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Identification of Novel MicroRNAs as Promising Therapeutics for SARS-CoV-2 by Regulating EGFR-ADAM17 axis: An *in-silico* Analysis

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Abstract- Cancer patients contracting SARS-CoV-2 encounter additional challenges due to inflammatory bursts and lymphopenia, which may aggravate breast cancer prognosis. In this *in-silico* analysis, we identified the potential of miRNAs as new therapeutic targets to treat breast cancer patients infected with COVID-19 via the regulation of ADAM17 and EGFR expression microRNAs.

Key words-SARS-CoV-2, microRNA, ADAM17, EGFR, Breast cancer

1. Introduction

The history of human coronaviruses began in the early 1960s, when David Tyrrell and Malcolm Bynoe began experimenting with several animal viruses and their human strains, including the infectious bronchitis virus, mouse hepatitis virus and the transmissible gastroenteritis virus of swine. This family of viruses was called coronavirus owing to the crown-like projections on their surface. Coronaviruses are a group of related RNA viruses that can cause upper respiratory tract infections in humans and birds. In the past 18 years, a minimum of five new human coronaviruses has been recognized, including the severe acute respiratory syndrome coronavirus (SARS), Middle East respiratory syndrome (MERS) in 2012, and the latest SARS-CoV-2 in December 2019, which caused substantial morbidity and death¹. This recent SARS-CoV-2 (coronavirus disease 2019 or COVID-19) outbreak devastated the scientific community with its unexpected severity and fast spread. Of late, genome sequencing and homology modeling studies have revealed angiotensin-converting enzyme 2 (ACE2) as the functional host receptor for SARS-CoV-2². After SARS-‘S’ binds to ACE2, the S-protein undergoes a proteolytic cleavage between the S1 and S2 subunits by the TMPRSS2 serine protease³. After interaction with the viral S-protein, ACE2 is internalized along with viral particles into endosomes, reducing the surface tissue expression of ACE2⁴. The SARS-CoV-2 infection of host cells is facilitated by the cleavage of spike proteins by other host cell proteases, such as cathepsin L (CTSL)⁵.

Disintegrin and Metalloproteases (ADAM), originally named metalloproteinases disintegrin cysteine-rich (MDC), are membrane-anchored cell surface proteins containing both disintegrin and metalloproteinase domains, which can specifically cleave the precursor of tumor necrosis factor α (pro-TNF- α)⁶. ADAM17 is well recognized for releasing ectodomains of a diverse variety of membrane-anchored cytokines, cell adhesion molecules, receptors, ligands and enzymes, such as ACE2⁶. Interestingly, the ACE2 tail and ADAM17 expression proved to be necessary for the SARS-CoV infection. A study by Lambert et al. revealed that TMPRSS2 and ADAM17 cleave ACE2 differentially by the SARS-CoV-2 spike protein. Moreover, the increased activity of the ADAM17 sheddase may also lead to the cleavage of TNF- α , IL6R and other pro-inflammatory molecules, reinforcing the inflammatory process during the SARS-CoV-2 infection. Therefore, the inhibition of ADAM17 by decreasing ACE2 shedding may have a protective effect on SARS-CoV infections by reducing the viral load⁴.

Over the last decade, the role ADAM17 plays in breast cancer, including cell proliferation, invasion, angiogenesis, apoptosis, trastuzumab resistance, and breast cancer promotion through EGFR-PI3K-AKT activation has received a great deal of attention⁷. McGowan et al. demonstrated that the overexpression of ADAM-17 in MCF-7 breast cancer cells amplified their *in vitro* invasion and proliferation, whereas the down-regulation of ADAM-17 expression in MDA-MB-435 cells decreased the cells' invasion and proliferation⁸. Furthermore, ADAM17-mediated degradation of interferon-gamma (IFN- γ) was able to block the anti-tumorigenic and anti-osteoclastogenic effects of IFN- γ in breast cancer cells. ADAM17 inhibition may be useful for the treatment of attenuated cancer immune surveillance and/or bone metastases⁹. The current SARS-CoV-2 pandemic, and the association of the virus with ADAM17 expression, poses considerable challenges to oncology patients due to inflammatory bursts and lymphopenia, which may aggravate cancer prognosis; for instance, in the Veneto study, breast and hematological cancers were associated with a higher risk of both hospitalization and death¹⁰.

MicroRNAs (miRNAs) are small nucleotides having a length of 22–25 nt, which play a role in the regulation of gene expression at the translational level by selectively interacting with a single gene or several target genes¹¹. A current study has shown that miRNAs as epigenetic regulators can differentiate between SARS-CoV and SARS-

CoV-2, which might clear some of the ambiguity surrounding the pathogenicity and diverse clinical characteristics of the COVID-19 pandemic. miRNAs can be an essential antiviral tool not only to fight against the virus, but also to trigger the host's immune system. Another recent study has demonstrated that complementary miRNA (cc-miRNA) complexes were expected to bind and inhibit the translation of corona viral proteins and the replication of SARS-CoV-2, and MERS-CoV genomes¹².

In the context of the current pandemic, gaining a detailed understanding of the molecular mechanisms involved in the pathophysiology of COVID-19 is crucial to identifying risk factors and developing new effective therapeutic approaches. In this study, we used different microRNA target prediction software programs to identify potential miRNAs that can directly bind to ACE2 and TMPRSS2 with a focus on the expression of ADAM17 and EGFR, as these regulate the expression of ADAM17 and maintain the delicate balance with ACE2, TMPRSS2 and EGFR interactions in each specific pathophysiological condition of SARS-CoV-2.

2. Materials and Methods

To determine potential miRNAs that can directly bind to ACE2 and TMPRSS2, multiple target bioinformatic prediction algorithms were used, including miRbase (<http://www.mirbase.org>), miRanda (www.microRNA.org), Target scan (http://www.targetscan.org/vert_72), and miRWalk2.029 (<http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/cellpub.html>). Computational approaches involve associating miRNA and mRNA nucleotide sequences in a special format that governs the binding characteristics between the exact beginning sequence followed by 7 to 11 nucleotide miRNA binding sites on the mRNA, which is known as 'seed match.' The location of the miRNA binding site within the specified ADAM17 gene can be either an untranslated region (3'UTR, 5'UTR) or a coding sequence (CDS). An miRNA binding to an mRNA, and resulting in a stable structure is considered the most likely target of that miRNA. Moreover, Gibb's free energy is indicative of the stability of the miRNA structure; as reactions with a more negative ΔG are less likely to occur, and thus have higher stability. The hybridization of miRNA with its target mRNA provides information about the high and low free energy regions, while ΔG predicts the strength of the bond between the miRNA and its target mRNA. ΔG [kJ/mole] further determines the collaboration pattern among nucleotides of miRNAs and mRNAs, which plays a crucial role during the intercellular gene silencing phenomenon, which needs to be critically selected. Furthermore, the ratio of $\Delta G/\Delta G_m$ (%) was calculated for each binding site, where ΔG_m is the free energy binding of miRNA with its full complementary nucleotide sequence.

Since each prediction analysis tool is equipped with different algorithms, it is difficult to know for certain which tool is the best choice for a particular study. Therefore, in this study, we only used miRNAs that were common in the three software programs mentioned above and had the following characteristics: (1) a 7–8 seed match, (2) GU Wobble seed match, which calculates the chances of a G pairing with a U instead of a C, (3) position contribution, (4) seed pairing stability, (5) target-site abundance, which determines the number of sites occurring in the 3'-UTR, (6) local AU, which flanks in the corresponding seed region, and (7) 3'-compensatory pairing, which is the pairing region (12–17 nts) in which the base pairs match with miRNA nucleotides.

3. Results

3.1. Identification of potential miRNAs involved in the regulation of ADAM17

The Initial analysis revealed that several miRNAs are associated with ADAM17, directly or indirectly through some pathways, thereby inhibiting its oncogenic properties. In our present study, we have taken the approach of altering the expression of ADAM17 by miRNAs. Our preliminary *in silico* miRNA prediction analysis yielded almost 19 miRNAs having potential binding efficiency against the ADAM17 gene, thus can be linked with breast cancer. These miRNAs might enhance or suppress the expression of ADAM17 by binding to them directly or through some other pathways, which can be used as a therapeutic strategy against SARS-Cov-2 infection by altering its attachment potential inside the host's system. Table I presents a list of potential miRNA candidates against the ADAM17 gene, which were not studied previously.

Table I. miRNAs having strong binding potential against ADAM17

No.	miRNA	Sequence (5' to 3')
1	hsa-miR-26a-5p	TTCAAGTAATCCAGGATAGGCT
2	hsa-miR-26b-5p	TTCAAGTAATCCAGGATAGGCT
3	hsa-miR-101b-3p	TTCAAGTAATCCAGGATAGGCT
4	hsa-miR-125a-5p	TCCCTGAGACCCTTTAACCTGTGA
5	hsa-miR-125b-5p	TCCCTGAGACCCTAACCTGTGA
6	hsa-miR-140-3p	TACCACAGGGTAGAACCACGG
7	hsa-miR-145-5p	GTCCAGTTTTCCCAGGAATCCCT
8	hsa-miR-148a-3p	TCAGTGCACACTACAGAACTTTGT
9	hsa-miR-148b-3p	TCAGTGCATCACAGAACTTTGT
10	hsa-miR-152-3p	TCAGTGCATGACAGAACTTGG
11	hsa-miR-329-3p	AACACACCTGGTTAACCTCTTT
12	hsa-miR-329-3p	AACACACCTGGTTAACCTCTTT
13	hsa-miR-340-5p	TTATAAAGCAATGAGACTGATT
14	hsa-miR-362-3p	AACACACCTATTCAAGGATTCA
15	hsa-miR-582-5p	TTACAGTTGTTCAACCAGTACT
16	hsa-miR-1297	TTCAAGTAATTCAGGTG
17	hsa-miR-4319	TCCCTGAGCAAAGCCAC
18	hsa-miR-4465	CTCAAGTAGTCTGACCAGGGGA
19	hsa-miR-5195-3p	ATCCAGTTCTCTGAGGGGGCT

3.2. Identification of prospective miRNAs involved in the regulation of EGFR

Our preliminary *in silico* miRNA prediction analysis revealed almost 27 miRNAs having a potential binding efficiency against EGFRs that can be linked to breast cancer. These miRNAs might alter EGFR expression by binding to them directly, or regulate EGFR using other pathways such as ERK/ AKT/Pi3K/ MEK. Moreover, we found common miRNAs, namely miR-26a, miR148a, miR-148b, miR-152, and miR-329 (marked in red in Table I and Table II) which can regulate both ADAM17 and EGFR, suggesting crosstalk among them.

Table II. Potential miRNA candidates against EGFR which were not studied previously.

No.	miRNA	Sequence (5' to 3')
1	hsa-miR-7-5p	TGGAAGACTAGTGATTTTGTGTGT
2	hsa-miR-17-5p	CAAAGTGCTTACAGTGCAGGTAG
3	hsa-miR-20a-5p	TAAAGTGCTTATAGTGACAGGTAG
4	hsa-miR-20b-5p	CAAAGTGCTCATAGTGCAGGTAG
5	hsa-miR-26a-5p	TTCAAGTAATCCAGGATAGGCT
6	hsa-miR-106a-5p	AAAAGTGCTTACAGTGCAGGTAG
7	hsa-miR-106b-5p	TAAAGTGCTGACAGTGCAGAT
8	hsa-miR-133a-3p	TTTGGTCCCCTTCAACCAGCTG
9	hsa-miR-133b	TTTGGTCCCCTTCAACCAGCTA
10	hsa-miR-1375p	ACGGGTATTCTTGGGTGGATAAT
11	hsa-miR-138-5p	AGCTGGTGTGTGAATCAGGCCG
12	hsa-miR-144-3p	TACAGTATAGATGATGTACT
13	hsa-miR-148a-3p	TCAGTGCACACTACAGAACTTTGT
14	hsa-miR-148b-3p	TCAGTGCATCACAGAACTTTGT
15	hsa-miR-152-3p	TCAGTGCATGACAGAACTTGG
16	hsa-miR-221-3p	AGCTACATGTCTGCTGGGTTC
17	hsa-miR-222-3p	AGCTACATCTGGCTACTGGGT
18	hsa-miR-302a-5p	ACTTAAACGTGGATGTACTTGCT
19	hsa-miR-302b-5p	ACTTTAACATGGAAGTGCTTTC
20	hsa-miR-329-3p	AACACACCTGGTTAACCTCTTT
21	hsa-miR-373-3p	GAAGTGCTTCGATTTTGGGGTGT
22	hsa-miR-411-3p	TATGTAACACGGTCCACTAACC
23	hsa-miR-421	ATCAACAGACATTAATTGGGCGC
24	hsa-miR-455-3p	GCAGTCCATGGGCATATACAC
25	hsa-miR-520a-3p	AAAGTGCTTCCCTTTGGACTGT
27	hsa-miR-520b-3p	AAAGTGCTTCCCTTTAGAGGG

4. Discussion

SARS-CoV-2 causes a severe respiratory and systemic infection mostly in aged and immunosuppressed individuals; that is why cancer patients are considered more susceptible to the SARS-CoV-2 infection than healthy individuals¹. Cancer type, staging, and specific therapies are additional risk factors for COVID-19 contraction in this patient population. Patients with hematological, lung or breast cancers are more vulnerable than those with other cancers. In the Veneto study, patients with breast and hematological cancers were at a higher risk of hospitalization and death¹⁰. Additionally, several studies have reported that SARS-CoV-2 seriously interferes with tumor therapy and hinders attempts to treat cancer patients properly. In our study, we utilized bioinformatic approaches to investigate the interaction between SARS-CoV-2 and miRNAs associated with breast cancer, with a focus on ADAM17 and EGFR genes using miRBase, Target scan and miRWalk2.029 algorithms. Various mechanisms based on the miRNA-mediated regulation of viruses have been reported. We searched for miRNAs that shared a 100% identity of the 8mer seed region against respective genes. This analysis resulted in a network of miRNA-mRNA interactions illustrated in Tables I and II.

Multiple pathways have been proposed to describe the SARS-CoV-2 viral attachment to the host and its subsequent spreading. In 2005, tumor necrosis factor-alpha convertase (ADAM17) was found to facilitate the ectodomain shedding of the SARS-CoV receptor, angiotensin-converting enzyme-2 (ACE2)⁴. Additionally, the intonation of the “TNF-alpha-converting enzyme ADAM17 by the spike protein of SARS-CoV and ACE2 could enhance TNF-alpha production and facilitate viral entry”⁶. A recent study by Zipeto et al. established that interactions between ACE2/ADAM17/TMPRSS2 play a crucial role in the SARS-CoV-2 attachment and progression. Therefore, the inhibition of ADAM17 might exert a protective effect on SARS-CoV-2 mediated breast cancer complications⁴.

Decades of research in molecular oncology have brought about promising new therapies designed to target specific molecules that promote tumor growth and survival. The epidermal growth factor receptor (EGFR) is considered one of the first identified targets, and the EGFR/PI3K/PTEN/Akt/mTORC1 pathway has been explored extensively in the framework of breast cancer treatment. Additionally, the expression of MAPK and EGFR is closely associated with tumor invasion and the metastasis of triple-negative breast cancer (TNBC), and therefore may be used as an indicator of poor prognosis in patients with TNBC. Similarly, the role of ADAM17 in breast cancer has been clearly identified, as it promotes cell proliferation, invasion, angiogenesis, apoptosis, and trastuzumab resistance. In 2010, ADAM17 was found to control EGFR expression through Notch1 stimulation in non-small cell lung cancer. This study introduced a new pathway linking ADAM17 and EGFR that could be used in developing novel therapeutics. Another study revealed that a transforming oncogene Src mutant could enhance the bioavailability of EGFR ligands through the activation of ADAM17 in breast cancer cells. In 2016, the regulatory mechanism of ADAM17 and EGFR was established using the IL-6 receptor and amphiregulin of chronic obstructive pulmonary disease (COPD) patient cells exposed to cigarette smoke. Stolarczyk et al. confirmed the link between the EGFR and ADAM17 axis in COPD and cystic fibrosis lung pathology¹³. These studies cumulatively suggest the significant role of ADAM17 in EGFR dependent tumor malignancy. Recently, researchers have shown that ADAM17 silencing could impede cell invasion and proliferation in breast cancer through the EGFR/PI3K/AKT and EGFR/MEK/ERK signaling pathways. The significance of this pathway was re-iterated by another research study that showed that ADAM17 contributed to tumor progression through the activation of the EGFR/PI3K/AKT pathway in breast cancer. These findings were our drive to explore the crosstalk among ADAM17/EGFR/miRNA and how it can be used against SARS-CoV2 and breast cancer.

The potential role of miRNAs in immunity regulation and in the modulation of viral receptors and co-receptors should be considered as a crucial factor to understand the different pathogenicity and lethality of the SARS-CoV-2 pandemic. miR-145 was found to suppress ADAM17 in renal cell cancer and in nasopharyngeal carcinoma cells. Furthermore, miR-338-3p was shown to inhibit the proliferation and migration of gastric cancer cells by targeting ADAM17. miR-152 act as tumor suppressors in non-small cell lung cancer (NSCLC) via targeting ADAM17. Concurrently, the ability to modulate several miRNA activities suggests a novel therapeutic possibility, one that is able to overcome resistance mechanisms to conventional and EGFR-targeted therapies. miR-539 acts as a tumor suppressor by targeting EGFR in breast cancer. miR-326 has been identified as a tumor suppressor in breast cancer that targets the EGFR/PI3K pathway. Overexpression of miR-361-5p in TNBC inhibits migration and invasion by targeting RQCD1 and inhibiting the EGFR/PI3K/Akt pathway. Additionally, miR-21 suppresses tumor cell migration and invasion by reducing PI3K/AKT signaling and reversing EMT in breast cancer. However, so far, there is no study that has established a relationship between the ADAM 17/EGFR axis, and how this relationship could launch therapeutics for SARS-Cov-2 associated with breast cancer.

Our present *in-silico* analysis was able to provide novel miRNA networks targeting the two genes (ADAM17 and EGFR) and their signaling pathways in response to the SARS-CoV-2 infection. This potentially acts as an important immune-modulatory mechanism. These findings predict a highly immunogenic viral-host interaction, which represents a promising opportunity to identify novel therapeutic targets for SARS-CoV-2. Moreover, we found some miRNAs that can regulate both ADAM17 as well as EGFR, signifying a novel relationship between

these 2 genes associated with SARS-CoV-2 and breast cancer. Finally, we believe that the outcomes of the present study represent an initial step towards directing the attention to miRNAs as vital contributors in the development of diagnostics and therapeutic targets for SARS-CoV-2 associated breast cancer pathogenesis.

5. Conclusions

Recent progress in diagnostic and interventional medicine includes using miRNAs to identify regulatory networks of new and unconventional therapeutic targets against the SARS-CoV-2 infection. The use of antisense oligonucleotides to block the viral genome is considered an important way towards new therapeutic strategies. In our present study, we summarized the potentials of miRNAs in controlling SARS-CoV-2 breast cancer progression by regulating the expression of two genes, namely ADAM17 and EGFR.

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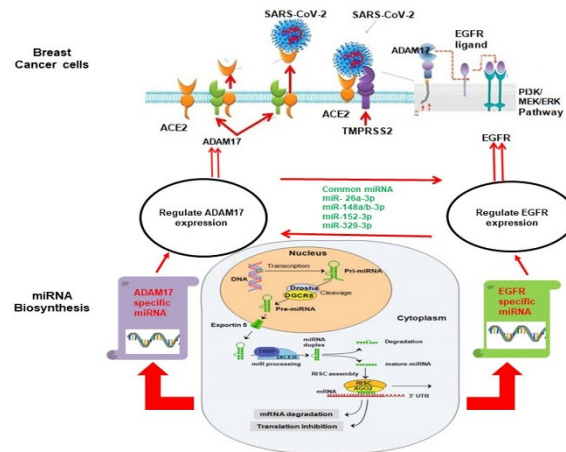
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Identification of Novel MicroRNAs as Promising Therapeutics for SARS-CoV-2 by Regulating EGFR-ADAM17 axis: An *in-silico* Analysis

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The pathophysiological aspects of angiotensin-converting enzyme 2 (ACE2), desintegrin and metalloproteinase domain 17 (ADAM17), along with specific genetic factors mainly associated with type II transmembrane serine protease (TMPRSS2) expression host proteins in COVID-19 could be decisive for the clinical outcome of SARS-CoV-2 comorbidities. miRNAs possess potential as new therapeutic targets to treat breast cancer patients associated with COVID-19 infection via regulation of ADAM17 and EGFR expression microRNAs.