Review Article

Vulnerability of The Male Reproductive System to SARS-CoV-2 Invasion: Potential Role for The Endoplasmic Reticulum Chaperone Grp78/HSPA5/BiP

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Abstract
Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) may adversely affect male reproductive tissues and male fertility. This concern is elicited by the higher susceptibility and mortality rate of men to the SARS-CoV-2 mediated coronavirus disease-19 (COVID-19), compared to the women. SARS-CoV-2 enters host cells after binding to a functional receptor named angiotensin-converting enzyme-2 (ACE2) and then replicates in the host cells and gets released into the plasma. SARS-CoVs use the endoplasmic reticulum (ER) as a site for viral protein synthesis and processing, as well as glucose-regulated protein 78 (Grp78) is a key ER chaperone involved in protein folding by preventing newly synthesized proteins from aggregation. Therefore, we analyzed Grp78 expression in various human organs, particularly male reproductive organs, using Broad Institute Cancer Cell Line Encyclopedia (CCLE), the Genotype-Tissue Expression (GTEx), and Human Protein Atlas online datasets. Grp78 is expressed in male reproductive tissues such as the testis, epididymis, prostate, and seminal vesicle. It can facilitate the coronavirus entry into the male reproductive tract, providing an opportunity for its replication. This link between the SARS-CoV-2 and the Grp78 protein could become a therapeutic target to mitigate its harmful effects on male fertility.

Keywords: COVID-19, Endoplasmic Reticulum, Grp78, Male Infertility, SARS-CoV-2

Introduction

The World Health Organization declared coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), as a global pandemic on March 11, 2020 (1). According to data from Johns Hopkins University's Center for Systems Science and Engineering, there have been >400 million reported cases of COVID-19 worldwide, with >5 million deaths until July 2021 (https://coronavirus.jhu.edu/map.html) (2). Interestingly, clinical data emerging from COVID-19 demonstrate that male patients constitute 56-73% of the infected population (3). In addition, higher morbidity and mortality rates of SARS-CoV-2- infected males than age-matched females suggest sex-based differences in COVID-19 outcomes (4).

Severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2 invade human cells through the angiotensin-converting enzyme-2 (ACE2) receptor and

transmembrane serine protease 2 (TMPRSS2) (5, 6). As the 79% amino acid sequence identity of SARS-CoV-2 is similar to SARS-CoV (7), both viruses are thought to utilize the same receptor, ACE2, as a gate to mediate virus entry to target host cells (5, 8, 9). The higher affinity of SARS-COV-2 spike protein for binding to ACE2 (approximately 10 to 15 folds) compared to that of SARS-COV, which is one of the reasons for the more impactful pathogenicity of the latter (10). The subunit S1 of the S protein, which includes the receptor-binding domain, directly attaches to the peptidase domain of ACE2, while the membrane fusion is in control of the S2 subunit (11). In addition to ACE2, TMPRSS2, the cell-surface membrane protein, is required to integrate the virus with the cell membrane through cleaving SARS-COV spike proteins (10).

ACE2 is a metallopeptidase enzyme attached to the membranes of cells located in different

organs, particularly the lungs, heart, kidneys, and testes. Interestingly, ACE2 protein expression is evident only in specific tissues despite the demonstration of ACE2 mRNA in virtually all body organs (5). The physiological relevance of ACE2 in most tissues is yet unknown. However, ACE2 is regarded to be an important regulator of cardiac function and blood pressure control (12), probably through functioning as a natural counterpart to ACE1 (13). The testis is also one of the few organs with high levels of ACE2 expression (14, 15). ACE2 functions to convert Angiotensin II to Angiotensin 1-7 in Leydig cells and adjust testosterone production and consequently contribute to spermatogenesis modulation, which reveals their influence on male fertility (16). Overall, ACE2 mediates the activation of the Renin-Angiotensin-Aldosterone System (RAAS) (5) as a cell signaling system to regulate spermatogenesis (17).

Human spermatozoa include a number of RAAS family ligand enzymes and receptors, including the angiotensin receptor type 1 and 2 and the angiotensin mitochondrial assembly receptor (18). SARS-CoV-2 binding to this sperm surface signaling system may impair the functional capacity of the affected sperm. Specifically, the virus's impact on spermatozoa ACE2 activity may enhance angiotensin II levels and increase reactive oxygen species (ROS) production resulting in oxidative stress (19). Excessive ROS production may cause sperm membrane and DNA damage and ultimately affect the sperm's fertilizing potential (20).

Viruses such as human immunodeficiency virus (HIV), mumps, hepatitis B virus (HBV), influenza A virus subtype H1N1 (A/H1N1), and Ebola virus (21) had been incriminated in the pathogenesis of orchitis, infertility, and testicular tumors (22, 23). Orchitis was also reported as a complication of SARS, an outbreak in 2003 caused by another member of the coronavirus family known as SARS-CoV-1. In another study, histopathological evaluation of testicular biopsy specimens from six men infected with SARS-CoV-1 indicated the findings of increased thickness of the basement membrane, destruction of germ cells, and low density of sperm in the seminiferous tubules (24). In this regard, a systematic review on the presence of SARS-CoV-2 in semen, including fourteen studies, suggested that the virus is rarely found in the semen of infected men and probably COVID-19 affects male fertility by making a deleterious effect on testicular structure (25). In line with this study, Khalili et al. (26) explained that despite the limited data on the detection of SARS-CoV-2 in the semen of infected patients, there is some evidence that the virus may play a role in testicular damage, abnormal sex hormone secretion, and infertility, which could be due to direct viral invasion through receptors or secondary immunological and inflammatory effects (26, 27). Hence, more studies are needed to evaluate all the possibilities.

The endoplasmic reticulum (ER) is the intracellular site where almost one-third of protein synthesis and protein folding occurs. Increased protein synthesis and excessive accumulation of unfolded/misfolded proteins in the ER lumen activate the unfolded protein response (UPR) and consequently drive the cell into ER stress (28). Under these circumstances, glucose-regulated protein 78 (Grp78), an ER chaperon protein, cooperates with three types of ER stress sensors such as Activating Transcription Factor 6 (ATF6), Protein Kinase R-like ER Kinase (PERK), and Inositol-requiring enzyme 1 (IRE1) to decrease unfolded/misfolded protein levels and avoid unfolded protein accumulation, thereby promoting cell survival. However, UPR may also activate the apoptotic response if ER homeostasis could not be regained (29). Therefore, Grp78 is more likely found within the ER lumen (30).

Under some circumstances, Grp78 can be moved towards the cell surface and act as a receptor to adjust different pathways (31). Grp78, also known as a binding immunoglobulin protein (BiP) or heat shock protein A5 (HSPA5) that is a member of the heat shock protein 70 (HSP70) family. This protein plays an essential role in resistance to apoptotic death in somatic cells and the response to chemical or physical cellular stress induced by cancer, malnutrition, and hypoxia (32, 33). Grp78 is highly expressed in human testicular tissue and mature spermatozoa, contributing to the physiology of spermatogenesis and fertilization (34, 35). Interestingly, previous results have confirmed that Grp78 protein contributes to the intrusion of different viruses like Ebola virus, dengue virus, influenza virus (36), Middle East respiratory syndrome coronavirus (MERS-CoV), Zika virus (37), and coronaviruses (38) into host cells. Moreover, recently the first experimental study proved that in addition to ACE2, the main receptor for virus entry, Grp78 as a host auxiliary factor for SAR-CoV-2 can simplify control virus entry (39).

Spike proteins of SARS-CoV-2 are one of the most important virulent factors of viruses to attach and penetrate host cells. For this purpose, host cell receptors like ACE2 and Grp78 are considered the target for viruses (5). Ibrahim et al. (40) have reported that the attachment of Grp78 Substrate Binding Domain β (SBD β) with the receptor-binding domain of the coronavirus spike protein is required to identify and help the virus to enter the host cells.

It has also been demonstrated that Grp78 protein expression increases in SARS-CoV infection, reflecting its role in virus entry into cells (41, 42). The present study aims to investigate the Grp78 expression in male reproductive organs using the findings of recent studies up to July 2021 and discuss the potential implications for SARS-CoV-2 invasion of the male reproductive tract.

In order to investigate the Grp78 expression in the male reproductive system, RNA and protein expression data of HSPA5/Grp78 in various human tissues and cancer, particularly male reproductive organs such as the testis and prostate were retrieved online using The Human Protein Atlas (http://www.proteinatlas.org/), Genotype-Tissue Expression (GTEx) (https://www.gtexportal.org), and

the Broad Institute Cancer Cell Line Encyclopedia (CCLE) (https://www.portals.broadinstitute.org/ccle) portals. All the data is available online. Protein expression scores are based on the best estimate of the "true" protein expression from a knowledge-based annotation. Immunohistochemistry (IHC) images of normal and cancer tissue of male reproductive organs such as testes, epididymis, and accessory sex glands from the tissue and pathology atlas on the Human Protein Atlas portal were used to evaluate the protein expression of HSPA5/Grp78 in the specific cells of these organs.

Data obtained from CCLE and the GTEx portal showed a high level of Grp78 mRNA expression in the male tissues such as the testis and prostate. The mRNA expression is also relatively high in the upper respiratory, digestive tracts, and lungs (Figs.1, 2). Data obtained from the Human Protein Atlas portal showed highly expressed in male tissues, including testis, epididymis, seminal vesicle, and prostate (Fig.3A, B). Moreover, IHC staining shows an increased level of Grp78 expression in cells of testis seminiferous tubules and the glandular cells of the epididymis, seminal vesicle, and prostate (Fig.3C). Data obtained from the Human Protein Atlas portal to assess Grp78 expression in various cancer organs, including the testis and prostate, showed high levels of this protein in these cancer organs (Fig.4).
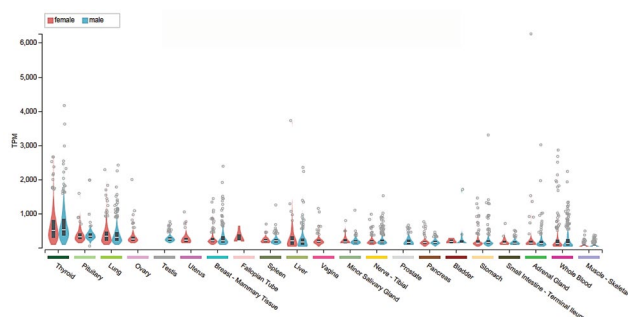

Fig.1: Male and female gene expression for heat shock protein A5 (HSPA5)/ glucose-regulated protein 78 (Grp78) (ENSG00000044574.7) obtained from the genotype-tissue expression (GTEx) portal in different tissues (https://www. gtexportal.org).
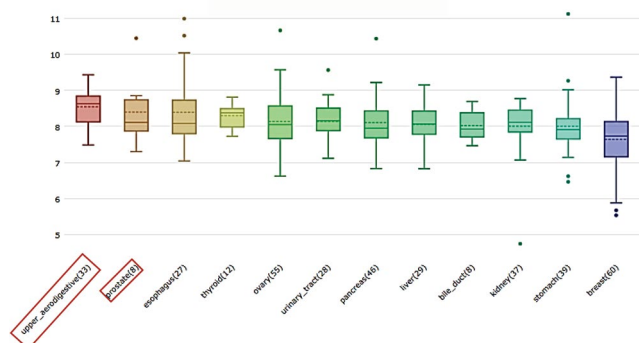

Fig.2: The mRNA expression level of HSPA5/Grp78 in diverse human tissues from the broad institute cancer cell line encyclopedia (CCLE) shows the expression of GRP78 in the upper aerodigestive tract and prostate (https:// www.portals.broadinstitute.org/ccle).
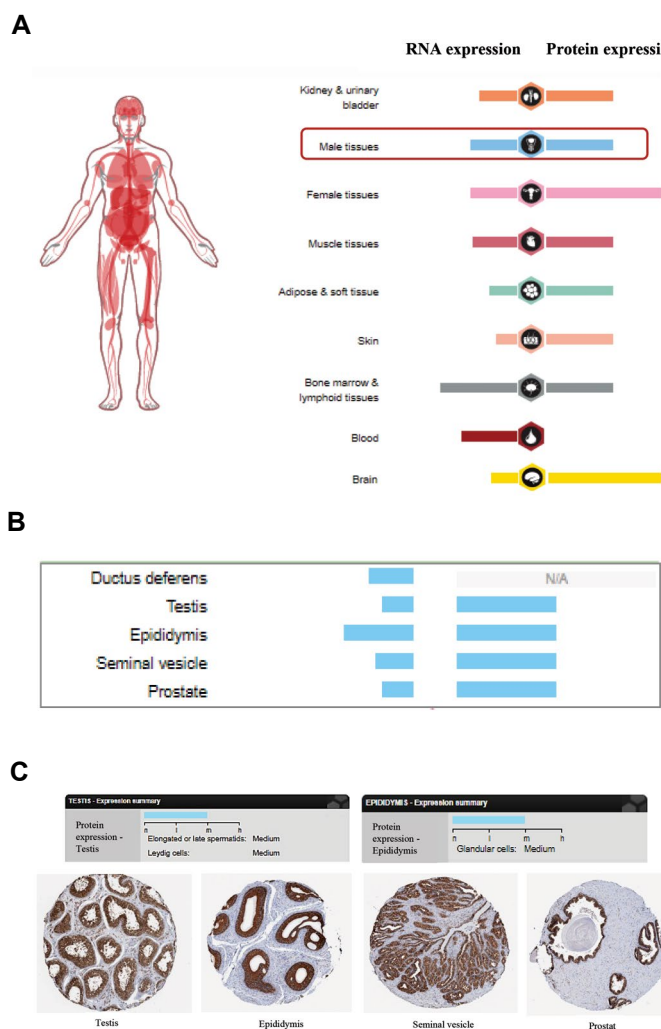

Fig.3: Data of HSPA5/Grp78 protein expression level in different human tissues from HPA portal. **A.** Each bar shows the highest expression score found in a particular group of tissues. Note the notable protein expression of HSPA5/Grp78 in male tissues (red box). **B.** HSPA5/Grp78 protein expression level in different part of male tissues (Human Protein Atlas portal). **C.** IHC staining of HSPA5/Grp78 in normal testis, epididymis, seminal vesicle, and prostate (Human Protein Atlas portal) (https://www. portals.broadinstitute.org/ccle).

Grp78 is overexpressed under pathological stresses like cancer, cellular malnutrition, hypoxia, and viral infections and is translocated from the ER to the plasma membrane (31, 32). This protein acts as a multifunctional receptor to interact with various proteins (29); therefore, it may be a gate for viruses to penetrate host cells (43, 44). In this regard, Grp78 has been introduced as a receptor to facilitate coronaviruses' entrance into host cells in humans and bats (38). Thus, Grp78 seems to be an essential factor in helping virus protein folding, and its internalization to the host cells as well as protecting them from host immunity (Fig.5).

Recent studies proposed interaction between host cell Grp78 and the particular region of the COVID-19 spike model and considered this receptor a probable vaccination target (40, 45, 46). A series of recent studies have indicated that Pep42, a cyclic peptide, binds to the overexpressed Grp78 in the cancer cell membrane and by which enters the cancer cell (47, 48).
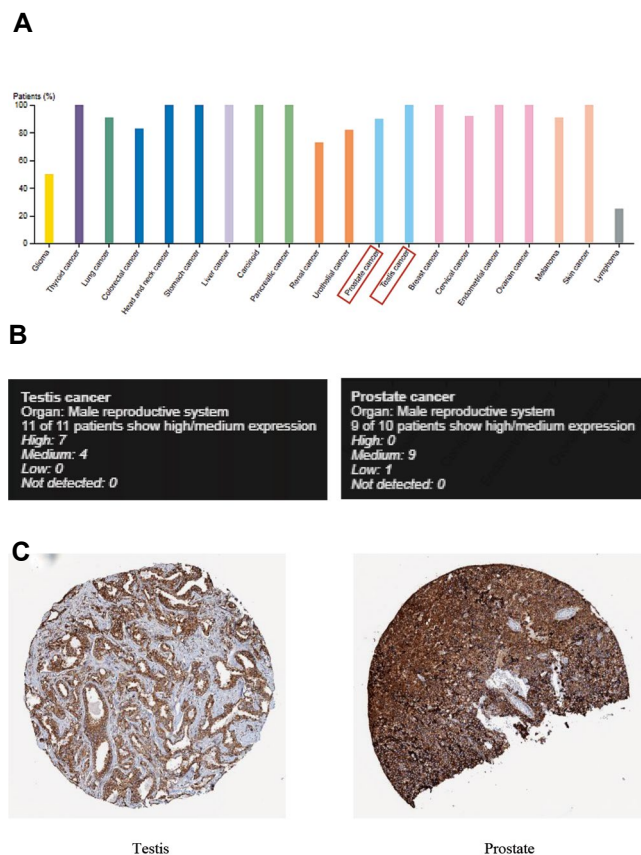
A

B

Testis cancer
Organ: Male reproductive system
11 of 11 patients show high/medium expression
High: 7
Medium: 4
Low: 0
Not detected: 0

Prostate cancer
Organ: Male reproductive system
9 of 10 patients show high/medium expression
High: 0
Medium: 9
Low: 1
Not detected: 0

C

Testis   Prostate

Fig.4: Data of HSPA5/Grp78 protein expression level in cancers. **A.** For each cancer, color-coded bars represent the percentage of patients (maximum 12 patients) with high and medium protein expression levels. **B.** Note the black box showing 100 percent of patients with testis cancer indicate high/medium expression and 90 percentage of patients with prostate shows a medium expression of GRP78. **C.** IHC staining of GRP78 in the testicular and prostatic cancer (IHC staining, Human Protein Atlas portal).
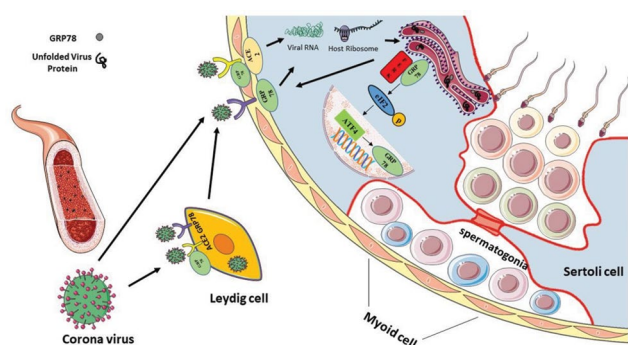

Fig.5: Hypothesized model for SARS-CoV-2 testicular infection. In addition to Leydig cell as one of the main targets for SARS-CoV-2 due to high expression of ACE2, Sertoli cell contributes to virus infection. The virus binds to the ACE2 receptor of the Sertoli cell and releases the viral RNA genome into the cytoplasm. Released and uncoated RNA is translated by the host ribosome to produce viral fundamental protein. Translated proteins are inserted into the endoplasmic reticulum for processing. Accumulation of unfolded SARS-CoV proteins in the lumen of E.R activates E.R. stress, UPR, and consequently PERK pathway by enhancing GRP78 expression as an important E.R. chaperon for folding of SARS-CoV proteins. Increased GRP78 expression and pathological stress induced by virus translocate GRP78 from E.R lumen to cell membrane and act either as a direct receptor or a host co-factor for ACE2 to accelerate virus entry genome.

That is why Pep42 is considered a vehicle for tumor cell-specific chemotherapy (48). Ibrahim et al. (40) assessed the binding features of spike proteins of SARS-CoV-2 with Grp78. Interestingly, he also encountered 13 different cyclic regions in this spike model that were matched with the cyclic Pep42 structure and demonstrated the contribution of the spike protein model (regions III and IV) to binding with Grp78. Furthermore, a recent experimental study by Carlos et al. (39) revealed a new aspect of Grp78 role in the coronavirus infection. They presented some evidence that Grp78 functions not only as a cofactor to aid viral spike binding to ACE2, but also as a regulator of ACE2 protein expression, which highlight the great contribution of this protein to viral entry.

In the context of male reproduction, Grp78 expression has been confirmed by various studies in germ cells of humans and mice during spermatogenesis (49, 50). Investigation of Grp78 cellular localization in human testis revealed its expression in spermatocytes, round spermatids, and nick region of ejaculated spermatozoa as well as principal cells of the epididymis (51). A similar observation was found using an animal model to measure the Grp78 gene expression in testicular tissue of two groups of 2-month and 4-month-old rats (52).

Based on the high level of Grp78 expression in male tissues, including spermatogenesis cells, epididymis cells, vesicle seminal, and prostate (Fig.3), we speculate that in addition to ACE2 and TMPRSS2, Grp78 can act as a receptor to intermediate coronavirus entrance to male reproductive cells. Leydig and Sertoli cells may be considered a target for SARS-CoV-2 due to the high expression of ACE-2 (8, 15). This may result in testicular destruction depending on the disease's severity as immune and inflammatory responses (25). In this case, the virus attaches to the ACE2 receptor of the Sertoli cell and releases the viral RNA genome into the cytoplasm. The host ribosome translates the released RNA to produce fundamental viral protein and is finally inserted into the ER for processing (53).

On the other hand, several studies failed to demonstrate SARS-CoV-2 in the semen of COVID-19 patients, whether those patients were tested during an acute attack of the disease (54) or at different stages of recovery (55, 56). Pan et al. (55), and Stanley et al. (57), attributed the lack of SARS-Co-2 in the semen of COVID-19 patients to the fact that less than 1% of testis cells (spermatozoa, spermatogonia, and Leydig and Sertoli cells) express both ACE2 and TMPRSS2 receptors, which may reduce the virus's ability to penetrate these cells. Accordingly, SARS-CoV-2 is unlikely to be sexually transmitted by men. However, it is difficult to rely on those observations to exclude the potential of sexual transmission of SARS-Cov-2 due to: i. Small sample of COVID-19 patients included in those studies and the heterogeneity of inclusion criteria, ii. Lack of data regarding the viral load of COVID-19- infected patients, iii. Difficulties

that surround the collection of semen samples from COVID-19 patients during acute attacks, and iv. lack of a standard protocol of RT-PCR technique that is used for detection of seminal SARS-CoV-2.

The genome of SARS-CoV, like other coronaviruses, replicates in the host cell cytoplasm and is highly dependent on ER function for the preparation of proteins. This induces ER stress owing to the accumulation of unfolded yet synthesized SARS-CoV proteins in the ER lumen (28). Under these circumstances, UPR is activated by multiple cell-signaling pathways to maintain cellular homeostasis. However, if this condition continues and damages ER function severely, the UPR triggers cellular apoptosis (30). Interestingly, viruses apply various strategies to regulate UPR for ER preservation. In the case of SARS-CoV, UPR modulation is accomplished by the PERK pathway and eIF2α phosphorylation. This leads to the transcriptional activation of Grp78 as the intraluminal ER chaperones increase the processing and folding of expressed SARS-CoV proteins through viral replication and protect cells from apoptosis, at least in the early stage of infection (Fig.5) (41, 58).

Moreover, upon ER stress activation, IRE1as an ER transmembrane sensor, starts generating X box-binding protein 1 (XBP1), acting as a transcriptional activator of genes involved in UPR to maintain ER and cellular function (30). It has been reported that SARS-CoV has been shown to cause a slight increase in XBP1 expression, which is likely important for increased virus protein folding and avoiding the harmful effects of ER stress-induced apoptosis (41). Therefore, SARS-CoV by selective modulation of the ER stress pathways provides the time and opportunity for viral replication before the infected host cell is sensitized to apoptosis.

It is notable that, *in vitro* treatment of lung epithelial cells with a humanized monoclonal antibody (hMAb159) with high affinity and specificity against GRP78, caused decreased cell surface GRP78 and cell surface ACE2 expression, as well as viral entry and SARS-CoV-2 infection. This finding showed that targeting host chaperones such as GRP78, which are necessary for viral entry and even production, might provide novel techniques for repressing SARS-CoV-2 and perhaps future coronavirus strains (39).

The connection between reproductive tissue cancers and increased Grp78 expression as a central part of UPR has been explored in prior studies. The results indicate a role of Grp78 in protein folding to guarantee survival, proliferation, and invasion of various cancer cells (59, 60) like the endometrial, gastric, renal cell, pancreatic, and prostate cancer (29, 61). These observations agree with our data extracted from the Human Protein Atlas portal (Fig.4). This may explain the relation of blocking Grp78 in different types of cancer cells and their apoptosis (62). Also, recently, some literature has proved the association

between up-regulation and relocation of Grp78 to the tumor cell surface and some features like aggressive and invasive growth patterns of these cells (63, 64). This feature of cancer cells has turned the Grp78 at the cell surface into a useful prognostic marker and a target for cancer therapy (31).

A recent study by Liang et al. (65) concluded that patients with cancer have a higher risk of getting infected with SARS-CoV-2 compared to individuals without cancer. Although cancer patients are at a higher risk of getting infected as a result of immunocompromised states (66), the observation that Grp78 is overexpressed and translocated to the cancer cell membrane (67) may explain why tumor cells are more likely vulnerable to virus entrance and thereby virus propagation in cancer patients. Similarly, in diabetic and obese individuals, Grp78 is translocated to the cell membrane due to cellular glucose starvation (68), thus explaining why those individuals are at higher risk and severely affected (69). Based on these reports, increased Grp78 at the cell membrane may increase the severity of viral infection in specific tissue and individuals with higher Grp78 in their serum. Furthermore, it has been demonstrated that dysregulation of male hormonal sex can result from acute SARS-CoV-2 infection (70). Since Grp78 can play a prominent role in the steroidogenesis regulation of various reproductive mammalian cells (60), It seems that the hormonal defect is dependent on Grp78 function in testicular tissues infected with SARS-CoV-2.

Conclusion

In this study, we reviewed the present studies regarding the possible role of Grp78/HSPA5/BiP to facilitate the virus entry into host cells. However, it remains unclear how and when SARS-CoV-2 can impair male fertility potential. Given the global importance of fertility, all aspects of the impact of SARS-CoV-2 infection on the male reproductive system should be further assessed. A better understanding of the infection routes and target cells of the male reproductive system is critical for predicting some effective methods to treat or avoid likely consequences of infection in the male reproductive system.

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Authors' Contributions

N.S.; Main search, collection, and study of published papers, writing, and approval of the manuscript. M.T., A.Sh., P.S., K.L., R.S., A.A., M.H.N.-E.; Reviewed the

manuscript, provided comments and suggestions, and finally approved the manuscript.

References

1. Zanke AA, Thenge RR, Adhao VS. COVID-19: A pandemic declare by world health organization. IP Int J Compr Adv Pharmacol. 2020; 5(2): 49-57.
2. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis. 2020; 20(5): 533-534.
3. Xu XW, Wu XX, Jiang XG, Xu KJ, Ying LJ, Ma CL, et al. Clinical findings in a group of patients infected with the 2019 novel corona-virus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. BMJ. 2020; 368: m606.
4. Teixeira TA, Bernardes FS, Oliveira YC, Hsieh MK, Esteves SC, Duarte Neto AN, et al. SARS-CoV-2 and multi-organ damage–what men's health specialists should know about the COVID-19 patho-physiology. Int Braz J Urol. 2021; 47(3): 637-646.
5. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen 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.
6. Abdel-Moneim A. COVID-19 pandemic and male fertility: Clinical manifestations and pathogenic mechanisms. Biochemistry (Mosc). 2021; 86(4): 389-396.
7. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic charac-terisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020; 395(10224): 565-574.
8. Wang Z, Xu X. scRNA-seq profiling of human testes reveals the presence of the ACE2 receptor, a target for SARS-CoV-2 infection in spermatogonia, leydig and sertoli cells. Cells. 2020; 9(4): 920.
9. Tian Y, Zhou LQ. Evaluating the impact of COVID-19 on male repro-duction. Reproduction. 2021; 161(2): R37-R44.
10. Stopsack KH, Mucci LA, Antonarakis ES, Nelson PS, Kantoff PW. TMPRSS2 and COVID-19: Serendipity or opportunity for interven-tion? Cancer Discov. 2020; 10(6): 779-782.
11. Hallak J, Teixeira TA, Bernardes FS, Carneiro F, Duarte SA, Pariz JR, et al. SARS-CoV-2 and its relationship with the genitourinary tract: Implications for male reproductive health in the context of COVID-19 pandemic. Andrology. 2021; 9(1):73-79.
12. Crackower MA, Sarao R, Oudit GY, Yagil C, Kozieradzki I, Scanga SE, et al. Angiotensin-converting enzyme 2 is an essential regulator of heart function. Nature. 2002; 417(6891): 822-828.
13. Yagil Y, Yagil C. Hypothesis: ACE2 modulates blood pressure in the mammalian organism. Hypertension. 2003; 41: 871–873.
14. Hezavehei M, Shokoohian B, Nasr-Esfahani MH, Shpichka A, Timashev P, Shahverdi AH, et al. Possible male reproduction com-plications after coronavirus pandemic. Cell J. 2021; 23(4): 382-388.
15. Tabar AN, Sojoudi K, Henduei H, Azizi H. Review of sertoli cell dysfunction caused by COVID-19 that could affect male fertility. Zy-gote. 2021; 30(1): 17-24.
16. Lee W, Mok A, Chung JP. Potential effects of COVID-19 on re-productive systems and fertility; assisted reproductive technology guidelines and considerations: a review. Hong Kong Med J. 2021; 27: 118-126.
17. Edenfield RC, Easley CA. Implications of testicular ACE2 and the renin–angiotensin system for SARS-CoV-2 on testis function. Nat Rev Urol. 2022; 19(2): 116-127.
18. Valdivia A, Cortés L, Beitia M, Totorikaguena L, Agirregoitia N, Cor-costegui B, et al. Role of angiotensin-(1–7) via MAS receptor in hu-man sperm motility and acrosome reaction. Reprod. 2020; 159(3): 241-249.
19. Aitken RJ. COVID-19 and human spermatozoa-Potential risks for infertility and sexual transmission? Andrology. 2021; 9(1): 48-52.
20. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen spe-cies in the pathophysiology of human reproduction. Fertil Steril. 2003; 79(4): 829-843.
21. Roychoudhury S, Das A, Sengupta P, Dutta S, Roychoudhury S, Choudhury AP, et al. Viral pandemics of the last four decades: pathophysiology, health impacts and perspectives. Int J Environ Res Public Health. 2020; 17(24): 9411.
22. Schuppe HC, Meinhardt A, Allam JP, Bergmann M, Weidner W, Haidl G. Chronic orchitis: a neglected cause of male infertility? Andrology. 2008; 40(2): 84-91.
23. Mahé D, Matusali G, Deleage C, Alvarenga RL, Satie AP, Pagliuzza

A, et al. Potential for virus endogenization in humans through tes-ticular germ cell infection: the case of HIV. J Virol. 2020; 94(24): e01145-20.
24. Xu J, Qi L, Chi X, Yang J, Wei X, Gong E, et al. Orchitis: a compli-cation of severe acute respiratory syndrome (SARS). Biol Reprod. 2006; 74(2): 410-416.
25. Yao Y, Yuan X, Wu L, Guo N, Yin L, Li Y. COVID-19 and male reproduction: Current research and unknown factors. Andrology. 2021; 9(4): 1027-1037.
26. Khalili MA, Leisegang K, Majzoub A, Finelli R, Selvam MK, Henkel R, et al. Male fertility and the COVID-19 pandemic: systematic re-view of the literature. World J Mens Health. 2020; 38(4): 506-520.
27. Rastrelli G, Di Stasi V, Inglese F, Beccaria M, Garuti M, Di Costanzo D, et al. Low testosterone levels predict clinical adverse outcomes in SARS-CoV-2 pneumonia patients. Andrology. 2021; 9(1): 88-98.
28. Almanza A, Carlesso A, Chintha C, Creedican S, Doultsinos D, Leuzzi B, et al. Endoplasmic reticulum stress signalling–from basic mechanisms to clinical applications. FEBS J. 2019; 286(2): 241-278.
29. Ibrahim IM, Abdelmalek DH, Elfiky AA. GRP78: a cell's response to stress. Life Sci. 2019; 226: 156-163.
30. Karna KK, Shin YS, Choi BR, Kim HK, Park JK. The role of endo-plasmic reticulum stress response in male reproductive physiol-ogy and pathology: a review. World J Mens Health. 2020; 38(4): 484-494.
31. Casas C. GRP78 at the centre of the stage in cancer and neuro-protection. Front Neurosci. 2017; 11: 177.
32. Ni M, Zhang Y, Lee AS. Beyond the endoplasmic reticulum: atypi-cal GRP78 in cell viability, signalling and therapeutic targeting. Bio-chem J. 2011; 434(2): 181-188.
33. Xing X, Lai M, Wang Y, Xu E, Huang Q. Overexpression of glu-cose-regulated protein 78 in colon cancer. Clin Chim Acta. 2006; 364(1-2): 308-315.
34. Boilard M, Reyes-Moreno C, Lachance C, Massicotte L, Bailey JL, Sirard MA, et al. Localization of the chaperone proteins GRP78 and HSP60 on the luminal surface of bovine oviduct epithelial cells and their association with spermatozoa. Biol Reprod. 2004; 71(6): 1879-1889.
35. Lachance C, Bailey JL, Leclerc P. Expression of Hsp60 and Grp78 in the human endometrium and oviduct, and their effect on sperm functions. Hum Reprod. 2007; 22(10): 2606-2614.
36. Booth L, Roberts JL, Cash DR, Tavallai S, Jean S, Fidanza A, et al. GRP78/BiP/HSPA5/Dna K is a universal therapeutic target for human disease. J Cell Physiol. 2015; 230(7): 1661-1676.
37. Turpin J, Frumence E, Harrabi W, Haddad JG, El Kalamouni C, Desprès P, et al. Zika virus subversion of chaperone GRP78/BiP expression in A549 cells during UPR activation. Biochimie. 2020; 175: 99-105.
38. Chu H, Chan CM, Zhang X, Wang Y, Yuan S, Zhou J, et al. Mid-dle East respiratory syndrome coronavirus and bat coronavirus HKU9 both can utilize GRP78 for attachment onto host cells. J Biol Chem. 2018; 293(30): 11709-11726.
39. Carlos AJ, Ha DP, Yeh DW, Van Krieken R, Tseng CC, Zhang P, et al. The chaperone GRP78 is a host auxiliary factor for SARS-CoV-2 and GRP78 depleting antibody blocks viral entry and infec-tion. J Biol Chem. 2021; 296: 100759.
40. Ibrahim IM, Abdelmalek DH, Elshahat ME, Elfiky AA. COVID-19 spike-host cell receptor GRP78 binding site prediction. J Infect. 2020; 80(5): 554-562.
41. Chan CP, Siu KL, Chin KT, Yuen KY, Zheng B, Jin DY. Modulation of the unfolded protein response by the severe acute respiratory syndrome coronavirus spike protein. J Virol. 2006; 80(18): 9279-9287.
42. Elgohary AM, Elfiky AA, Barakat K. GRP78: A possible relationship of COVID-19 and the mucormycosis; in silico perspective. Comput Biol Med. 2021; 139: 104956.
43. Jindadamrongwech S, Thepparit C, Smith DR. Identification of GRP 78 (BiP) as a liver cell expressed receptor element for den-gue virus serotype 2. Arch Virol. 2004; 149(5): 915-927.
44. Honda T, Horie M, Daito T, Ikuta K, Tomonaga K. Molecular chap-erone BiP interacts with Borna disease virus glycoprotein at the cell surface. J Virol. 2009; 83(23): 12622-12625.
45. Elfiky AA. SARS-CoV-2 spike-heat shock protein A5 (GRP78) rec-ognition may be related to the immersed human coronaviruses. Front Pharmacol. 2020; 11: 577467.
46. Sabirli R, Koseler A, Goren T, Turkcuer I, Kurt O. High GRP78 lev-els in Covid-19 infection: a case-control study. Life Sci. 2021; 265: 118781.
47. Kim Y, Lillo AM, Steiniger SC, Liu Y, Ballatore C, Anichini A, et al.

Targeting heat shock proteins on cancer cells: selection, characterization, and cell-penetrating properties of a peptidic GRP78 ligand. Biochemistry. 2006; 45(31): 9434-9444.

48. Yoneda Y, Steiniger SC, Čapková K, Mee JM, Liu Y, Kaufmann GF, et al. A cell-penetrating peptidic GRP78 ligand for tumor cell-specific prodrug therapy. Bio Med Chem Lett. 2008; 18(5): 1632-1636.

49. Huo R, Zhu YF, Ma X, Lin M, Zhou ZM, Sha JH. Differential expression of glucose-regulated protein 78 during spermatogenesis. Cell Tissue Res. 2004; 316(3): 359-367.

50. Aguilar-Mahecha A, Hales BF, Robaire B. Expression of stress response genes in germ cells during spermatogenesis. Biol Reprod. 2001; 65(1): 119-127.

51. Wang W, Wang X, Zhu P, Sun CM, Jin S, Liu J, et al. Expression and location of glucose-regulated protein 78 in testis and epididymis. WIMJ Open. 2014; 1: 14-7.

52. Rahmani M, Tavalaee M, Hosseini M, Eskandari A, Shaygannia E, Sadeghi N, et al. Deferasirox, an iron-chelating agent, improves testicular morphometric and sperm functional parameters in a pat model of varicocele. Oxid Med Cell Longev. 2021; 2021: 6698482.

53. Yesudhas D, Srivastava A, Gromiha MM. COVID-19 outbreak: history, mechanism, transmission, structural studies and therapeutics. Infection. 2021; 49(2): 199-213.

54. Song C, Wang Y, Li W, Hu B, Chen G, Xia P, et al. Absence of 2019 novel coronavirus in semen and testes of COVID-19 patients. Biol Reprod. 2020; 103(1): 4-6.

55. Pan F, Xiao X, Guo J, Song Y, Li H, Patel DP, et al. No evidence of severe acute respiratory syndrome–coronavirus 2 in semen of males recovering from coronavirus disease 2019. Ferti Steril. 2020; 113(6): 1135-1139.

56. Ma L, Xie W, Li D, Shi L, Ye G, Mao Y, et al. Evaluation of sex-elated hormones and semen characteristics in reproductive-aged male COVID-19 patients. J Med Virol. 2021; 93(1):456-462.

57. Stanley KE, Thomas E, Leaver M, Wells D. Coronavirus disease-19 and fertility: viral host entry protein expression in male and female reproductive tissues. Fertil Steril. 2020; 114(1):33-43.

58. Luo S, Baumeister P, Yang S, Abcouwer SF, Lee AS. Induction of Grp78/BiP by translational block: activation of the Grp78 promoter by ATF4 through an upstream ATF/CRE site independent of the endoplasmic reticulum stress elements. J Biol Chem. 2003; 278(39): 37375-37385.

59. Guzel E, Arlier S, Guzeloglu-Kayisli O, Tabak MS, Ekiz T, Semerci N, et al. Endoplasmic reticulum stress and homeostasis in reproductive physiology and pathology. Int J Mol Sci. 2017; 18(4): 792.

60. Hebert-Schuster M, Rotta BE, Kirkpatrick B, Guibourdenche J, Cohen M. The interplay between glucose-regulated protein 78 (GRP78) and steroids in the reproductive system. Int J Mol Sci. 2018; 19(7): 1842.

61. Gifford JB, Huang W, Zeleniak AE, Hindoyan A, Wu H, Donahue TR, et al. Expression of GRP78, master regulator of the unfolded protein response, increases chemoresistance in pancreatic ductal adenocarcinoma. Mol Cancer Ther. 2016; 15(5): 1043-1052.

62. Rasche L, Duell J, Morgner C, Chatterjee M, Hensel F, Rosenwald A, et al. The natural human IgM antibody PAT-SM6 induces apoptosis in primary human multiple myeloma cells by targeting heat shock protein GRP78. PLoS One. 2013; 8(5): e63414.

63. Lee AS. Glucose-regulated proteins in cancer: molecular mechanisms and therapeutic potential. Nat Rev Cancer. 2014; 14(4): 263-276.

64. Ferrara F, Staquicini DI, Driessen WH, D'Angelo S, Dobroff AS, Barry M, et al. Targeted molecular-genetic imaging and ligand-directed therapy in aggressive variant prostate cancer. Proc Natl Acad Sci USA. 2016; 113(45): 12786-12791.

65. Liang W, Guan W, Chen R, Wang W, Li J, Xu K, et al. Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. Lancet Oncol. 2020; 21(3): 335-337.

66. Gosain R, Abdou Y, Singh A, Rana N, Puzanov I, Ernstoff MS. COVID-19 and cancer: a comprehensive review. Curr Oncol Rep. 2020; 22(5): 1-5.

67. Toyoda Y, Akarlar B, Sarov M, Ozlu N, Saitoh S. Extracellular glucose level regulates dependence on GRP 78 for cell surface localization of multipass transmembrane proteins in HeLa cells. FEBS lett. 2018; 592(19): 3295-3304.

68. Girona J, Rodríguez-Borjabad C, Ibarretxe D, Vallvé JC, Ferré R, Heras M, et al. The circulating GRP78/BiP is a marker of metabolic diseases and atherosclerosis: bringing endoplasmic reticulum stress into the clinical scenario. J Clin Med. 2019; 8(11):1793.

69. Santos A, Magro DO, Evangelista-Poderoso R, Saad MJ. Diabetes, obesity, and insulin resistance in COVID-19: molecular inter-relationship and therapeutic implications. Diabetol Metab Syndr. 2021; 13(1): 23.

70. Verma S, Saksena S, Sadri-Ardekani H. ACE2 receptor expression in testes: implications in coronavirus disease 2019 pathogenesis. Biol Reprod. 2020; 103(3): 449-451.