 2020; Chan et al., 2020; Zhou et al., 2020; Matsuyama et al., 2020) | ATCC Cat #CRL-1586 |
| Vero         | Cercopithecus Aethiops, Kidney | Kidney                          | Remdesivir (Caly et al., 2020; Weston et al., 2020; Liu et al., 2020; Andreani et al., 2020) | Viral Enhanced Virus Isolation (Matsuyama et al., 2020) | |
Table 1 (continued)

| Organ/ Tissue | Experimental Models: Cell Lines | Origin/ Details of culture model | Drug/vaccine Evaluated with reference | Availability | Remark |
|---------------|--------------------------------|---------------------------------|--------------------------------------|--------------|--------|
|               |                                |                                 | Drug Screening                       | Vaccine/Antibodies Production/Immune Response/Genes Expression | Virus Isolation, Expression /Other purpose |
| VeroE6/       | Cercopithecus aethiops, Kidney | Enhanced Virus Isolation        |                                      | ATCC Cat #CRL-1586 |        |
| TMPRSS2       |                                |                                 |                                      |               |        |
| Vero/hSLAM    | Cercopithecus aethiops, Kidney | Ivermectin (Sheahan et al., 2020a) |                                      | ATCC Cat #CRL-1586 |        |
| HEK 293       | Homo sapiens, Embryonic Kidney | Vaccine Production              |                                      | ATCC Cat #CRL-1573 |        |
| 293 T         | Homo sapiens, Embryonic Kidney | Teicoplanin (Wu et al., 2020)    | Vaccine Production                   | ATCC# CRL-11,268 |        |
|               |                                |                                 |                                      |               |        |
| 293 FT        | Homo sapiens, Embryonic Kidney | DHODH Inhibitors                | Vaccine Production                   | ATCC CCL-7.1  |        |
| 293 T-ACE2    | Homo sapiens, Embryonic Kidney |                                |                                      |               |        |
| LLC-MK2       | Macaca mulatta, Kidney         | Virus susceptibility             |                                      | ATCC Cat# CRL-2936; RRID: CVCL_B034 |        |
| MDCKII        | Canine, Kidney                 |                                |                                      |               |        |
| BHK-21        | Mesocricetus auratus, Kidney fibroblast | MAb screening                  |                                      | ATCC Cat# CCL-10; RRID: CVCL_1915 |        |

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Table 1 (continued)

| Organ/ Tissue | Experimental Models: Cell Lines | Origin/ Details of culture model | Drug/vaccine Evaluated with reference | Availability | Remark |
|---------------|---------------------------------|---------------------------------|--------------------------------------|--------------|--------|
|               |                                 |                                 | Drug Screening | Vaccine/Antibodies Production/Immune Response/Genes Expression | Virus Isolation, Expression/Other purpose | |
| CO5-7 cells   | Cercopithecus aethiops, Kidney Fibroblast, SVO Transformed | | | Antibodies Production (Pu et al., 2020) | ATCC CRL-1651 |
| PaKi          | Chiroptera, Kidney, Primary Pteropus alecto kidney cells | | | MTHFD1 as a target of Antiviral Therapy (Xing et al., 2020) | NA |
| 293 F         | Derivative of HEK 293 Cells | | | MAb Production (Li et al., 2020; Sun et al., 2020) | ThermoFisher, Cat #R79007 |
| HK2           | Homo sapiens, Human papillomavirus 16 (HPV-16) transformed | | | Remdesivir (Hamming et al., 2004) | ATCC® CRL-2190 |
| NRK-49F       | Rattus norvegicus, Kidney Fibroblast Normal | | | Remdesivir (Hamming et al., 2004) | ATCC® CRL-1570 |
| Calu-3        | Adenocarcinoma Epithelial Type II Cells | | | EIDD-2801 (Yoshikawa et al., 2010) | ATCC Cat# HTB-55; RRID: CVCL_0699 |
| Calu-3 2B4    | Homo sapiens, Lung | | | ATAZANAVIR (Gr et al., 2020) | ATCC Cat# HTB-55; RRID: CVCL_0699 |
| A549          | Homo sapiens, Lung | | | ACE2 Gene Expression (Zhai et al., 2020b) | ATCC Cat# CRM-CCL-185; RRID: CVCL_0023 |
| NCI-H1299     | Homo sapiens, Lung | | | | ATCC Cat# CRL-5803; RRID: CVCL_0060 |
| Respiratory   | Homo sapiens, Lung epithelial virus transformed | | | | ATCC Cat# CRL-9659; RRID: CVCL_0168 |
| BEAS-2B       | Homo sapiens, Lung | | | | Most commonly used cell lines in case of SARS-CoV-2 are Calu-3 and Calu-3 2B4 |
| Hep2          | HeLa contaminant, Carcinoma | | | Vaccine Production (Manlto et al., 2020) | ATCC CCL-23 |
| Primary culture of type II alveolar cells (AT2) | Homo sapiens, Lung Alveolar Tissue | | | ACE2 Gene Expression (Pouchet et al., 2020) | N/A |
| IB3–1 and its derivative S9 | Homo sapiens, Immortalized Bronchial Epithelial Cells | | | | ATCC CRL-2777 |
| KMB17         | Homo sapiens, Lung, human embryonic lung fibroblast-like cells | | | | http://www.biofeng.com/xibao/xibaozh u/KMB-17.html |
| Liver         | Huh-7 | | | | Most commonly used cell lines in case of SARS-CoV-2 is Huh-7 |

(continued on next page)
Table 1 (continued)

| Organ/ Tissue | Experimental Models: Cell Lines | Origin/ Details of culture model | Drug/vaccine Evaluated with reference | Availability | Remark |
|---------------|--------------------------------|----------------------------------|--------------------------------------|--------------|--------|
|               |                                |                                  | Drug Screening                       |              |        |
|               |                                |                                  | Vaccine/Antibodies Production        |              |        |
|               |                                |                                  | Immune Response/Genes Expression     |              |        |
|               |                                |                                  | Virus Isolation, Expression/Other Purpose |              |        |

- HepG-2
  - Homo sapiens, Hepatocellular carcinoma
  - Hep1–6: Mus musculus, Hepatoma Epithelial Cells
  - ATCC® CRL-1830
  - HL7702: Homo sapiens, Liver-Cancer Cell Line

- X9.0
  - NA: Scandentia, Immortalized Tree shrew liver cells

- X9.5
  - Scandentia, Immortalized tree shrew liver cells

- C3A
  - Homo sapiens, Derivative of Hep-G2

- Intestinal
  - Caco-2: Homo sapiens, Colorectal adenocarcinoma Epithelial Cells

- Immune Cells
  - MT-2 cell: Homo sapiens, T cell leukemia cells

- Stems Cells
  - DYR0100: Homo sapiens, Human Induced Pluripotent Stem (IPS) Cells

- Embryo
  - NIH3T3: Mus musculus, Embryo Fibroblast

- Ovary
  - CHO-K1: Cricetulus griseus [Hamster]

- skin
  - A357: Homo sapiens, melanoma

- Leukemia
  - LR7: Ratus norvegicus, Murine LR7 cells

- Cervix
  - HeLa-CEACAM1a cells

- BALB/c immortalized cell line
  - 17C1: Mus musculus, Breed/ subspecies: BALB/c

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ACE-2 has been quantified organ wise explaining these organ/tissue-specific cells as potential in-vitro models for drug screening against COVID-19 as its entry into the target cells occur through S-protein-ACE receptor binding (Xu et al., 2020a). ACE-2 is a type I transmembrane metallocarboxypeptidase enzyme that plays a key role in the renin-angiotensin system (RAS), and the majority of it expresses in the renal tubular epithelium of the kidney, lungs, gastrointestinal tract, vascular endothelium, and Leydig cells of testes (Kuba et al., 2013; Jiang et al., 2014; Siazeck et al., 2003; Harmer et al., 2002). SARS-CoV-2 and target cells binding occur through spike protein–ACE-2 receptor interaction similar to SARS-CoV (Hoffmann et al., 2020; Li et al., 2003). Most of the used in-vitro models rely on the expression of ACE-2 and TMPRSS-2 by the cellular system and isolation, pathogenesis and all the drugs/new chemical entities evaluated upon them (Table 1).

### Table 1 (continued)

| Organ/ Tissue | Experimental Models: Cell Lines | Drug/vaccine Evaluated with reference | Availability | Remark |
|---------------|--------------------------------|--------------------------------------|--------------|--------|
| | | Drug Screening | Vaccine/Abodities Production/Immune Response/Genes Expression | Virus Isolation, Expression / Other purpose | |
| Spontaneously immortalized cell line | | Snijder et al., 2020a,b |

3.2.1. Kidney cell lines as in vitro model system for Covid19

3.2.1.1. Vero and vero E6 cell lines. Vero and Vero E6 cells are one of the most extensively used kidneys based epithelial cell lines for the culture of SARS-CoV-2 due to the presence of high expression of ACE-2 receptors as we have already discussed that SARS-CoV-2 entry in the cells takes place through S-protein–ACE2 receptors binding. The Vero cell line was established by Japanese scientists Yasumura and Kawakita in 1962 from Chlorocebus sp. of African green monkeys. Vero E6 or Vero 1008 cell line is the clone of Vero 76 cells and is a better option than Vero cells as it shows some contact inhibition and imitates properties more like primary cells. Immediately after the outbreak of SARS-CoV-2, several scientific groups used Vero and Vero E6 cells for their identification, pathogenesis as well as transmission studies (Hoffmann et al., 2020; Chan et al., 2020b; Zhou et al., 2020; Matsuyama et al., 2020), isolation (Chan et al., 2020b; Xiao et al., 2020; Caly et al., 2020a; TT-Y et al., 2020; Harcourt et al., 2020; Chan et al., 2020c; Capobianchi et al., 2020; Nie et al., 2020), and virus susceptibility (Thevarajan et al., 2020). Vero and Vero E6 cell lines are used for the immune response (Pan et al., 2020; Fukushi et al., 2006; Zheng et al., 2020), antibodies production (Pu et al., 2020), vaccine production (Mantlo et al., 2020), Type I IFNs as a target of antiviral therapy (Anderson et al., 2020), MTHFD1 as a target of Anti-viral Therapy (Xing et al., 2020), virus-induced genes expression reversal (Xiong et al., 2020a), and mAbs screening (Fintelman-Rodrigues et al., 2020). Vero cells were found to be the best screening models for new drugs as well as for already available anti-viral drugs for their repositioning as shown in Table 1 (Xiong et al., 2020a; Ge et al., 2020; Weston et al., 2020; Yao et al., 2020; Touret et al., 2020; Choy et al., 2020; Liu et al., 2020; Jin et al., 2020; Dai et al., 2020; Liu et al., 2020b; Zbijian et al., 2020; Xiong et al., 2020b; Wang et al., 2020a; Caly et al., 2020b; Sheahan et al., 2020a; Runfeng et al., 2020; Andreani et al., 2020; Su et al., 2020; Ohashi et al., 2020; Yamamoto et al., 2004; Abbott et al., 2020). Some of the drugs, such as Hydroxychloroquine, Ivermectin, Nelfinavir, lopinavir, emetine, Remdesivir, and homoharringtonine have shown effectiveness against SARS-CoV-2 in Vero and Vero E6 cells. TMPRSS-2 is another recently discovered mode of SARS-CoV-2 entry into the target cells and further spread to other organs (Hoffmann et al., 2020). In another recent study on Vero and Vero E6 cells, where Vero E6 cells expressing TMPRSS-2, enhanced the isolation of SARS-CoV-2 (Matsuyama et al., 2020). However, this is a new and unexplored area, and more studies are required in this direction to target the TMPRSS-2 based viral spread and might be a potential therapeutic target for drug development against SARS-CoV-2.

3.2.1.2. HEK293 and its variant cell lines. Other important kidney-specific cell lines that have been used for SARS-CoV2 studies include the HEK293 cell line for vaccine production (Mantlo et al., 2020), viral pathogenesis, and transmission (Hoffmann et al., 2020). 293 T cell line is derived from HEK293 cell line. It is very useful for various studies such as antiviral CRISPR (Wang et al., 2020b), virus isolation, (Dai et al., 2020), MTHFD1 as a target of anti-viral therapy (Xing et al., 2020), virus susceptibility (Thevarajan et al., 2020), viral pathogenesis/transmission (Gao et al., 2020), vaccine production (Mantlo et al., 2020), immune response (Ye et al., 2020; Ju et al., 2020), mAb production (Li et al., 2020; Sun et al., 2020), mAbs screening (Fintelman-Rodrigues et al., 2020), vaccine production and antibodies response (Mossel et al., 2005), ACE-2 gene expression in target cells (Zhang et al., 2020a), for drug screening like Teicoplanin (Wu et al., 2020). Similarly, 293 FT cell line is used for screening of DHODH Inhibitors (Wang et al., 2020a), MAb production (Li et al., 2020), and 293 F used for monoclonal antibodies production (Li et al., 2020; Sun et al., 2020); whereas, 293 T-ACE-2 cell line for vaccine production and antibodies response (Mossel et al., 2005) and viral pathogenesis (Gao et al., 2020).

3.2.1.3. Other kidney cell lines as in vitro model system. Other kidney-specific cell lines used for SARS-CoV-2 study include LLC-MK2 for virus susceptibility (Chan et al., 2013); MDCKII cell line for the screening of DHODH Inhibitors (Wang et al., 2020a), virus susceptibility (Pan et al., 2020), and viral pathogenesis and transmission (Hoffmann et al., 2020); BHK-21 cell line for MAb screening (Fintelman-Rodrigues et al., 2020); COS-7 cells for antibodies production (Pu et al., 2020); PaKi cell line for MTHFD1 as a target of Antiviral Therapy (Xing et al., 2020); and HK2 as well as NRK-49 F cell lines for Remdesivir drug screening (Hamming et al., 2004).

3.2.2. Respiratory cell lines as in vitro model system for Covid19

In the lungs, the majorities of the ACE-2-expressing cells (~83 %) are alveolar type II cells and may serve as in vitro Model system for Covid 19 studies (Zhao et al., 2020a; Zhang et al., 2020b). Similarly, oral and nasal epithelial cells also showed higher expression of ACE-2 receptors of SARS-CoV-2 (Sungnak et al., 2020; Sheahan et al., 2020a).

3.2.2.1. Calu-3 and Calu-3 2B4 cell line. Calu-3 is a lung adenocarcinoma epithelial cell line used for various SARS-CoV-2 studies (Matsuyama et al., 2020; Yoshikawa et al., 2010). Among Calu-3 and its clonally derived Calu-3 2B4, ACE-2 expression was checked, and comparatively, 2B4 have higher ACE2 expression (Lokugamage et al., 2020), suggesting them a better model system for SARS-CoV2. It has been used to check the viral pathogenesis and transmission (Hoffmann et al., 2020; Matsuyama et al., 2020; Hikmet et al., 2020) and for drug screening of EIDD-2801 (Yoshikawa et al., 2010).

3.2.2.2. Other respiratory cell lines. Other respiratory systems based in
3.2.5. Other cells as in vitro model system for Covid19

There are several other cell lines that have been used for SARS-CoV-2 studies such as T cell leukemia (MT-2) cells derived from co-culturing of human cord leukocytes and leukemia T-cells, shown susceptibility to SARS-CoV2 infection (Wang et al., 2020b); Stems Cells based cell line DYR0100 for Differentiation assay (Zebin et al., 2020); Embryo specific NIH3T3 cell line for vaccine production (Pu et al., 2020); Ovary specific CHO-K1 cell line for vaccine production (Pu et al., 2020) and virus susceptibility (Nie et al., 2020); skin based A357 and HACAT cell lines for vaccine production (Pu et al., 2020); NIH3T3 cell line for vaccine production (Mantlo et al., 2020); Ovary specific DYR0100 for Differentiation assay (Zebin et al., 2020); Embryo specific SARS-CoV2 infection (Wang et al., 2020b); Stems Cells based cell line 3.2.5. Other cells as in vitro model system for Covid19

Together In a nut-shell, cells expressing the ACE2 receptor and TMPRSS-2 could be potential In-vitro models for all types of studies against SARS-Cov-2, including molecular or biochemical studies of a virus, repurposing of drugs and their dose standardization, vaccine production, efficacy, and safety profile of newly identified lead molecules. In this regard, the knowledge of suitable cell lines becomes essential, and Vero E6, Huh-7, 293 T, Calu-3, and Caco-2 cell lines have shown high potential as in-vitro models to combat the COVID-19.

4. Conclusion

Together In a nut-shell, cells expressing the ACE2 receptor and TMPRSS-2 could be potential In-vitro models for all types of studies against SARS-Cov-2, including molecular or biochemical studies of a virus, repurposing of drugs and their dose standardization, vaccine production, efficacy, and safety profile of newly identified lead molecules. In this regard, the knowledge of suitable cell lines becomes essential, and Vero E6, Huh-7, 293 T, Calu-3, and Caco-2 cell lines have shown high potential as in-vitro models to combat the COVID-19.

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Authors Contributions

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Disclosure of Competing Interest

None of the authors declared any conflict of interest.
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