doi: 10.53446/actamednicomedia.1253701

Acta Medica Nicomedia

Cilt: 6 Sayı: 2 Haziran 2023 / Vol: 6 Issue: 2 June 2023
https://dergipark.org.tr/tr/pub/actamednicomedia

Research Article | Araştırma Makalesi

EXPRESSION LEVELS OF ACE2 AND TMPRSS2 IN DIFFERENT CELL LINES

ACE2 VE TMPRSS2 GENLERİNİN FARKLI HÜCRE HATLARINDAKİ İFADE DÜZEYLERİ

Merve Gulsen Bal Albayrak[1*], Sevinc Yanar[2], Murat Kasap[1], Gurler Akpinar[1]

[1]Kocaeli University, Medical School, Department of Medical Biology, Kocaeli, Türkiye. [2]Sakarya University, Medical School, Department of Histology and Embryology, Sakarya, Türkiye.

ABSTRACT

Objective: ACE2 and TMPRSS2 proteins have received increased attention with the outbreak of pandemic COVID-19. These proteins have roles in respiratory and hypertension disorders as well as cardiovascular and renal diseases. The objective of this work was to examine the mRNA and protein levels of ACE2 and TMPRSS2 in cell lines derived from various tissue origins.

Methods: After the growth of 14 different cell lines, protein and mRNA were isolated from the cell pellets. The amounts of mRNAs and proteins were then determined and quantified using qRT-PCR and ELISA.

Results: Findings showed that VERO, HEK293T, and VERO E6 cell lines significantly differed from others in the mRNA levels of both the ACE2 and TMPRSS2 genes. In protein levels obtained using ELISA, PNT1A cell line had the highest level of ACE2 protein expression, while for TMPRSS2, A549 had the highest level of protein expression.

Conclusions: It has found in this study how the expressions levels of ACE2 and TMPRSS2 depend on the cell type. This may be an explanation for why virulence entrance differs in different types of tissues. It is speculated that HEK293T cells with high levels of both genes may be a suitable option for studies at the RNA level by using these two genes. MCF7 may be a good candidate for studies at the protein level. Given the high levels of mRNA expression of both genes, it may be inferred that cells derived from the kidney were among those that were most susceptible to virus entry.

Keywords: ACE2, TMPRSS2, COVID19

ÖZ

Amaç: ACE2 ve TMPRSS2 proteinleri, COVID-19 pandemisi ile birlikte önem kazanmıştır. Bu proteinlerin solunum ve hipertansiyon bozukluklarının yanı sıra kardiyovasküler ve renal hastalıklarda rolleri vardır. Bu çalışmanın amacı, çeşitli dokulardan üretilen hücre hatlarında ACE2 ve TMPRSS2'nin mRNA ve protein seviyelerini belirlemektir.

Yöntem: 14 hücre hattı kültürde çoğaltıldıktan sonra, hücre pelletlerinden protein ve mRNA izole edildi. Ardından mRNA seviyeleri qRT-PCR ve protein seviyeleri ise ELISA metotları kullanılarak ölçüldü.

Bulgular: Çalışmanın sonuçları, ACE2 ve TMPRSS2 genlerinin mRNA seviyelerinin VERO, HEK293T ve VERO E6 hücre hatlarında, diğer hücre hatlarına kıyasla, önemli ölçüde yüksek olduğunu göstermiştir. ELISA kullanılarak elde edilen protein seviyelerinde, PNT1A hücre hattı en yüksek ACE2 protein ifadesine sahipken, TMPRSS2 için A549 en yüksek protein ekspresyonuna sahip olarak bulunmuştur.

Sonuç: Bu çalışmada ACE2 ve TMPRSS2 gen ifadelerinin hücre tipine göre farklılaşabileceği gösterilmiştir. Bu sonuç, farklı doku tiplerinde virüs girişinin neden farklı olduğunun bir açıklaması olabilir. Her iki genin de yüksek düzeyde bulunduğu HEK293T hücrelerinin, bu iki gen kullanılarak RNA düzeyinde yapılacak çalışmalar için uygun bir seçenek olabileceği düşünülmektedir. MCF7 hücre hattı ise protein seviyesindeki çalışmalar için iyi bir seçenek olabilir. Her iki genin yüksek mRNA ekspresyon seviyeleri göz önüne alındığında, böbrekten üretilen hücre hatlarının virüs girişine en duyarlı olanlar arasında olduğu sonucuna varılabilir.

Anahtar Kelimeler: ACE2, TMPRSS2, COVID19

*** Corresponding author/İletişim kurulacak yazar:** Merve Gulsen Bal Albayrak; Department of Medical Biology, Medical School, Kocaeli University, 41001 Kocaeli, Türkiye
Phone/Telefon: +90 (537) 984 72 04 **e-mail/e-posta:** mervegulsenbal@gmail.com
Submitted/Başvuru: 20.02.2023 • **Accepted/Kabul:** 10.05.2023 • **Published Online/ Online Yayın:** 30.06.2023

Introduction

In late 2019, a novel coronavirus, named lately as coronavirus disease 2019 (COVID-19), has started to outbreak and affected many people throughout the world. More than 762 million people were affected so far in the World and 6.9 million patients were died due to the disease.[1] Because of the growing number of studies examining the disease's mechanism and how the coronavirus 2 (SARS-CoV-2) invades healthy cells, angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) proteins have gained prominence.

In healthy cells, by regulating blood pressure and maintaining electrolyte balance, ACE2 plays a key role in the renin-angiotensin system (RAS) of the regulation of cardiovascular and renal functions[3], In COVID-19, ACE2 was discovered as a gate in the virus entrance mechanism. It has been shown that the transmembrane protein ACE2 serves as a receptor for the spike (S) protein of the SARS-CoV-2 virus.[4-7] This S protein consists of two subunits as S1 and S2. When S1 binds to ACE2, S2 is cleaved by transmembrane serine protease 2 (TMPRSS2) and the virus is taken inside the cells by invagination of the cell membrane.[7-10]

Regulations in ACE2 protein levels have been linked to different diseases such as cardiovascular diseases[11], hypertension[12], lung injuries[13,14] and, respiratory system diseases.[3,15] It has been shown in studies that SARS-CoV, which caused more than 800 deaths in 2003, uses the ACE2 receptor for cell entry.[16-19] Since ACE2 is linked to many diseases including COVID-19, it is important to determine both RNA and protein levels of this enzyme in different types of cells.

TMPRSS2 is a type II transmembrane serine protease. Even though its biological function is not clear, it is thought that TMPRSS2 is responsible for the initiation of extracellular matrix (ECM) proteolysis.[21] Additionally, the androgen receptor (AR) is a transcription factor in for TMPRSS2 gene and it promotes gene expression. Upon increased expression of the gene, viruses primed into the cells with the help of protease activity of that gene.[22] TMPRSS2 proteins appear vital for the entrance of different types of viruses into cells such as H1N1 and H7N9 influenza viruses, SARS-CoV, MERS-CoV, and finally SARS-CoV 2 coronaviruses.[23] In androgen receptor-dependent prostate cancer cells, mRNA levels of TMPRSS2 gene were found to be increased.[24-26]mRNA expression level of TMPRSS2 was demonstrated in different tissues such as prostate, breast, kidney, and colon.[24,25,27]

Although there are many *in vivo* and clinical studies investigating the expression of ACE2 and TMPRSS2 in different types of tissues[16,28,29], *in vitro* studies on the expression of these genes are very limited. In the recent study, ACE2 and TMPRSS2 levels were compared with the RT-PCR method using various cell lines, it was shown that both genes were expressed in HUVEC, A549 and HaCaT cells.[30] In another study, it has been demonstrated that ACE2 expression was found in 16HBE and VeroE6 cells, while it was not expressed in BEAS2B and A549 cells.[31] In a different study, high-throughput mRNA sequencing was used to identify potential cell lines for ACE2 analysis. Nevertheless, expression could not be confirmed in some cell types including Alveolar type II cells such as A549.[32] In another study in which primary cells were used, it was reported that airway epithelial cells highly expressed ACE2 and TMPRSS2 proteins.[33] However, primary epithelial cells are not a good option for *in vitro* studies due to disadvantages such as being expensive and having a restricted proliferative capacity. Some other *in vitro* studies have used Caco-2[34], Calu-3[35], HEK293T[36], and Huh7[35] cells and have been shown to express varying levels of ACE2 and/or TMPRSS2. Studies with a limited number of cell lines have shown that the cell line with the highest expression level of ACE2 is VeroE6[16,30,36].

Studies on ACE2 and TMPRSS2 appear to focus more on tissues than cells. Additionally, most studies seem to be predominantly conducted at the RNA level. It is noteworthy that protein expression levels of those genes are typically not verified. Since the results collected from various studies are both insufficient and conflicting, it is clear that determining both RNA and protein levels of these genes in different cell types is necessary in order to provide data for future *in vitro* investigations.

In this study, it was aimed to compare the levels of ACE2 and TMPRSS2 in cell lines of different tissue origins at both mRNA and protein levels. The results of this research will contribute to studies those investigating the roles of ACE2 and TMPRSS2 in diseases such as COVID-19 either separately or together. Additionally, this research may be used as a benchmark when investigating the processes or mechanisms connected to these genes. These findings will contribute to studies examining the involvement of ACE2 and TMPRSS2 in diseases, separately or together, as well as contribute to the elucidation of the pathways and mechanisms related to these genes.

Methods

Briefly, after fourteen different cell lines were cultured, proteins and mRNAs were isolated from the cell pellets. Then, the levels of mRNAs and proteins were determined by using qRT-PCR and ELISA, respectively (Figure 1).

Cell Lines and Culture Conditions

Fourteen different cell lines were used in this study (Table 1). Vero, Vero E6, HEK293, MRC5, A549, DMS114, MCF7, HeLa, SHSY5Y, BJ and HUVEC cells were incubated in Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific, USA), PNT1A, DU145, LNCaP cells were incubated in Roswell Park Memorial Institute 1640 medium (RPMI, Thermo Fisher Scientific, USA). All media contained 10% fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific, USA) and 1% penicillin/streptomycin (Gibco, Thermo Fisher Scientific, USA). Cells were cultured in a 37°C incubator (Thermo Fisher Scientific, USA) with 5% CO_2.

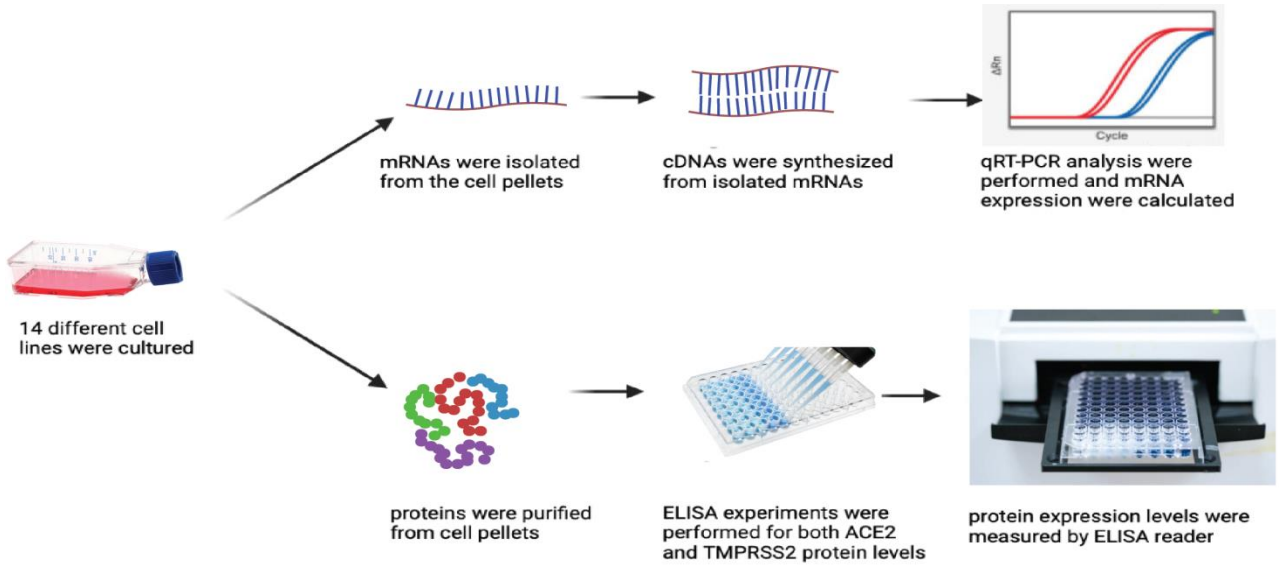

Figure 1. Schematic workflow of experimental methodology

Table 1. Properties of the cell lines that were used in the study

Cell line	Organism	Tissue	Cell type	Disease
Vero	*Cercopithecus aethiops*	Kidney	Epithelial	Normal
Vero E6	*Cercopithecus aethiops*	Kidney	Epithelial	Normal
HEK293	*Homo sapiens*	Embryonic Kidney	Epithelial	Normal
PNT1A	*Homo sapiens*	Prostate	Epithelial	Normal
DU145	*Homo sapiens*	Prostate	Epithelial	Carcinoma
LNCaP	*Homo sapiens*	Prostate	Epithelial	Carcinoma
MRC5	*Homo sapiens*	Lung	Fibroblast	Normal
A549	*Homo sapiens*	Lung	Epithelial-Like	Carcinoma
DMS114	*Homo sapiens*	Lung	Epithelial	Carcinoma
MCF7	*Homo sapiens*	Mammary Gland	Epithelial	Adenocarcinoma
HeLa	*Homo sapiens*	Cervix	Epithelial	Adenocarcinoma
SHSY5Y	*Homo sapiens*	Bone Marrow	Epithelial	Neuroblastoma
BJ	*Homo sapiens*	Skin	Fibroblast	Normal
HUVEC	*Homo sapiens*	Umbilical	Endothelial	Normal

RNA Isolation and Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from the pellets of cultured cells using the QIAGEN ® RNeasy Mini Kit (QIAGEN, USA) according to described by Yanar and her colleagues.[37] The purity and concentration of the isolated RNAs were determined by Nanodrop (Thermo Scientific, USA). Then, cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). From the obtained cDNAs, a qRT-PCR reaction was set up using ACE2 and β -actin primers (QIAGEN, USA). Using SYBR™ Green PCR Master Mix (ThermoFisher Scientific, USA) and the commercially obtained primers listed in Table 2, reactions were performed in accordance with the manufacturer's instructions using a Roche Light Cycler 480 System (Roche, USA). β-actin was used as a reference gene. Each experiment was set up as three replicates.

Table 2. Primers used in qRT-PCR experiments

Primer	Source	Annealing Temperature	Product size (Bp, length)	Gene Globe ID
ACE2	NM_021804 RT² qPCR Primer Assay, QIAGEN, USA	60˚C	3519	PPH 02572A-200
TMPRSS2	NM_005656 RT² qPCR Primer Assay, QIAGEN, USA	60˚C	3212	PPH 02262C-200
β-actin	NM_001101 RT² qPCR Primer Assay, QIAGEN, USA	60˚C	1582	PPH 00073G-200

Protein Isolation and Enzyme-Linked Immunosorbent Assay (ELISA)

Protein isolation was performed according to as described by Albayrak with small modifications.[38] Cell pellets were suspended in MPER buffer (Pierce Inc., USA) and then SSB14B 1.4 mm stainless steel beads and protease inhibitor cocktail were added. After they were homogenized with Next Advance homogenizer (Bullet Blender, Next Advance, Troy, NY, USA) the samples were centrifuged at 20,000 ×g for 30 min at 4°C and the supernatant containing the proteins was taken into fresh tubes. Concentrations of protein samples were measured and calculated using the Bradford Assay (Bio-Rad, USA) using a Nano Drop ND-1000 spectrophotometer (Thermo Scientific, USA). Proteins were stored at -80°C until used in experiments.

ELISA tests were performed using 200 µg of each protein sample. The amount of ACE2 and TMPRSS2 proteins were analyzed using commercial ELISA kits (Elabscience, E-EL-H0281 and E-EL-H1418 USA) according to the manufacturer's instructions.

Each experiment was performed in three replicates. Briefly, 100 µl sample was added to each well and incubated at 37°C for 90 minutes. After the liquid was removed, 100 µl of biotinylated detection solution was added to each well and incubated at 37°C for 1 hour. After washing for three times, 100 µl of HRP conjugate was added to each well this time and incubated at 37°C for 30 minutes. After the liquid was taken out, washing was done for five times and 90 µl of substrate solution was added and incubated at 37°C for 15 minutes. Finally, 50 µl of stop solution was added and the OD at 450 nm was measured with an ELISA plate reader. The results were evaluated for further studies.

Results

The aim of the study was to determine the expression levels of the ACE2 and TMPRSS2 genes in fourteen different type of cell lines. For the investigation of transcription levels, mRNA expressions were determined by qRT-PCR analysis. In that analysis, β-actin gene was used as reference gene and the mRNA expression was calculated based on their CT values (Figure 2).

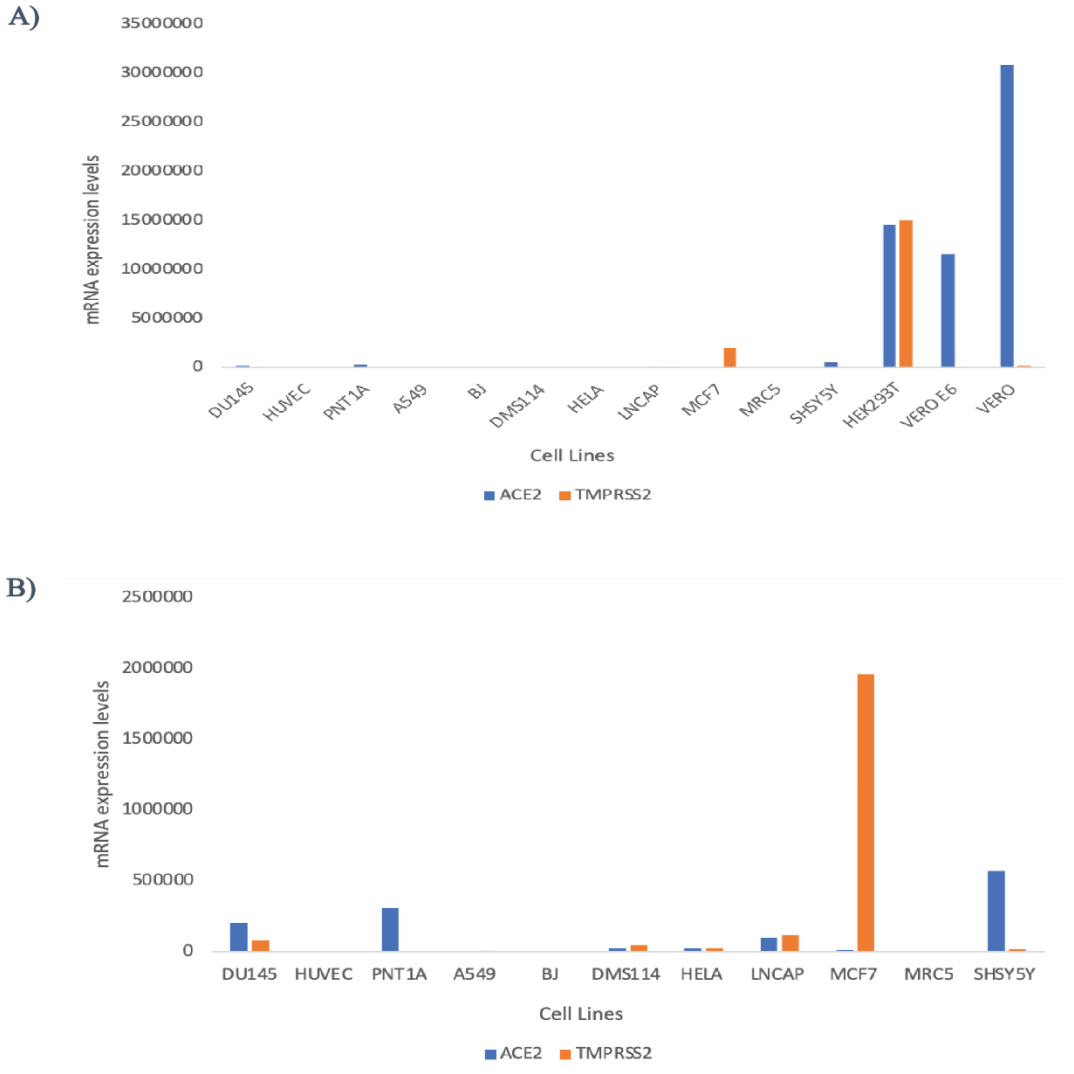

Figure 2. Bar graph of mRNA levels of cell lines A) mRNA levels of ACE2 and TMPRSS2 in 14 cell lines. B) mRNA levels of ACE2 and TMPRSS2 in 11 cell lines after Vero, Vero E6 and HEK293T were excluded) (Blue bars: ACE2, orange bars: TMPRSS2)

According to the results, there was a dramatic difference in the mRNA levels of both ACE2 and TMPRSS2 genes in VERO, HEK293T and VERO E6 cell lines, compared to the other eleven cell lines. When these fold increases were displayed in a single bar graph, other cell lines were hardly detected (Figure 2A). When these three cell lines were removed and a bar graph was generated again, the mRNA levels continued in order as SHSY5Y, PNT1A, and DU145 for ACE2 and MCF7, and LNCaP for TMPRSS2 (Figure 2B). The mRNA levels of both genes were highest in the HEK293T cell line when two genes were evaluated together.

After the mRNA levels of the genes were determined by using qRT-PCR, determination and calculation of the protein levels of ACE2 and TMPRSS2, ELISA experiments were performed afterwards protein purifications from all cell lines (Figure 3). According to Figure 3, ACE2 protein expression was highest in PNT1A cell line and MCF7, HEK293T, HELA and SHSY5Y were following in order whereas for TMPRSS2, A549 has the highest in protein expression and MCF7 and PNT1A were following.

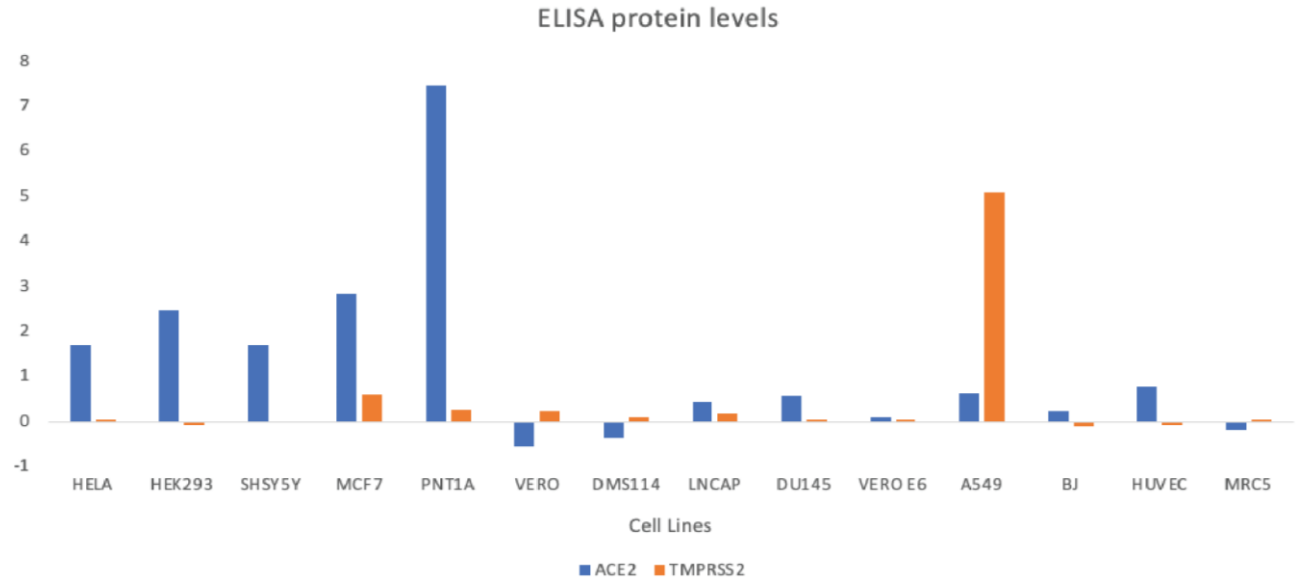

Figure 3. ACE2 and TMPRSS2 protein levels of the cell lines detected by ELISA (blue bars for ACE2 protein levels while red bars for TMPRSS2)

Since TMPRSS2 is a key enzyme in prostate cancer[39], prostate cancer cell lines were evaluated separately. As seen in figure 4, while TMPRSS2 expression was higher in LNCaP cells compared to DU145 cells, ACE2 expression was the opposite (Figure 4A). Same trend was observed for the protein level in ELISA (Figure 4B).

Discussion

With the global COVID-19 disease outbreak, researches related to the mechanism of how SARS-CoV-2 virus enters and spreads within cells has been accelerated in speed and increased in tendency. According to the findings, ACE2 and TMPRSS2 proteins estimated to have a role in the virus entrance to the cells; however, it is still a bottleneck to make conclusion from these studies[40]. These proteins function in different cellular mechanisms in healthy cells and their expression levels have been associated with diseases like hypertension, cardiac or lung injuries.[24] The studies investigating the protein expression levels are mostly in tissue samples not in cell lines. Additionally, there are some inconsistences in the expression levels of ACE2 and TMPRSS2 between different studies.[41-44] To make contribution to the literature and offer data for future *in vitro* studies, protein and mRNA levels of ACE2 and TMPRSS2 in fourteen different cell lines were investigated in this study.

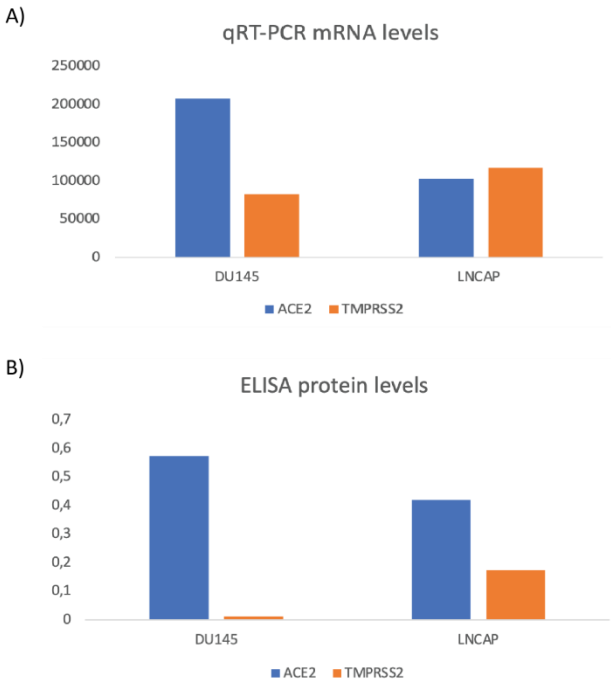

Figure 4. ACE2 and TMPRSS2 expression levels for DU145 and LNCaP cell lines A) mRNA levels detected by qRT-PCR, B) Protein levels detected by ELISA

We have demonstrated using our experimental study that how the expressions of ACE2 and depend on the cell

type. Some of the cell lines did not express one or even both of the proteins, while some others showed significant expression levels. This may be an explanation for why virulence entrance differs in different types of tissues. In COVID-19 Disease, for SARS-COV2 virus to enter the cells, ACE2 protein functions as a gate for the virus. TMPRSS2 seems to act as protease responsible for the S2 protein of the virus cleavage. However, TMPRSS2 route is not the only way for the virus to invade. Apart from the TMPRSS2, the SARS-CoV-2 fusion can be activated via cathepsin-mediated systems together with ACE2 receptor binding.[45] It can be speculated that, the expression of TMPRSS2 is not vital for virus infection however the presence of both ACE2 and TMPRSS2 at the same time may increase the pathogenicity and susceptibility of the virus infection.[46] For that reason, it is important to determine which type of cells express ACE2 or TMPRSS2 alone or together.

According to our findings, highest mRNA expression of ACE2 gene was found in Vero, Vero E6 and HEK293T cells. Vero and Vero E6 cell lines originated from the kidney tissue of a species of monkey called Cercopithecus aethiops. Those cell lines are mostly used in virus replication and investigation studies. Especially, Vero E6 is often preferred for replication of viruses because of its fast growth rate, low probability of malignancy, their sensitivity to a variety of viruses.[47,48] However, the mRNA expression of TMPRSS2 gene is significantly low in these cell lines. If mRNA levels of both proteins are expected to be higher amounts for investigations of the functions of those genes or associated mechanisms, it might not be suitable to use those cell lines for a model cell. Additionally, employing such cell lines to research diseases with human origins may not produce accurate results due to their dissimilar origin, it might be required confirmation using cell lines having human origins. In the ELISA data in Figure 3, protein expression levels of the genes in these cell lines are not found significant. Moreover, since those cell lines were originated from different species, the results of the mRNA levels of those genes do not represent the human kidney data. HEK293T cell lines may possible candidate for as represent the human kidney data, since both ACE2 and TMPRSS2 genes were expressed in roughly comparable amounts in mRNA level and at maximum level compared to other cell lines originated from human tissues. For that reason, HEK293T cells might be suitable for to investigate mechanisms related to mRNA of ACE2 and/or TMPRSS2 genes. However, TMPRSS2 is much lower in protein level than ACE2, and this discrepancy may be explained by the possibility that TMPRSS2 protein turnover is higher than that of ACE2 protein. Furthermore, since highest mRNA levels of both genes were found in cell lines originated from kidney tissues in, it can be estimated that that kidney tissue is much more susceptible for virus entrance. According to meta-analysis data of Gkogkou, it is also indicated that both ACE2 and TMPRSS2 are highly expressed in kidney tissues.[49] According to the study investigating RT-PCR expression levels of ACE2 and TMPRSS2 genes in mouse ocular and systemic tissues, kidney had the highest expression levels for both genes.[30] Our observations are consistent with the literature; therefore, it can be assumed that kidney-originating cells were among those that were most vulnerable to virus entry because of the high levels of both genes' mRNA expression.

TMPRSS2 is a frequently altered gene in prostate cancer mostly in fused form with ERG gene.[50,51] TMPRSS2 expression was significantly increased in response to androgens in cDNA microarray analysis.[25] Similarly, it was reported that androgen regulates both ACE2 and TMPRSS2 expression levels in mouse tissues and androgen levels may be targeted for alternative COVID-19 treatment.[52] In this study, in order to reveal the correlation of androgen receptors with ACE2 and TMPRSS2 expression levels, prostate cancer cell lines were evaluated separately. Besides a healthy prostate cell line PNT1A, we have used two prostate cancer cell line as LNCaP for androgen dependent[53] and DU145 for androgen independent.[54] According to our findings, which are in line with the literature, TMPRSS2 mRNA levels in the LNCaP cells were higher than those in the DU145 cells. The situation was the opposite for ACE2 mRNA levels, though. The level was higher in DU145 cell line compared to LNCaP (Figure 4). This is because there is no clear evidence that ACE2 gene expression is altered by androgen receptor. However, apart from androgen receptor expression, there might be other factors affecting ACE2 expression. In the study of Baratchian and his colleagues, it is reported that the suppression of ACE2 expression with anti-androgen drugs depended on sex differences.[55] Mjaess and his colleagues also stated that because of the androgen dependence, the risk for cardiovascular diseases or COVID-19 may increase in male patients.[56]

It is quite expected that protein expression levels might differ from mRNA levels. The amount of protein produced from an mRNA molecule varies depending on many factors such as translation rates, post-translational modifications such as alternative splicing and RNA methylations, protein damage or degradation, protein half-lives, and stability of the proteins. In addition, another reason for the lack of correlation in RNA-protein levels may be related to the fact that ACE2 protein is a membrane protein. Membrane proteins are mostly flexible and unstable. Therefore, they cause difficulties in studies such as expression, solubilization, purification, and crystallization.[57] On the other hand, in general, the obtained data from the cell lines is consistent with the data obtained from the tissues in the literature. Generally, both in the tissues of kidney, prostate and breast and the cell lines obtained from those adjacent tissues, expressions of both genes were elevated. The differences might be due to the difference between cell lines and the characteristics of the tissues.[49]

In conclusion, in light of the data we have obtained, it is thought that HEK293 cells with high levels of both genes may be a suitable option for studies to be carried out at the RNA level by using these two genes. MCF7 may be a good option for studies at the protein level. Although the

TMPRSS2 protein level in A549 cells is higher than MCF7, its usage is not recommended because the ACE2 level remains too low compared to MCF7. It would be appropriate to decide which gene is at the forefront for studies to be conducted at both the RNA and protein levels, in which both genes will be studied. We think that it may be appropriate to use HEK293 cells in studies where ACE2 will be at the forefront, and MCF7 cells in studies where TMPRSS2 will be at the forefront. Based on the literature, ACE2 and TMPRSS2 may be a gate for the entrance of coronavirus species. Therefore, the data obtained here will be a source for future studies not only for COVID-19 but also possible future coronavirus species. Additionally, ACE2 and TMPRSS2 became popular with COVID-19 pandemic, yet those genes are related to severe diseases like lung, renal or cardiac disorders. For this reason, it is important to obtain protein and mRNA levels of them in different tissues for further studies related to those diseases. This study is offering the data in which the protein and mRNA levels of ACE2 and TMPRSS2 genes in different types of cell lines for the researchers who would like to study either virus related studies like COVID-19 or the genes related diseases like cardiac, renal or lung diseases. For researchers who are interested in studying either virus-related studies like COVID-19 or gene-related disorders like cardiac, renal, or lung ailments, this study is providing data on the protein and mRNA levels of ACE2 and TMPRSS2 in various types of cell lines. It will be appropriate to support these data with further studies such as investigating protein stability, crystallography, or function to validate mRNA-protein consistency.

Compliance with Ethical Standards

Since this study was in vitro cell line study, there were no need for ethical approval.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contribution

MGBA, SY: Study idea, hypothesis, study design; MGBA, SY, MK, GA: Material preparation, data collection and analysis; MGBA, SY: Writing the first draft of the article; GA, MK: Critical review of the article finalization and publication process.

Financial Disclosure

Financial disclosure none.

References

1. Ritchie H, Mathieu E, Rodés-Guirao L, et al. Coronavirus Pandemic (COVID-19). Accessed April 17, 2023. https://ourworldindata.org/coronavirus
2. Imai Y, Kuba K, Ohto-Nakanishi T, Penninger JM. Angiotensin-Converting Enzyme 2 (ACE2) in Disease Pathogenesis. *Circ J*. 2010;74(3):405-410. doi:10.1253/circj.cj-10-0045
3. Imai Y, Kuba K, Rao S, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature*. 2005;436(7047):112-116. doi:10.1038/nature03712
4. Gurwitz D. Angiotensin receptor blockers as tentative SARS-CoV-2 therapeutics. *Drug Develop Res*. 2020;81(5):537-540. doi:10.1002/ddr.21656
5. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet Lond Engl*. 2020;395(10224):565-574. doi:10.1016/s0140-6736(20)30251-8
6. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. *J Virol*. 2020;94(7):e00127-20. doi:10.1128/jvi.00127-20
7. Rezaei M, Ziai SA, Fakhri S, Pouriran R. ACE2: Its potential role and regulation in severe acute respiratory syndrome and COVID-19. *J Cell Physiol*. 2021;236(4):2430-2442. doi:10.1002/jcp.30041
8. Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. *Nat Rev Mol Cell Biology*. 2022;23(1):3-20. doi:10.1038/s41580-021-00418-x
9. Reindl-Schwaighofer R, Hödlmoser S, Eskandary F, et al. ACE2 Elevation in Severe COVID-19. *Am J Resp Crit Care*. 2021;203(9):1191-1196. doi:10.1164/rccm.202101-0142le
10. Beyerstedt S, Casaro EB, Rangel ÉB. COVID-19: angiotensin-converting enzyme 2 (ACE2) expression and tissue susceptibility to SARS-CoV-2 infection. *Eur J Clin Microbiol*. 2021;40(5):905-919. doi:10.1007/s10096-020-04138-6
11. Crackower MA, Sarao R, Oudit GY, et al. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature*. 2002;417(6891):822-828. doi:10.1038/nature00786
12. Zhong J, Yan Z, Liu D, et al. Association of angiotensin-converting enzyme 2 gene A/G polymorphism and elevated blood pressure in Chinese patients with metabolic syndrome. *J Laboratory Clin Medicine*. 2006;147(2):91-95. doi:10.1016/j.lab.2005.10.001
13. Burrell LM, Risvanis J, Kubota E, et al. Myocardial infarction increases ACE2 expression in rat and humans. *Eur Heart J*. 2005;26(4):369-375. doi:10.1093/eurheartj/ehi114
14. Goulter AB, Goddard MJ, Allen JC, Clark KL. ACE2 gene expression is up-regulated in the human failing heart. *Bmc Med*. 2004;2(1):19-19. doi:10.1186/1741-7015-2-19
15. Marshall RP, Webb S, Bellingan GJ, et al. Angiotensin Converting Enzyme Insertion/Deletion Polymorphism Is Associated with Susceptibility and Outcome in Acute Respiratory Distress Syndrome. *Am J Resp Crit Care*. 2002;166(5):646-650. doi:10.1164/rccm.2108086
16. Sato T, Ueha R, Goto T, Yamauchi A, Kondo K, Yamasoba T. Expression of ACE2 and TMPRSS2 proteins in the upper and lower aerodigestive tracts of rats. *Biorxiv*. Published online 2020:2020.05.14.097204. doi:10.1101/2020.05.14.097204
17. Song H, Seddighzadeh B, Cooperberg MR, Huang FW. Expression of ACE2, the SARS-CoV-2 receptor, and TMPRSS2 in prostate epithelial cells. *Biorxiv*. Published online 2020:2020.04.24.056259. doi:10.1101/2020.04.24.056259
18. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS

coronavirus. *Nature*. 2003;426(6965):450-454. doi:10.1038/nature02145

19. Xu J, Chu M, Zhong F, et al. Digestive symptoms of COVID-19 and expression of ACE2 in digestive tract organs. *Cell Death Discov*. 2020;6(1):76. doi:10.1038/s41420-020-00307-w
20. Kuba K, Imai Y, Rao S, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury. *Nat Med*. 2005;11(8):875-879. doi:10.1038/nm1267
21. (NCATS) B (MD): NC for ATS. TMPRSS2 enzymatic activity. SARS-CoV-2 Assays [Internet]. Published 2020. Accessed September 29, 2022. https://www.ncbi.nlm.nih.gov/books/NBK579897/
22. Mohamed MS, Moulin TC, Schiöth HB. Sex differences in COVID-19: the role of androgens in disease severity and progression. *Endocrine*. 2021;71(1):3-8. doi:10.1007/s12020-020-02536-6
23. Shen LW, Mao HJ, Wu YL, Tanaka Y, Zhang W. TMPRSS2: A potential target for treatment of influenza virus and coronavirus infections. *Biochimie*. 2017;142:1-10. doi:10.1016/j.biochi.2017.07.016
24. Singh H, Choudhari R, Nema V, Khan AA. ACE2 and TMPRSS2 polymorphisms in various diseases with special reference to its impact on COVID-19 disease. *Microb Pathogenesis*. 2021;150:104621-104621. doi:10.1016/j.micpath.2020.104621
25. Lin B, Ferguson C, White JT, et al. Prostate-localized and androgen-regulated expression of the membrane-bound serine protease TMPRSS2. *Cancer Res*. 1999;59(17):4180-4184.
26. Afar DE, Vivanco I, Hubert RS, et al. Catalytic cleavage of the androgen-regulated TMPRSS2 protease results in its secretion by prostate and prostate cancer epithelia. *Cancer Res*. 2001;61(4):1686-1692.
27. Jacquinet E, Rao NV, Rao GV, Hoidal JR. Cloning, genomic organization, chromosomal assignment and expression of a novel mosaic serine proteinase: epitheliasin. *Febs Lett*. 2000;468(1):93-100. doi:10.1016/s0014-5793(00)01196-0
28. Zhang H, Rostami MR, Leopold PL, et al. Expression of the SARS-CoV-2 ACE2 Receptor in the Human Airway Epithelium. *Am J Resp Crit Care*. 2020;202(2):219-229. doi:10.1164/rccm.202003-0541oc
29. Dong M, Zhang J, Ma X, et al. ACE2, TMPRSS2 distribution and extrapulmonary organ injury in patients with COVID-19. *Biomed Pharmacother*. 2020;131:110678. doi:10.1016/j.biopha.2020.110678
30. Ma D, Chen CB, Jhanji V, et al. Expression of SARS-CoV-2 receptor ACE2 and TMPRSS2 in human primary conjunctival and pterygium cell lines and in mouse cornea. *Eye*. 2020;34(7):1212-1219. doi:10.1038/s41433-020-0939-4
31. Kam YW, Okumura Y, Kido H, Ng LFP, Bruzzone R, Altmeyer R. Cleavage of the SARS Coronavirus Spike Glycoprotein by Airway Proteases Enhances Virus Entry into Human Bronchial Epithelial Cells In Vitro. *Plos One*. 2009;4(11):e7870. doi:10.1371/journal.pone.0007870
32. Hikmet F, Méar L, Edvinsson Å, Micke P, Uhlén M, Lindskog C. The protein expression profile of ACE2 in human tissues. *Biorxiv*. Published online 2020:2020.03.31.016048. doi:10.1101/2020.03.31.016048
33. Takayama K. In vitro and Animal Models for SARS-CoV-2 research. *Trends Pharmacol Sci*. 2020;41(8):513-517. doi:10.1016/j.tips.2020.05.005
34. Elfiky AA. Anti-HCV, nucleotide inhibitors, repurposing against COVID-19. *Life Sci*. 2020;248:117477. doi:10.1016/j.lfs.2020.117477
35. Ji Y, Ma Z, Peppelenbosch MP, Pan Q. Potential association between COVID-19 mortality and health-care resource availability. *Lancet Global Heal*. 2020;8(4):e480. doi:10.1016/s2214-109x(20)30068-1
36. Harcourt J, Tamin A, Lu X, et al. Isolation and characterization of SARS-CoV-2 from the first US COVID-19 patient. *Biorxiv*. Published online 2020:2020.03.02.972935. doi:10.1101/2020.03.02.972935
37. Yanar S, Kasap M, Kanli A, Akpinar G, Sarihan M. Proteomics analysis of meclofenamic acid-treated small cell lung carcinoma cells revealed changes in cellular energy metabolism for cancer cell survival. *J Biochem Mol Toxicol*. Published online 2022:e23289. doi:10.1002/jbt.23289
38. Albayrak MGB, Simsek T, Kasap M, Akpinar G, Canturk NZ, Guler SA. Tissue proteome analysis revealed an association between cancer, immune system response, and the idiopathic granulomatous mastitis. *Med Oncol*. 2022;39(12):238. doi:10.1007/s12032-022-01845-2
39. Vaarala MH, Porvari K, Kyllönen A, Lukkarinen O, Vihko P. The TMPRSS2 gene encoding transmembrane serine protease is overexpressed in a majority of prostate cancer patients: Detection of mutated TMPRSS2 form in a case of aggressive disease. *Int J Cancer*. 2001;94(5):705-710. doi:10.1002/ijc.1526
40. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*. 2020;181(2):271-280.e8. doi:10.1016/j.cell.2020.02.052
41. Bertram S, Heurich A, Lavender H, et al. Influenza and SARS-Coronavirus Activating Proteases TMPRSS2 and HAT Are Expressed at Multiple Sites in Human Respiratory and Gastrointestinal Tracts. *Plos One*. 2012;7(4):e35876. doi:10.1371/journal.pone.0035876
42. Hamming I, Timens W, Bulthuis M, Lely A, Navis G, Goor H van. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathology*. 2004;203(2):631-637. doi:10.1002/path.1570
43. Ren X, Glende J, Al-Falah M, et al. Analysis of ACE2 in polarized epithelial cells: surface expression and function as receptor for severe acute respiratory syndrome-associated coronavirus. *J Gen Virol*. 2006;87(6):1691-1695. doi:10.1099/vir.0.81749-0
44. Ortiz ME, Thurman A, Pezzulo AA, et al. Heterogeneous expression of the SARS-Coronavirus-2 receptor ACE2 in the human respiratory tract. *Ebiomedicine*. 2020;60:102976. doi:10.1016/j.ebiom.2020.102976
45. Ou T, Mou H, Zhang L, Ojha A, Choe H, Farzan M. Hydroxychloroquine-mediated inhibition of SARS-CoV-2 entry is attenuated by TMPRSS2. *Plos Pathog*. 2021;17(1):e1009212. doi:10.1371/journal.ppat.1009212
46. Iwata-Yoshikawa N, Okamura T, Shimizu Y, Hasegawa H, Takeda M, Nagata N. TMPRSS2 Contributes to Virus Spread and Immunopathology in the Airways of Murine Models after Coronavirus Infection. *J Virol*. 2019;93(6):e01815-18. doi:10.1128/jvi.01815-18
47. Ammerman NC, Beier-Sexton M, Azad AF. Growth and Maintenance of Vero Cell Lines. *Curr Protoc Microbiol*. 2008;11(1):A.4E.1-A.4E.7. doi:10.1002/9780471729259.mca04es11

48. Shen CF, Guilbault C, Li X, et al. Development of suspension adapted Vero cell culture process technology for production of viral vaccines. *Vaccine*. 2019;37(47):6996-7002. doi:10.1016/j.vaccine.2019.07.003
49. Gkogkou E, Barnasas G, Vougas K, Trougakos IP. Expression profiling meta-analysis of ACE2 and TMPRSS2, the putative anti-inflammatory receptor and priming protease of SARS-CoV-2 in human cells, and identification of putative modulators. *Redox Biol*. 2020;36:101615. doi:10.1016/j.redox.2020.101615
50. Tomlins SA, Rhodes DR, Perner S, et al. Recurrent Fusion of TMPRSS2 and ETS Transcription Factor Genes in Prostate Cancer. *J Urology*. 2006;175(5):1707. doi:10.1016/s0022-5347(06)00096-6
51. Wang Z, Wang Y, Zhang J, et al. Significance of the TMPRSS2:ERG gene fusion in prostate cancer. *Mol Med Rep*. 2017;16(4):5450-5458. doi:10.3892/mmr.2017.7281
52. Deng Q, Rasool R ur, Russell RM, Natesan R, Asangani IA. Targeting androgen regulation of TMPRSS2 and ACE2 as a therapeutic strategy to combat COVID-19. *Iscience*. 2021;24(3):102254. doi:10.1016/j.isci.2021.102254
53. Siciliano T, Sommer U, Beier AMK, et al. The Androgen Hormone-Induced Increase in Androgen Receptor Protein Expression Is Caused by the Autoinduction of the Androgen Receptor Translational Activity. *Curr Issues Mol Biol*. 2022;44(2):597-608. doi:10.3390/cimb44020041
54. Minamiguchi K, Kawada M, Someno T, Ishizuka M. Androgen-independent prostate cancer DU145 cells suppress androgen-dependent growth of prostate stromal cells through production of inhibitory factors for androgen responsiveness. *Biochem Bioph Res Co*. 2003;306(3):629-636. doi:10.1016/s0006-291x(03)01023-4
55. Baratchian M, McManus JM, Berk MP, et al. Androgen regulation of pulmonary AR, TMPRSS2 and ACE2 with implications for sex-discordant COVID-19 outcomes. *Sci Rep-uk*. 2021;11(1):11130. doi:10.1038/s41598-021-90491-1
56. Mjaess G, Karam A, Aoun F, Albisinni S, Roumeguère T. COVID-19 and the male susceptibility: the role of ACE2, TMPRSS2 and the androgen receptor. *Prog Urol*. 2020;30(10):484-487. doi:10.1016/j.purol.2020.05.007
57. Carpenter EP, Beis K, Cameron AD, Iwata S. Overcoming the challenges of membrane protein crystallography. *Curr Opin Struc Biol*. 2008;18(5):581-586. doi:10.1016/j.sbi.2008.07.001