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Potential Approach for Fighting Against Corona Virus Disease

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Abstract

A viral disease (or viral infection, or infectious disease) occurs when pathogenic viruses invade an organism's body, and infectious virus particles (virions) attach to and enter susceptible cells [1]. There are many types of viral diseases affecting the human body as explained below. But in this approach focus on a noved viral infectious disease belongs to corona viruses group as this dangerous viruses showed a wide spread activity all over the globe. The virus has a characteristic genetic instability means genetic mutations occurs in high rate such a phenomena make the virus replicates with different genetic sequences and spread rapidly therefore, it is not easy to be controlled neither by vaccines nor by drugs. This research looking for methods to fight against corona virus through certain mechanisms that have a relationships with transforming growth factor (TGF-beta). Here comes the idea that if we could control TGF-beta, with which the virus interacts, it would be possible to get over the virus either directly or indirectly and at the same giving a chance for the immune system in terms of humeral and cell mediated immunity of the human body to be active against the disease. Taking into account that TGF-beta is a growth factor found everywhere in the body.

Key words: MERS-CoV; (Middle East Rispiratory Syndrome-corona virus); BSAAs; (Broad-Spectrum Antiviral Agents); PCR; (Polymerase Chain Reaction); Q-PCR; (Quantitative Polymerase Reaction); SARS; (Severe acute respiratory syndrome); SARS-CoV; (Severe acute respiratory syndrome corona virus); siRNA; (short interfering ryboneocliac acid); SMAD; (small.mothers against decapentaplegic family of genes); PRRSV; (Reproductive and Respiratory syndrome); EMT; (Endothelial Mesenchymal Transition).

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1. Introduction

Viruses are very small infectious agents. They are made up of a piece of genetic material, such as DNA or RNA that is enclosed in a coat of protein. Viruses invade cells in your body and use components of those cells to help them multiply.(figure.1). Unlike cellular organisms, viruses do not contain all the biochemical mechanisms for their own replication; they replicate by using the biochemical mechanisms of a host cell to synthesize and assemble their separate components. (Some do contain or produce essential enzymes when there is no cellular enzyme that will serve.) When a complete virus particle (virion) comes in contact with a host cell, only the viral nucleic acid and, in some viruses, a few enzymes are injected into the host cell. Within the host cell the genetic material of a DNA virus is replicated and transcribed into messenger RNA by host cell enzymes, and proteins coded for by viral genes are synthesized by host cell ribosomes. These are the proteins that form the capsid (protein coat); there may also be a few enzymes or regulatory proteins involved in assembling the capsid around newly synthesized viral nucleic acid, in controlling the biochemical mechanisms of the host cell, and in lysing the host cell when new virions have been assembled. Some of these may already have been present within the initial virus and others may be coded for by the viral genome for production within the host cell. Because host cells do not have the ability to replicate “viral RNA” but are able to transcribe messenger RNA, RNA viruses must contain enzymes to produce genetic material for new virions. Viruses the RNA is replicated by a viral enzyme (transcriptase) contained in the virion, or produced by the host cell using the viral RNA as a messenger. In other viruses a reverse transcriptase contained in the virion transcribes the genetic message on the viral RNA into DNA, which is then replicated by the host cell. Reverse transcriptase is actually a combination of two enzymes: a polymerase that assembles the new DNA copy and an RNase that degrades the source RNA. In viruses that have membranes, membrane-bound viral proteins are synthesized by the host cell and move, like host cell membrane proteins, to the cell surface. When these proteins assemble to form the capsid, part of the host cell membrane is pinched off to form the envelope of the virion. Some viruses do not produce rapid lysis of host cells, but rather remain latent for long periods in the host before the appearance of clinical symptoms (carrier manifestations). In viral latency, most of the host cells may be protected from infection by immune mechanisms involving antibodies to the viral particles or interferon. Cell-mediated immunity is essential, especially in dealing with infected host cells. Cytotoxic lymphocytes may also act as antigen-presenting cells to better coordinate the immune response. Containment of virus in mucosal tissues is far more complex, involving follicular dendritic cells and Langerhans cells. Because viral reproduction is almost completely carried out by host cell mechanisms, there are few points in the process where stopping viral reproduction will not also kill host cells. For this reason, there are no chemotherapeutic agents for most viral diseases. acyclovir is an antiviral that requires viral proteins to become active. Some viral infections can be prevented by vaccination (active immunization), and others can be treated by passive immunization with immune globulin, although this has been shown to be effective against only a few dozen viruses. State can take any of several different forms. The term latency is used to denote the interval from infection to clinical state.

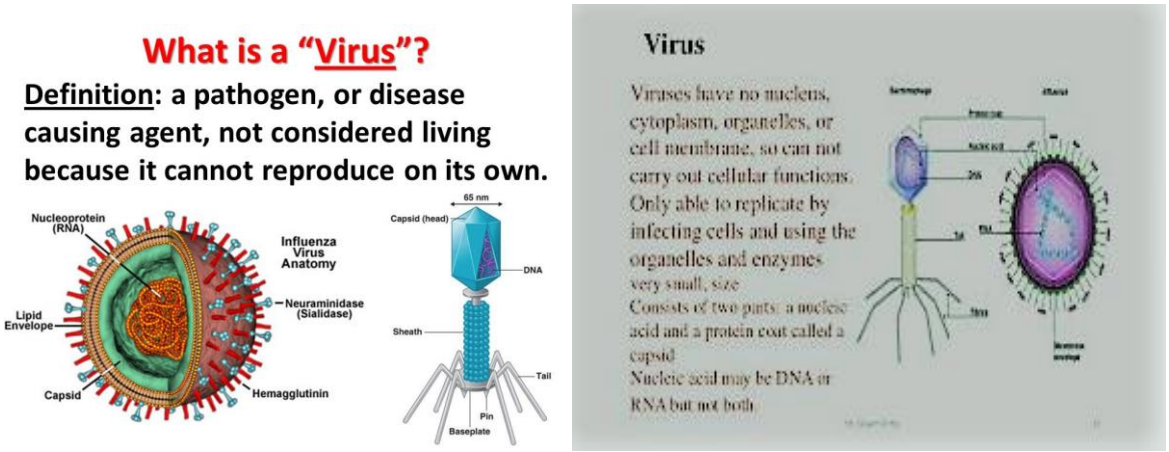

Figure 1.1: Virus

https://www.quora.com/Does-virus-have-cell-membrane

2. Materials and Methods

1. Gene Silencing
2. Semi-Quantitative Polymerase chain reaction.
3. Quantitative Polymerase chain reaction (Q-PCR).
4. Western Blot Analysis.
5. Reporter Gene Analysis.
6. Statistical Analysis.

3. There are different types of viruses

1. Double-stranded DNA families: three are non-enveloped adenoviridae, enveloped Herpesviridae
2. Single-stranded DNA viruses that infect humans eg. Parvoviridae. These viruses are non-enveloped
3. Single-stranded RNA families: three non-enveloped eg.Astroviridae and enveloped (Coronoviridae).
4. Single-stranded RNA families eg. Arenaviridae, enveloped with helical nucleocapsids.
5. Double-stranded RNA genome: Reoviridae.

As a rule, DNA viruses replicate within the nucleus while RNA viruses replicate within the cytoplasm but with some exceptions. Only one family of enveloped viruses causes gastroenteritis (Coronaviridae). All other viruses associated with gastroenteritis are non-enveloped. Viral disease is usually detected by clinical presentation, for instance severe muscle chest tightness, cough and joint pains preceding fever, or skin rash and swollen lymph glands. Laboratory investigation is not directly effective in detecting viral infections, because they do not themselves increase the white blood cell count. There are several serology techniques that can be used depending on the antibodies being studied. These include: ELISA, agglutination, precipitation, complement-fixation, and fluorescent antibodies and more recently chemiluminescence. PCR technique considered quite helpful [2].

4. Corona Virus

Coronaviruses were first discovered in the 1960s [3]. The earliest ones discovered were infectious bronchitis virus in chickens and two viruses from the nasal cavities of human patients. Coronaviruses are named for the crown-like spikes on their surface. Typically range in size from 80 nm to 100 nm in diameter. There are four main sub-groupings of coronaviruses common cold. It is a novel virus named for the crown like spikes that protrude from its surface. The coronavirus can infect both animals and people and can cause a range of respiratory illnesses from the common cold to more dangerous conditions like Severe Acute Respiratory Syndrome, or SARS. known alpha, beta, gamma and delta (figure 2). These types of corona virus consists of alpha and beta that affect human, but gamma and delta affects birds. Kidneys are affected seriously most of the time as well.

4.1 The mechanism of injury

The mechanism of injury caused by SARS-CoV infection remains unknown. A SARS disease model was proposed, consisting of three phases: viral replication, immune hyperactivity, and pulmonary destruction. SARS pathology of the lung has been associated with diffuse alveolar damage, epithelial cell proliferation, and an increase of macrophages. Multinucleate giant-cell infiltrates of macrophage or epithelial origin have been associated with putative syncytium-like formation that is characteristic of many coronavirus infections

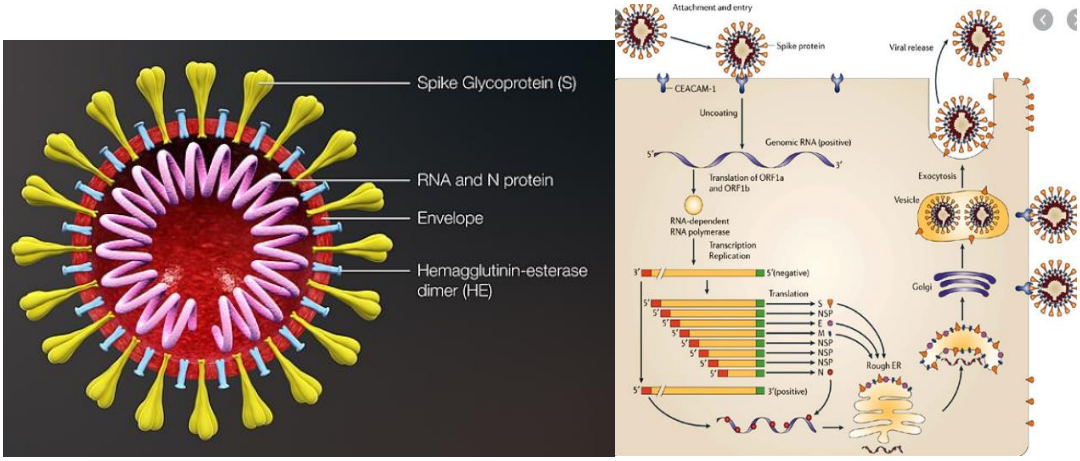

Figure 2: Cross-sectional model of a coronavirus and its mechanism of infection

SARS Coronavirus. sinobiological.com

4.2 Common human coronaviruses

229E (alpha coronavirus)

NL63 (alpha coronavirus)

OC43 (beta coronavirus)

HKU1 (beta coronavirus)

Other human coronaviruses

MERS-CoV (the beta coronavirus that causes Middle East Respiratory Syndrome or MERS)

SARS-CoV (the beta coronavirus that causes severe acute respiratory syndrome or SARS)

(SARS-CoV-2 (the novel coronavirus that causes coronavirus disease 2019, or COVID-19))

People around the world commonly get infected with human coronaviruses 229E, NL63, OC43, and HKU1.

Sometimes coronaviruses that infect animals can evolve and make people sick and become a new human coronavirus. Three recent examples of this are 2019-nCoV, SARS-CoV, and MERS-CoV [3]. Richard Neher: These coronaviruses tend to change their genome, they mutate, at a fairly high rate. These mutations allow us to group viruses into more closely related viruses and less closely related viruses. All the sequences on the site are super similar because they were closely related. As time goes on, the lineages pick up independent mutations, and then they cause outbreaks in different parts of the world. You can group these sequences together by genetic makeup and reconstruct the transmission tree of the virus. That is why a proper vaccine could not be prepared (Mutations). The mutations change codons and where they change amino acids. Most of the mutations are probably completely inconsequential. They just happen; it is at a rate of about one mutation per month. However, we are keeping an eye on mutations that might make a difference. Therefore, it was clearly shown that coronavirus have a characteristic phenomenon of genetic alteration and instability that is why a proper vaccine is not easy to be produced. Further, targeted recombination studies have confirmed the genetic flexibility of the coronavirus genome and the ability of coronaviruses to recover wild-type replication following deletions, mutations, substitutions, and gene order rearrangements in the structural and accessory genes [4]. On the other hand, The BSAAs inhibit viral or host factors and block viral replication, reduce the viral burden to a level at which host immune responses can deal with it or facilitate apoptosis of infected cells," the authors noted. They suggested that 31 of the SBAAs could represent possible. Such combinations of drugs could serve as front line therapeutics against poorly characterized emerging viruses or re-emerging drug-resistant viral strains." Kainov and colleagues [5]. Severe acute respiratory syndrome (SARS)–associated coronavirus (SARS-CoV) is the causative agent of SARS outbreak in 2003 [6,7]. SARS-CoV infection induces severe respiratory illnesses, such as bronchial epithelial denudation, loss of cilia, multinucleated syncytial cells, squamous metaplasia and transendothelial migration of monocytes/macrophages and neutrophils into lung tissue [8,9]. SARS-CoV triggers a pro-inflammatory cytokine storm that links with pulmonary fibrosis of SARS patients [10,11]. Near 20% of SARS patients recovered still have lung fibrosis 9 months post infection [12,13].

5. TGF-β1

Transforming growth factor β1 (TGF-β1), which is a multifunctional growth factor with an important role in development, cell proliferation, matrix deposition, tissue fibrosis, modulation of the, immunity, regulation of embryonic development and cellular differentiation and apoptosis (programmed cell death). Participation of

TGF-β signaling pathway on up regulation of TNC Tenascin C (TNC) is an extracellular matrix protein .which in turn support the idea that misregulation of this signaling pathway produces changes that may contribute to disease. TGF-β1 also plays a role in the immune system as it inhibits the function of T lymphocytes and large granular lymphocytes, which mediate natural killer cell activity[14]. TGF-β contributes greatly to maintain immune homeostasis and tolerance through regulating the differentiation, proliferation and activity of multiple leukocyte lineages, and inhibiting the production and signaling of effector cytokine in Smad-dependent and -independent mechanisms [15]. Recent developments have found that, using certain types of protein antagonists such as a receptor ligand or drug that does not induce a biological response itself upon binding to a receptor but sequential activation of two serine/threonine kinase receptors, the type I and type II receptors. A third receptor, (Betaglycan) serves as a co-receptor for TGF-β1.(figure 3). Increased TGF-β responsiveness with decreased betaglycan expression, or vice versa[16], these are physiologically relevant models for further studies investigating the role of betaglycan, as are cell types in which betaglycan expression changes dramatically during differentiation [17]. Recent reports indicate that membrane anchored betaglycan may play an inhibitory role in TGF-β1 signalling. This inhibitory role of betaglycan is a function of the heparan sulphate composition of the betaglycan glycosaminoglycan [18]. We can say that, the expression of betaglycan and its augmentation by cytokines or viral infection has been described. By concentrating on TGF-β activity, it is shown that knock-down by siRNA and inhibition of glycoslation of betaglycan antagonize signalling by TGF-β end production of EMT. Binding of transforming growth factor beta (TGF-β) to its cell surface receptor Type II leads to the phosphorylation of the Type I receptor by Type II. The Type I receptor is then able to phosphorylate and activate the SMAD2 protein. Smad2, with Smad4, is translocated to the nucleus where the activated Smad complex recruits other biological effects of TGF-b, the above-explained pathway called the classical signaling pathway of TGF-β (Figure 4). There are also SMAD-independent pathways activated by TGF binding its receptors as Rho,MAPK,PKC,Cross-talk TGF-β signalling and Wint.

Figure 3: structure of TGF-beta

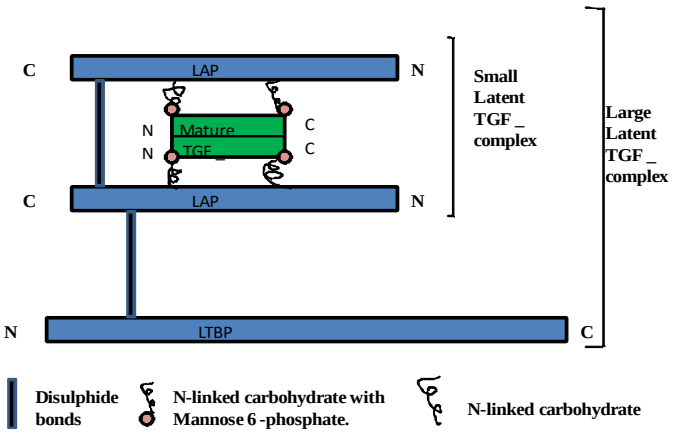

Figure 3: Shows the structure of Transforming Growth Factor Beta with its N and C terminals with small and large latent complexes. researchblogs.duke.edu/jas83.

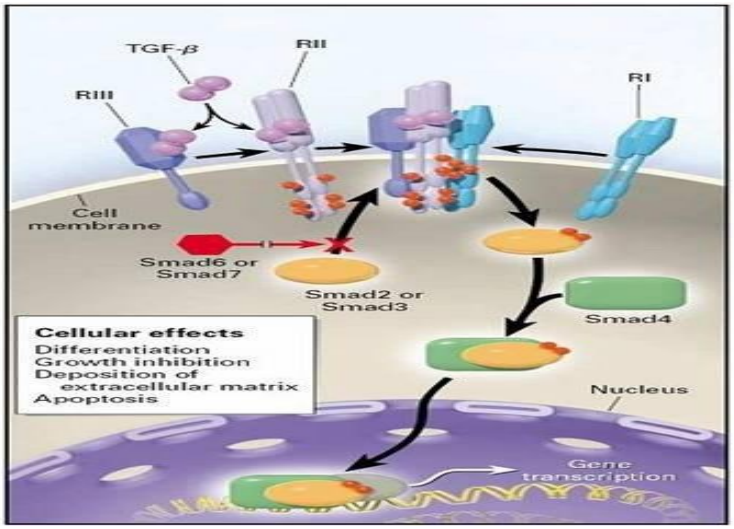

Figure 4: Signaling Pathways of TGF-β

Adapted from: www.biochemj.org/bj/352/0601/bj3520601f01.jpg, Rahmi ÖKLÜ, and Robin HESKETH

6. Betaglycan (TGF-β RIII)

Betaglycan is Transforming Growth Factor Beta receptor III (TGFBR3), (figure 5) and identified as an accessory receptor for the TGFβs is a cell-surface chondroitin sulfate / heparan sulfate proteoglycan, its molecular weight is about >300 kDa.(figure 3,10). It is a trans membrane proteoglycan with a large extracellular domain that binds TGF-β with high affinity but lacks a cytoplasmic signaling domain [19]. It is formed from heparin and chondroitin sulphate, core protein, ectodomain part, with its N and C terminals binding to ECM and cytoskeleton (Figure 3). Betaglycan binds to various members of the TGF-beta of ligands by its superfamily core protein, and bFGF (basic fibroblast growth factor), via its heparan sulfate chains. It is not involved directly in TGF-beta signal transduction but by binding to different member of the TGF-beta superfamily at the Cell surface, it acts as a reservoir of ligand for TGF-beta receptors[20]. It modulates some effects of TGF-β, it binds the three TGF-β isoforms through two binding sites on the core ectodomain also enhances TGF-β signaling by increasing affinity of the ligand for TGF-B. On the other hand, it has also an inhibitory function by binding to TGF-β members. Betaglycan undergoes ligand-independent ectodomain shedding and a stable soluble fragment is produced which sequesters TGF-β. its down regulation enhances epithelial to mesenchyme transition (EMT), betaglycan is down regulated during TGF-β differentiation of fibroblasts and this imply the anti fibrotic action of betaglycan [21]. Betaglycan appears to deliver TGF-β to the signaling receptors Betaglycan binds TGF-β through the core protein [22]. Betaglycan also forms a complex with syndcan-2 which is important in regulating TGF-β signaling and fibrosis, betaglcan species have opposing effects on TGF-β signaling whereby betaglycan serving as (molecular switch) .when isolated betaglycan cytoplasmic domain is inhibited it leads to decrease TGF-β signaling through activation of the p38/MAPK pathway. Therefore, betaglycan has an essential role in mediating the specificity of TGF-β signaling pathways.

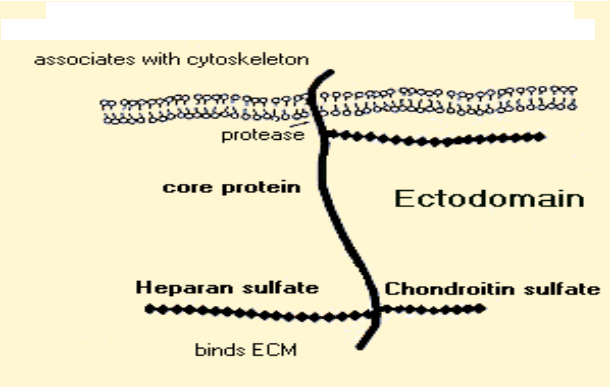

Figure 5: Structure of betaglycan

(Adapted online at www.med.unibs.it/%7Eairc/hspgs.htm).

In addition to a membrane-bound form, there is a soluble form of betaglycan as well, generated by the ectodomain shedding of the cell surface receptor. Although the membrane-bound form presents ligand, the soluble form is thought to sequester ligand from the serine/threonine TGF-ß signalling receptors. Furthermore, betaglycan, a cell surface heparan sulphate proteoglycan, is traditionally thought to function by binding transforming growth factor type β (TGF-β) via its core protein and then transferring the growth factor to its signalling receptor, the type II receptor.Betaglycan (BG) has a dual role in the modulation of TGF-β activities (Figure 4). BG, potentiates TGF-β actions when it is membrane bound. Shedding of BG extracellular region generates the soluble form of the receptor. Soluble BG still binds TGF-β with the high affinity of the membrane BG, but instead of presenting it to the type II receptor, soluble BG sequesters it and thus neutralizes its actions. Betaglycan undergoes ligand-independent ectodomain shedding and a stable soluble fragment is produced which sequesters TGF-β [23]. betaglycan also forms a complex with syndcan-2 which is important in regulating TGF-β signalling and fibrosis. Betaglcan species have opposing effects on TGF-β signalling whereby betaglycan serving as (molecular switch) . when isolated betaglycan cytoplasmic domain is inhibited it leads to decrease TGF-β signaling through activation of the p38/MAPK pathway, so betaglycan has got an essential role in mediating the specificity of TGF-β signaling pathways. Knocking down the action of betaglycan was achieved by a technique using betaglycan siRNA gene silencing. Betaglycan was knocked-down by transfection with specific siRNA. In the following experiment, determination of the optimal time of knock-down will help; namely at what time TGF-β can be best controlled by betaglycan.(figure 6.3). The degree of TGF-βIII knock-down was measured by Q-PCR. Cells were transfected with siRNA(figure 6.7). It was also found that P-Smad-III could be inhibited by betaglycan siRNA . So it is possible to control TGF-beta core-protein by knocking down using betaglycan siRNA gene silencing.

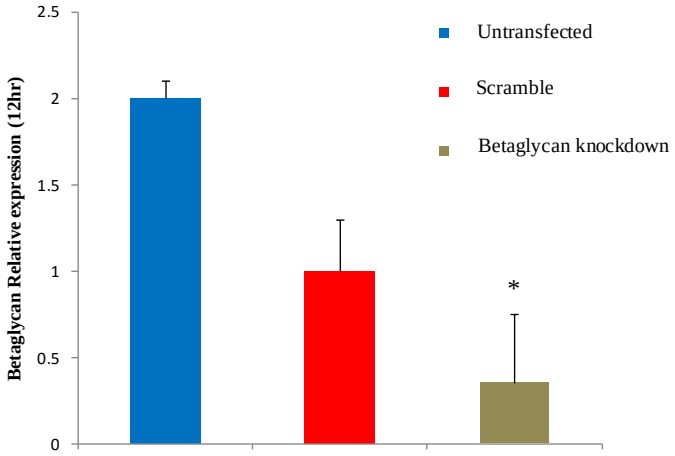

Figure 6.1: Betaglycan knock-down at 24 hours

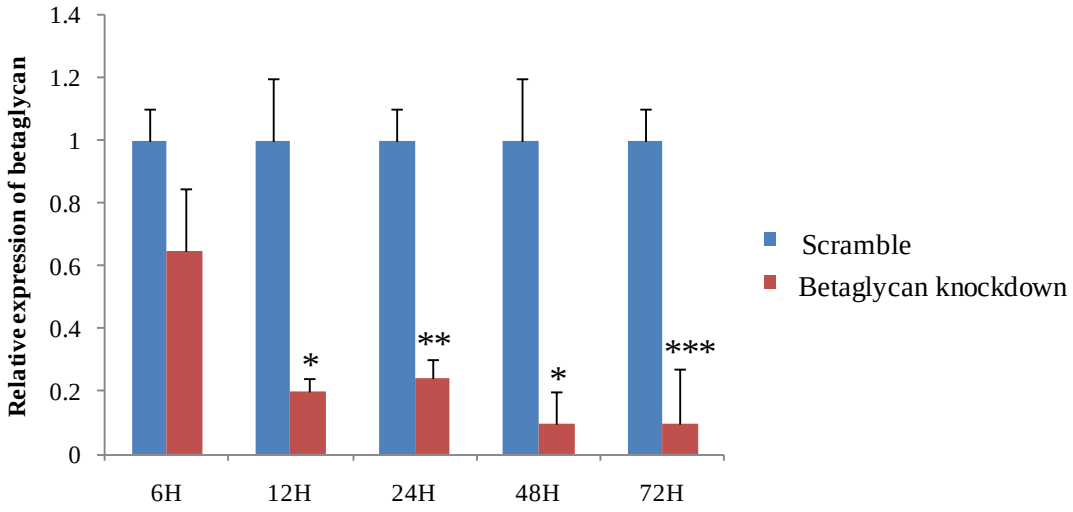

Figure 6.2: Optimizing Transfection Time

Cells were grown up to 50-60%, then kept with serum free medium overnight, then transfected with Lipofectamine2000 (Invitrogen), siRNA concentration was 40 pmol per µl that means 33nm/well was extracted, reverse transcribed and then Q-PCR carried out as described in methods. This experiment was done 3 times and the same result was achieved each time. S = scramble, ⊤ = Standard Error.* = p value < .05 = statistically significant. It was obvious that betaglycan knockdown has reached around 80%.

The bar graphs showing the best time for betaglycan knock-down, every group done individually and compared to its own scramble.

7. Demonstration of the HK2 cell functions in terms of betaglycan expression after being knocked-down then stimulated by TGF-β

This experiment, investigated what happened to TGF-β expression when betaglycan was knocked-down. HK2

cells were cultured in triplicate samples for 3 days until become confluent then growth arrested overnight. They were transfected with betaglycan siRNA and incubated for 24 hours before TGF-β stimulation for different time intervals was applied as shown in (Figure 6.3) mRNA was isolated, then reverse transcribed and finally quantified by Q-PCR as described in methods section. Samples were compared to the 1st scramble, and statistically a t-test was done to match each sample with its own scramble at that particular time, and all samples were found to be statistically significant.

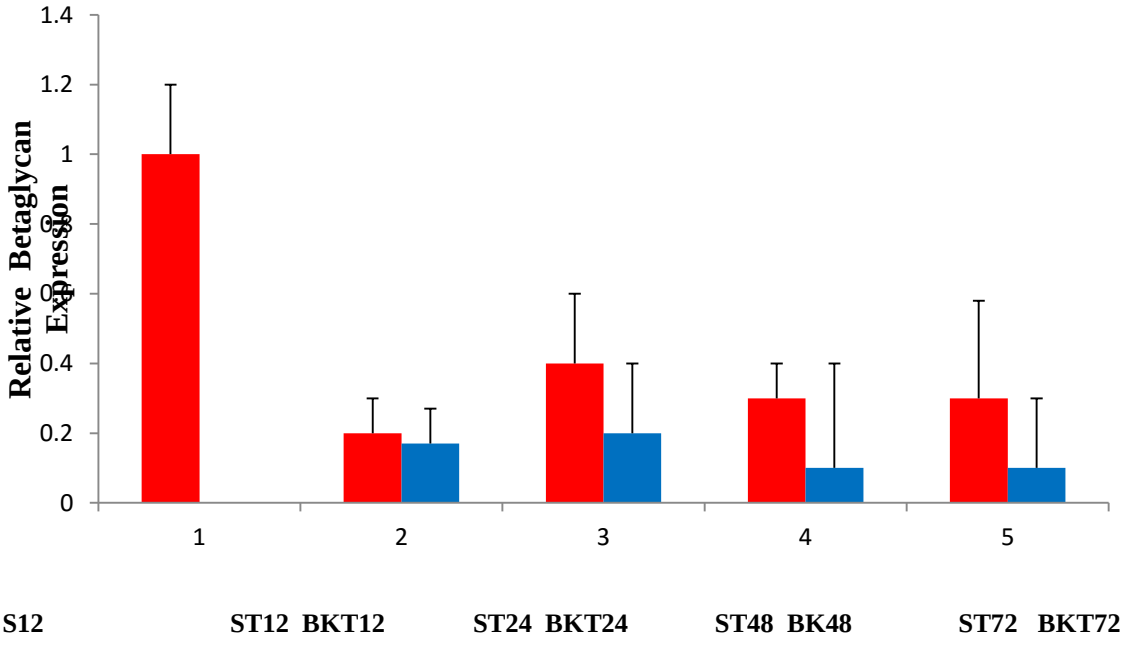

Figure 6.3: Expression of betaglycan after Knock-down and TGF-β stimulation

8. Time (Hours)

This Q-PCR bar graph illustrates betaglycan Expression after betaglycan knock-down then TGF-β stimulation at different time points, every sample compared to the 1st scramble one at 12 hour. T-test carried out to match each sample with its own scramble of that particular time. ⊤ = Standard Error. * = P = < 0.05 = Statistically Significant, ** = P = < 0.03 = highly Significant, *** = P = < 0.005 = very highly Significant. ST12H = Scramble at 12 hours then TGF-β stimulation, BKT12H = betaglycan knock-down at 12 hours then TGF-β stimulation. Samples were done in triplicate, and the experiment was repeated 3 times.

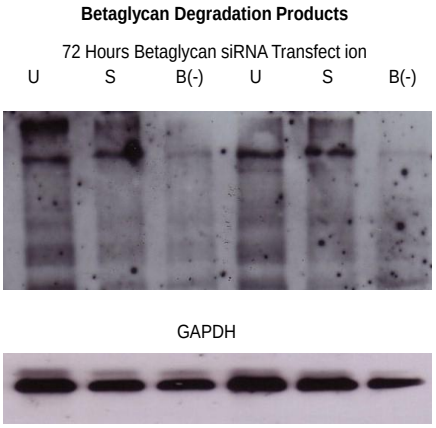

Figure 7: Betaglycan Bands after siRNA Transfection

The effect of betaglycan siRNA on Smad-III phosphorlation was also carried out by SDS-PAGE and Western blotting was revealed a significant change of P-smad-III phosphorylation .There was a marked reduction in betaglycan protein expression in knocked-down cells. Samples were compared to GAPDH. U = untransfected samples, S = Scrambled, B(-) = betaglycan knock-down.(figure 8).

Figure 8: Here betaglycan prevents P-smad III of being expressed using HK2 cells were transfected and 30 Western blotting carried out. GAPDH has been used for comparison. ST = Scramble + TGF-β, B(-)T = betaglycan knock-down + TGF-β. The relationship of betaglycan knock-down on TGF-β signalling in terms of P-smad-III phosphorylation changes which were used at different time intervals.

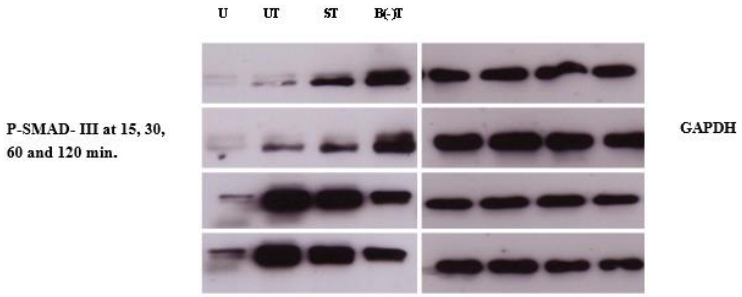

Figure 8: The effect of betaglycan siRNA on Smad-III phosphorlation

9. The relationship between betaglycan and TGF-β1 signalling following treatment with P-nitrophenyl-β-D-xylopyranoside (Dual Luciferase Enzyme Reporter)

The dual luciferase reporter assay (Promega) has been used to show how inhibition of betaglycan heparan sulphate with P-nitrophenyl-β-D-xylopyranoside regulates and affects TGF-β signalling (Figure 14). Removal

of betaglycan heparan sulphate in epithelial cells increases the ratio of TGF-β1 binding to TβR-II and TβR-I, attenuates degradation of TGF-β1 , and augments TGF-β1 induced cellular responses but this action depends on the magnitude of TGF-β as it has been known that around one-quarter of TGF-β has been shown to bind to heparan sulphate [24] (Figure 9).

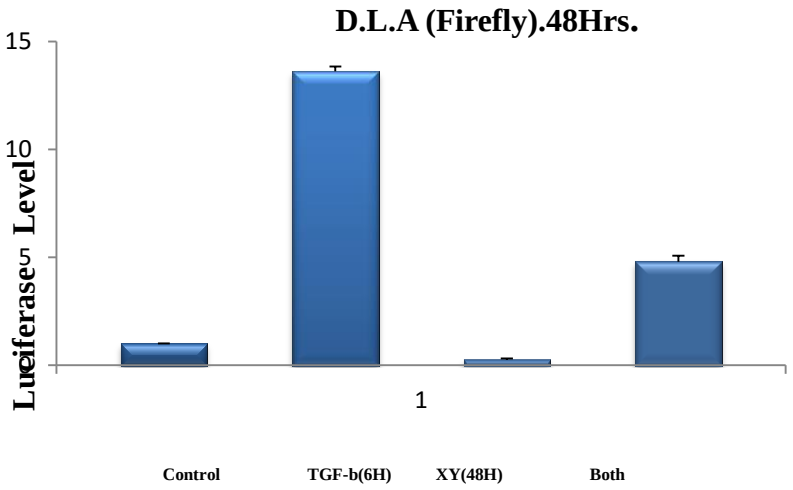

Figure 9: The relation between BG and TGF-β following P-N-Xpyranoside

Preparing the white 96 well plate, 12 wells + 2 blank + 1 extra = 15 wells, dose of P-nitro phenyl-β-D-xylopyranoside is 0.406 milligram per 500 microliter in each well, and for Tgf-β is 1 microlitre per ml which is equal to 1 ng per ml. Cells transfected with the CAGA-luciferase reporter construct, treated with P-nitro phenyl-β-D-xylopyranoside (single dose), Tgf-β stimulation was at 6 hours. Four groups used in the experiment (Control, Tgf-β, PXY, Both). The samples were incubated for 72 hours with the inhibitor. This experiment was repeated three times. ┬ = Standard deviation. * = P-value = < 0.05 = Statistically significant = < 0.05 , ** = P-value = highly significant = < 0.03 , Control = Neither XY nor TGF-β was applied. XY = P-nitro phenyl-β-D-xylopyranoside. The previous experiment (figure 9) shows that the inhibition of the active part of betaglycan (Heparan sulphate) has brought down TGF-β luminescence level. These results suggest that heparan sulfate modulates TGF-β1 responsiveness by decreasing the ratio of TGF-β1, thus diminishing signaling of TGF-β1 in the epithelial cells [25]. Generally betaglycan has an important impact on TGF-β signaling as it affects the core protein expression and subsequently the cell functions, when betaglycan blocked, its bands down-regulated and phosphorylation of P-smad III has changed, which shows how significant betaglycan (TGF-β-R III) could manage TGF-β signaling .

10. The effect of P-nitrophenyl-β-D-xylopyranoside treatment on betaglycan protein

Betaglycan and TGF-β1 signaling association were examined by Dual Luciferase Enzyme Reporter assay with P-nitrophenyl-β-D-xylopyranoside treatment. Betaglycan protein was down regulated as seen by the Western blotting experiment which was regulated by treatment with P-nitro phenyl-β-D-xylopyranoside (Figure 10)

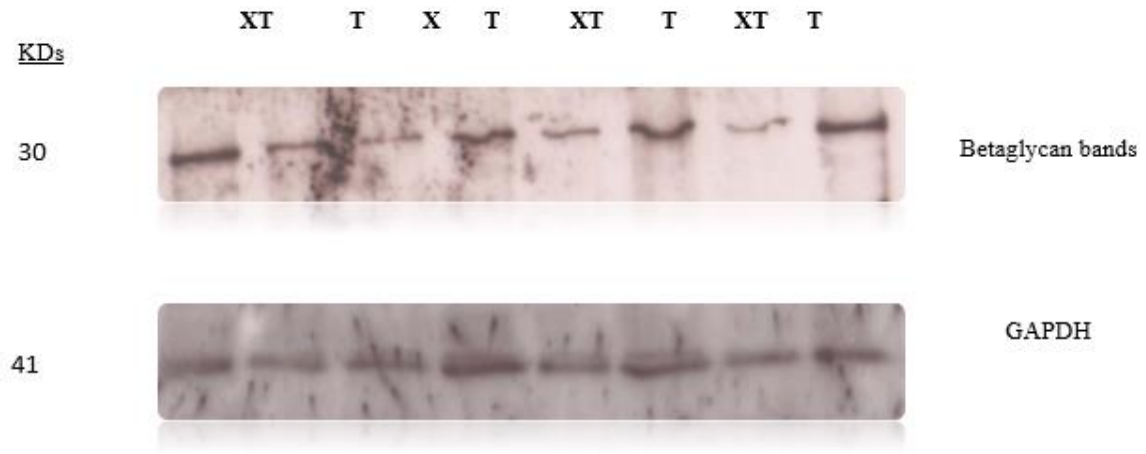

Figure 10: The effect of P-nitro phenyl-β-D-xylopyranoside treatment on betaglycan core-protein expression

Figure 10 : Incubation period of P-nitrophenyl-β-D-xylopyranoside was 72 hours. The effect of P-nitrophenyl-β-D-xylopyranoside treatment (The betaglycan functional part inhibitor, heparan sulphate), as it affects betaglycan protein expression, results assayed with GAPDH. High molecular weight ladder was used to visualize the high molecular weight betaglycan size. This experiment was repeated 3 times and gave the same result. T = TGF-β, XT = P-nitrophenyl-β-D-xylopyranoside + TGF-β.

11. Effect of P-nitrophenyl-β-D-xylopyranoside treatment on P-Smad-III

This experiment was to show how P-nitrophenyl-β-D-xylopyranoside could affect TGF-β core-protein (the P-Smad-III) which was visualised by Western blotting. As it has been shown above indicates that Betaglycan acts as apotential regulator of TGF- β1 signalling, TGF-β, the SBG sequester TGF-β, betaglycan can be knocked down by siRNA and affect affects the core protein expression P-smad III. Also inhibition of betaglycan heparan sulphate with P-nitrophenyl-β-D-xylopyranoside regulates and affects TGF-β signaling as explained [26].

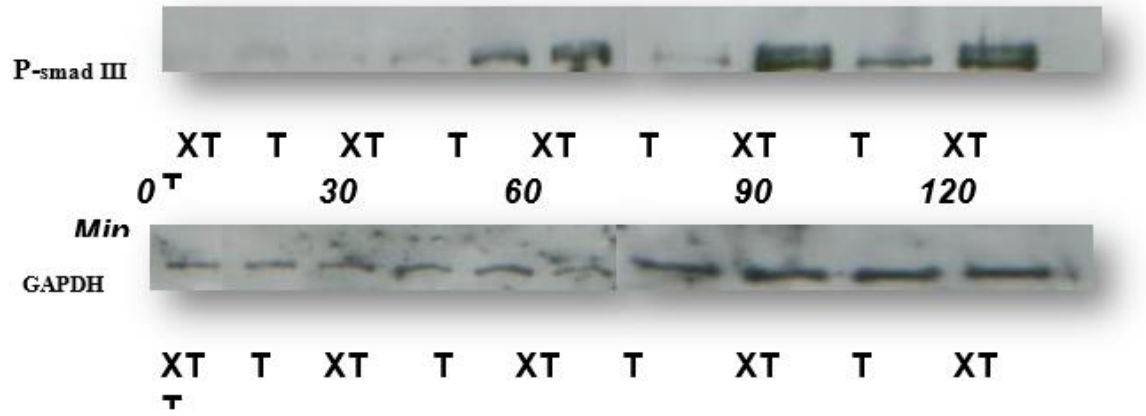

Figure 11: Effect of P-nitrophenyl-β-D-xylopyranoside treatment on P-smad-III . T = TGF-β, XT = Pnitrophenyl-β-D-xylopyranoside + TGF-β.

12. The Relationship between Corona virus and TGF-beta

TGF-β1 rises in plasma and lung tissues in patients during the early phase of SARS [27,28]. TGF-β1 is the major mediator of NF-κB, Egr-1, and STAT-3 trans activate TGF-β1 promoter, in which are regulated by various cellular kinases [29,30] The result demonstrated SARS-CoV PLpro initiating TGF-β1-dependent up-regulation of pro-fibrotic genes in human lung epithelial cells and, up regulating the expression of pro-fibrotic genes such as vimentin, type I and type III collagen Several transcription factors such as AP-1, Sp1, means how strong is the relationship between coronas viruses and TGF beta and that SARS-CoV PLpro plays an important role in triggering a significant increase of TGF-β1 mRNA and protein levels in human lung epithelial cells [31]. The knockdown of TGF-β1 gene expression by shRNA not only inhibits the replication of PRRSV but also improves immune responsiveness following viral infection, suggesting a novel way to facilitate the control of PRRSV infection in pigs..These results could inspire the development of novel promising way to prevent PRRSV infection of pig and promote the immunity of animal against viral disease [32]. In conclusion, SARS-CoV PLpro significantly induced the TGF-β1-mediated pro-fibrotic response via ROS/p38 MAPK/STAT3/Egr-1 pathway in vitro and in vivo. PLpro also triggered Egr-1 dependent transcription of TSP-1 as an important role in latent TGF-β1 activation. Therefore, Egr-1 plays a critical mediator in SARS-CoV PLpro induced pathogenesis, as a potential therapeutic target to prevent the pulmonary fibrosis induced by SARS-CoV. And finally come to the point that trying to block, reduce or counteract the action of TGF-β1 would inhibit or reduce the complication of viral spread and fibrosis as well as giving chance for cellular immunity to exert its effect and hence reduction of the viral yield in the infected cells. TGF-beta regulates an array of immune responses—both inflammatory and regulatory—however, its function is highly location- and context-dependent. We demonstrate that epithelial-derived TGF-β acts as a pro-viral factor suppressing early immune responses during influenza. [33]. (SARS-CoV) nucleocapsid (N) protein potentiates transforming growth factor-beta (TGF-beta)-induced expression of plasminogen activator inhibitor-1but attenuates Smad3/Smad4-mediated apoptosis of human peripheral lung epithelial HPL1 cells. The promoting effect of N protein on the transcriptional responses of TGF-beta is Smad3-specific. N protein associates with Smad3 and promotes Smad3-p300 complex formation while it interferes with the complex formation between Smad3 and Smad4. These findings provide evidence of a novel mechanism whereby N protein modulates TGF-beta signaling to block apoptosis of SARS-CoV-infected host cells and meanwhile promote tissue fibrosis. These results reveal a novel mode of Smad3 action in a Smad4-independent manner and may lead to successful strategies for SARS treatment by targeting the TGF-beta signaling molecules a novel mode of Smad3 action in a Smad4-independent manner and may lead to successful strategies for SARS treatment by targeting the TGF-beta signaling molecules.[34]. Silence of TGF-β1 gene expression reduces prrsv replication and potentiates immunity of immune cells of Tibetan pig. Epithelial-derived TGF-β acts to suppress early IFNβ responses leading to increased viral burden and pathology. Effective specific shRNA (short.haipin) tar gated to pig TGFβ1gene,it could significantly knockdown them RNA level of TGF-β1 gene and obviously increase the viability of PRRSV infected cells. The knockdown of TGF-β1 gene expression by shRNA could not only lead to significant inhibition of PRRSV replication in pig immune cells, but also enhance the antiviral immune responses and reduce the PRRSV yield in the infected cells.These results could inspire the development of novel promising way to prevent PRRSV infection of pig and promote the immunity of animal against viral disease [35].

13. Conclusion

As it was concluded that TGF-beta is a factor that has a strong interaction with corona virus mechanism of action and betaglycan is a potential regulator of TGF-beta, which is a multifunctional cytokine, belonging to the transforming growth factor superfamily that includes three different mammalian isoforms. TGF-beta causes cell growth, matrix deposition and eventually tissue fibrosis. Logically we could interfere or subside the biological effects of TGF-beta by reduction of pro-viral yields to facilitate a excellent chance for the human immune system to exert its defense actions. Therefore at this point, we can reach the main approach for protection.. To achieve such idea, we have to knockdown of TGF-β1 gene expression directly or to knock-down betaglycan, as betaglycan it is a potential regulator of TGF-beta by gene silencing of betaglycan genes or interfering with its Smad signaling pathways of TGF-beta and its consequences . Another method to block beatglycan is by blocking its heparan sulphate with P-nitrophenyl-β-D-xylopyranoside . In short blocking of Betaglycan brought down TGF-beta, which in turn enhance the antiviral immune responses of the human body and attenuate viral burden. At this time there will be enough time and chance for the immune system to get over most of the viral infection particularly Corona virus. This approach of management could be applied for most of the viruses irrespective of any genetic instability that the virus might have particularly corona virus. Finally, dealing with Betaglycan (TGF-beta III) is a Potential approach for fighting against Corona virus either by direct genetic interfering or by fighting to stop and treat its complication (eg. Tissue damage and fibrosis).

14. Recommendation

Corona virus is not easy to be killed directly but it needs to control the mechanisms that contribute to its mode of conjunction with Transforming growth factor β1 (TGF-β1) and its third receptor (Betaglycan)) during the infectious process (Methods explained above). Blocking this growth factor by the mentioned mechanisms definitely will give rise to a powerful weapon against corona virus and at the same time sparing the human own immune defense to work on its own combined with the current healthy and safety measures. I recommend the big recognized company to manufacture a suitable medicine based on this approach.

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