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Original Research Paper

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Virology

DNA METHYLATION AND MOLECULAR THERAPY OF LUNG CANCER LINKED WITH SARS-CoV-2

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ABSTRACT Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) contains several variants like Delta, Alpha, Beta and Omicron, that is linked to DNA methylation leading to lung cancers in humans. COVID-19 wave 1 and wave 2 are highly effected all around the world. Nine genes are identified in SARS-CoV-2 virus showing membrane protein with 669bp. The genes of normal and delta SARS COV-2 membrane proteins showed several mutations which is shown some effect with Remidesvir followed by Osimertinib and Curcumin. Normal, Beta and Alfa varients has shown in one branch. Delta varient (second wave) has shown in other branch which shown variation from first wave. Methylation frequency is 100% for PTGS2 followed by 77% for CDH1 for lung cancer. PTGS2 has shown good protein-protein interaction with ESR1, CDH1, APC, PTEN etc that are related for the cause of lung cancer. APC mediated PTCS2 from proliferation differentiation and survival can be controlled via drugs. The selected molecules like Remidesvir, Osimertinib and curcumin have shown control of mutated PTGS2 proteins of lung cancer. Osimertinib and Remidesvir found best with PTGS2 proteins.

KEYWORDS: lung cancer, SARS-CoV--2, DNA methylation, Molecular therapy

INTRODUCTION

Cancer is a growing and epidemic disease that affects millions of humans all around the world every year (Youlden et al., 2008; Kaladhar and Srinivasan, 2022). Lung cancer is the major cause of cancer deaths worldwide. Understanding the process of totipotency and cause of abnormal growth in cells and tissues due to cancer is an essential step used to managing cancer in the future (López-Lázaro, 2018). Lung cancer is a type of cancer that make abnormal growth in lungs. Greatest risk of lung cancer occurs in people who smoke regularly due to damage and mutation in genes from lung cells/tissues.

The COVID-19 (Lone and Ahmad, 2020) a pandemic coronavirus is an ongoing (as on 23-4-2022) global coronavirus disease 2019 (COVID-19) caused by a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) containing several variants like Delta, Alpha, Beta and Omicron (Kaladhar, 2021). The novel virus was identified in Wuhan, China, in December 2019 and as on 31 August 2021, more than 217 million cases and 4.51 million deaths have been confirmed with several waves due to viral mutations in modern era.

GISAID is a good SARS CoV-2 database was shown in figure 1.



Figure 1: GISAID, a SARS CoV-2 database (As on 31 August 2021)

DNA methylation is a biological process by which methyl groups are added to the DNA segment without changing the sequence (Bird, 2002). DNA methylation typically acts to repress normal development and gene transcription. and is associated with a number of key biological processes like Xchromosome inactivation, genomic imprinting, chromosomal instability, aging, repression of transposable elements, and carcinogenesis (Robertson and Wolffe, 2000). Alterations of DNA methylation inherited by daughter cells acquired by abnormal hypermethylation have been recognized as an important component of cancer development due to transcriptional silencing. oncogene suppressor silencing linked to hypermethylation associated with promoters might be a target for epigenetic therapy (Baylin and Ohm, (2006).

MATERIALS AND METHODS

Genome retrieval

The wave 1 normal genome of Severe acute respiratory syndrome coronavirus 2 (NC_045512.2 Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1) is retrieved from NCBI database. The genome is 29903 bp, ss-RNA and linear.

The wave 2 Beta corona virus genome of AY.4 (Pango v.3.1.11 2021-08-24 ; hCoV-19/India/AP-CCMB-CIA7806/2021 |EPI_ISL_3935286|2021-08-06), Delta (B.1.617.2-like) (Scorpio) type of VOC Delta GK/478K.V1 (B.1.617.2+AY.x) first detected in India was retrieved from GISAID(https:// www.gisaid.org/hcov19-variants/).

FGENESV0

FGENESV0 is the server that predicts genes present in viral genome. (http://www.softberry.com/berry.phtml?topic=virus0&group=programs&subgroup=gfindv)

BLASTp

BLASTp is a protein alignment server for protein identification that shows identification of genes for query protein.

MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0

The Molecular Evolutionary Genetics Analysis (MEGA) software is developed for comparative analyses of DNA and protein sequences that are aimed at inferring the molecular evolutionary patterns of genes, genomes, and species over time

System properties

Processor of Intel ® Core ™ i5 CPU 650@ 3.20GHz Processor with 8GB RAM and 64 bit operating system.

Pubmeth

Pubmeth is a cancer methylation database which is annotated and reviewed based on the automated textmining of literature in the databases. The database includes several reporting of genes that are methylated in several cancer types like oral cancer. The website for search for PUBMETH is

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http://pubmeth.biobix.be/search.html. The diseases that are related to lung cancer have been analyzed using pubmeth database.

String v11.0

STRING v11.0 is a database that is known to predicted protein-protein interactions based on the physical and functional associations from several databases. The search site for multiple protein interactions is https://stringdb.org/cgi/input?sessionId=byDwXWOC0ymQ&input_page active form=multiple identifiers.

Retrieval of ligands and proteins

The drug molecules are retrieved from Drugbank

Table 1: Drugs for Colorectal Cancer from DrugBank

S.No	Name	DrugBank
1	Curcumin	DB11672
2	Remdesvir	Db14761
3	Osimertinib	DB09330

The mutated protein of tumor gene PTGS2 (pdb id: 5f19) is retrieved from string via PDB database (https://www.rcsb.org/). The modeled structures of covid proteins conducted using swiss model (https://swissmodel.expasy.org/)

iGEMDOCK v2.1

iGEMDOCK is a Graphical Environment used for Recognizing Pharmacological Interactions and Virtual Screening processes. Pharmacological interactions are useful for understanding ligand binding mechanisms and identifying lead compounds for the therapeutic target against diseased gene or protein.

RESULTS AND DISCUSSION

Global mean methylation levels assessed by scientists did not differ much between COVID-19 patients and healthy prepandemic controls (Balnis et al., 2021). About 75% of acute illness are associated differentially in the methylated regions were identified near gene promoter regions. Gene ontology analyses associated with the immune response revealed with viral infections and the leukocyte activation (Joshi and Gangenahalli, 2020). The disease ontology analyses has shown a predominance of autoimmune disorders among COVID-19-positive patients associated with a prevailing hyper-methylation.

Table 2: Gene identification for COVID-19 (First wave) and Delta(Second wave)

Gene	COVII	0-19 (First w	/ave)	Delta(Delta(Second wave)										
	Start	End	Score	Start	End	Score									
1	266 -	13483	13218	235 -	13452	13218									

2	13768 -	21555	7788	13737 -	21524	7788
3	21536 -	25384	3849	21505 -	25353	3849
4	25393 -	26220	828	25362 -	26189	828
5	26245 -	26472	228	26214 -	26441	228
6	26523 -	27191	669	26492 -	27160	669
7	27394 -	27759	366	27363 -	27728	366
8	27894 -	28259	366	27863 -	28228	366
9	28274 -	29533	1260	28243 -	29502	1260

Table 3: Gene identification of normal and delta virus

Gene	Normal	Delta					
Number							
1	ORF1a polyprotein [Severe acute respiratory syndrome coronavirus 2]	ORF1a polyprotein [Severe acute respiratory syndrome coronavirus 2]					
2	ORF1ab polyprotein [Severe acute respiratory syndrome coronavirus 2]	ORF1ab polyprotein [Severe acute respiratory syndrome coronavirus 2]					
3	surface glycoprotein [Severe acute respiratory syndrome coronavirus 2]	surface glycoprotein [Severe acute respiratory syndrome coronavirus 2]					
4	ORF3a protein [Severe acute respiratory syndrome coronavirus 2]	ORF3a protein [Severe acute respiratory syndrome coronavirus 2]					
5	envelope protein [Severe acute respiratory syndrome coronavirus 2]	envelope protein [Severe acute respiratory syndrome coronavirus 2]					
6	membrane glycoprotein [Severe acute respiratory syndrome coronavirus 2]	membrane glycoprotein [Severe acute respiratory syndrome coronavirus 2]					
7	ORF7a protein [Severe acute respiratory syndrome coronavirus 2]	ORF7a protein [Severe acute respiratory syndrome coronavirus 2]					
8	ORF8 protein [Severe acute respiratory syndrome coronavirus 2]	ORF8 protein [Severe acute respiratory syndrome coronavirus 2]					
9	nucleocapsid phosphoprotein [Severe acute respiratory syndrome coronavirus 2]	nucleocapsid phosphoprotein [Severe acute respiratory syndrome coronavirus 2]					

Gene variation locations were shown in Figure 4.

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Species/Abb Group Name	***************************************
1. Delta	HVSGINGIKRFDNPVLPFNDGVYFASIEKSNIIRGWIFGTILDSKIQSLLIVNNAINVVI
2. Normal	HVSGINGIKRFDNPVLPFNDGVYFASIEKSNIIRGWIFGIILDSKIQSLLIVNNAINVVI
Species/Abb Group Name	******* *******************************
1. Delta	SKVGGNYNYRYRLFRKSNLKPFERDISTEIYQAGSKPCNGVEGFNCYFPLQSYGFQPING
2. Normal	SKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNG
Species/Abt Group Name	***************************************
1. Delta	VITPGTNTSNQVAVLYQGVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHV
2. Normal	VITPGINTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHV
Species/Abk Group Name	***************************************
1. Delta	ECDIPIGAGICASYQTQTNSRRRARSVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTI
2. Normal	ECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYIMSLGAENSVAYSNNSIAIPINFII
Species/Abb Group Name	***************************************
1. Delta	DRLNEVAKNINESLIDIQEIGKYDQYIKWPWYIWIGFIAGLIAIVMVTIMLCCMTSCCSC
2. Normal	DRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSC

Figure 5 : Surface protein comparision

The phylogenetic analysis for varients of SARS CoV-2 was shown in Figure 6. Normal, Beta and Alfa varients has shown in one branch. Delta varient (second wave) has shown in other branch which shown variation from first wave.



ב אומים א

Figure 6: Phylogenetic analysis for varients of SARS CoV-2

The genes relatyed to lung cancer with methylation was shown in Table 4.

Table 4: Methylated genes relatyed to lung cancer

Methylated Gene	Name as on Pubmeth (No of references 10 and above)
CDKN2A	Cyclin-dependent kinase inhibitor 2A, isoform 4 (p14ARF) (p19ARF)
RASSF1	Ras association domain-containing protein 1
MGMT	Methylated-DNAprotein-cysteine methyltransferase (EC 2.1.1.63) (6-O- methylguanine-DNA methyltransferase) (MGMT) (O-6-methylguanine-DNA- alkyltransferase)
CDH1	Epithelial-cadherin precursor (E-cadherin) (Uvomorulin) (Cadherin-1) (CAM 120/80) (CD324 antigen
MLH1	DNA mismatch repair protein Mlh1 (MutL protein homolog 1)
DAPK1	Death-associated protein kinase 1 (EC 2.7.11.1) (DAP kinase 1)
APC	Adenomatous polyposis coli protein (Protein APC)
GSTP1	Glutathione S-transferase P (EC 2.5.1.18) (GST class-pi) (GSTP1-1)
RARB	Retinoic acid receptor beta (RAR-beta) (RAR- epsilon) (HBV-activated protein)
CDKN2B	Cyclin-dependent kinase 4 inhibitor B (p14- INK4b) (p15-INK4b) (p15INK4B) (Multiple tumor suppressor 2) (MTS2)

KAEŐAIKM	PWYIWLGFIAGLIAIVMVTIMLCCMTSCCSC						
TIMP3	Metalloproteinase inhibitor 3 precursor (TIMP- 3) (Tissue inhibitor of metalloproteinases 3) (Protein MIG-5)						
FHIT	Bis(5'-adenosyl)-triphosphatase (EC 3.6.1.29) (Diadenosine 5',5'''-P1,P3-triphosphate hydrolase) (Dinucleosidetriphosphatase) (AP3A hydrolase) (AP3AASE) (Fragile histidine triad protein)						
ESR1	Estrogen receptor (ER) (Estradiol receptor) (ER- alpha)						
PTGS2	Prostaglandin G/H synthase 2 precursor (EC 1.14.99.1) (Cyclooxygenase- 2) (COX-2) (Prostaglandin-endoperoxide synthase 2) (Prostaglandin H2 synthase 2) (PGH synthase 2) (PGHS-2) (PHS II)						
CDH13	Cadherin-13 precursor (Truncated-cadherin) (T cadherin) (T-cad) (Heart-cadherin) (H- cadherin) (P105)						
RUNX3	Runt-related transcription factor 3 (Core- binding factor, alpha 3 subunit) (CBF-alpha 3) (Acute myeloid leukemia 2 protein) (Oncogene AML-2) (Polyomavirus enhancer-binding protein 2 alpha C subunit) (PEBP2-alpha C) (PEA2-alpha C) (SL3-3 enhancer factor						
PTEN	Phosphatidylinositol-3,4,5-trisphosphate 3- phosphatase and dual- specificity protein phosphatase PTEN (EC 3.1.3.67) (EC 3.1.3.16) (EC 3.1.3.48) (Phosphatase and tensin homolog) (Mutated in multiple advanced cancers 1)						
TMEFF2	Transmembrane protein with EGF-like and two follistatin-like domains 2 precursor (Transmembrane protein containing epidermal growth factor and follistatin domains) (Tomoregulin) (TR) (Hyperplastic polyposis protein 1)						
SCGB3A1	Secretoglobin family 3A member 1 precursor (Uteroglobin-related protein 2) (Cytokine HIN- 1) (High in normal 1) (Pneumo secretory protein 2) (PnSP-2)						
CADM1	immunoglobulin superfamily, member 4						
CHFR	E3 ubiquitin-protein ligase CHFR (EC 6.3.2) (Checkpoint with forkhead and RING finger domains protein) (RING finger protein 196)						
ZMYND10	Zinc finger MYND domain-containing protein 10 (BLu protein)						

DNA methylation in blood occur in the promoter regions of immune-related genes. Table 5 shows the genes responsible for lung cancer related to methylation. Methylation frequency is 100% for PTGS2 followed by 77% for CDH1 for lung cancer.

	responsible for l		-		
Gene	Total	Number of	Methylation	Number of	Details for methylation
	Number of	references in	frequency	samples	In lung
CDIDIO	references	lung cancer			
CDKN2A	205				non-small cell lung cancer (24); no
		31	32	2048	subtype specified (5),
					non-chromate lung cancer (1),
DAGGE	105	1.7	41	070	small cell lung cancer (1)
RASSF1	125	17	41	972	non-small cell lung cancer (11) small cell lung cancer (4) no subtype specified (2)
MGMT	86	7	24	422	non-small cell lung cancer (5) no subtype specified (2)
CDH1	81	3	77	84	no subtype specified (2) non-small cell
					lung cancer (1)
MLH1	69	1	7	75	non-small cell lung cancer (1)
DAPK1	68	7	32	404	non-small cell lung cancer (7)
APC	65	6	54	557	non-small cell lung cancer (6)
GSTP1	56	4	14	307	non-small cell lung cancer (4)
RARB	48	6	39	446	non-small cell lung cancer (3) no subtype
					specified (3)
CDKN2B	42	1	23	48	non-small cell lung cancer (1)
TIMP3	34	3	28	18	non-small cell lung cancer (2) small cell
					lung cancer (1)
FHIT	24	5	28	451	non-small cell lung cancer (3) small cell
					lung cancer (1)
					no subtype specified (1)
ESR1	24	3	3	130	non-small cell lung cancer (2) no subtype
					specified (1)
PTGS2	20	2	100	7	no subtype specified (1)
CDH13	17	6	37	846	non-small cell lung cancer (6)
		-			adenocarcinoma (1)
RUNX3	17	3	26	66	non-small cell lung cancer (2)
					small cell lung cancer (1)
PTEN	15	1	23	30	non-small cell lung cancer (1)
TMEFF2	13	2	30	56	non-small cell lung cancer (1)
agabat 1		2		10	small cell lung cancer (1)
SCGB3A1	12	3	2	16	small cell lung cancer (2)
CADM1	12	4	39	386	non-small cell lung cancer (4)
CHFR	11	1	10	20	non-small cell lung cancer (1)
ZMYND10	10	1	0	0	small cell lung cancer (1)

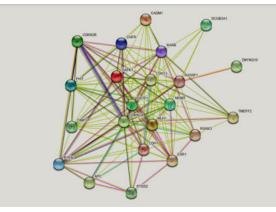


Figure 7: Protein-Protein interaction analysis

Figure 7 has shown that protein interactions for lung cancer. PTGS2 has shown good interaction with ESR1, CDH1, APC, PTEN etc that are related for the cause of lung cancer.

Table 6 has shown that Methylation genes with different types of diseases

Table 6: Diseases related to lung Cancer

S.No	Disease	Top Affiliating Genes (text searches by Pubmeth)
1	small cell lung cancer	CDKN2A; RASSF1; MGMT; TIMP3; FHIT; RUNX3;TMEFF2; SCGB3A1; ZMYND10

2	non-small cell lung cancer	CDKN2A; RASSF1; CDH1; MLH1; DAPK1; APC; GSTP1; TIMP3; RARB; CDKN2B; FHIT; ESR1; CDH13; RUNX3; PTEN; TMEFF2; CADM1; CHFR
3	adenocarcin oma	CDH13
4	no subtype specified	CDKN2A; RASSF1; MGMT; CDH1; RARB; FHIT; ESR1; PTGS2
5	non- chromate lung cancer	CDKN2A

Lung cancer

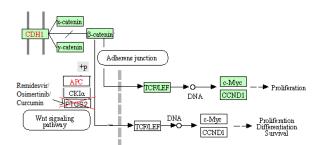


Figure 8: Mechanism of lung cancer via SARS-CoV-2 control by drugs

Figure 8 has shown that APC mediated PTCS2 from proliferation differentiation and survival can be controlled via

drugs.

Figure 9 shown modeled structures for $\ensuremath{\text{PTGS2}}$ and membrane proteins of $\ensuremath{\text{SARS}}$

Figure 9: Modeled/retrieved receptors in present study

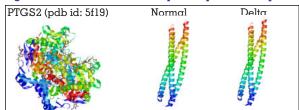


Figure 10 has shown the magic fit in SPDBV which shown 100 percent superimposition with normal and delta viruses.

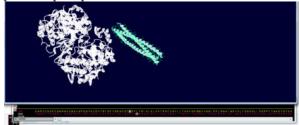


Figure 10: Protein superimposition

Figure 11 has shown ligands selected for the present work.

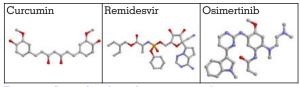


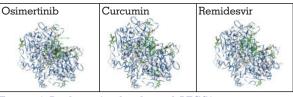
Figure 11: Ligands selected in present study

Table 7: Activity of drugs against PTGS2 gene as molecular therapy for lung cancer

S.No	Name of Drug	PTGS2		Delta	
		Total energy in Kcal/m ol	Active site	Total energ y in Kcal/ mol	Active site
1	Remide svir	-114.07	H-M-ALA-132 H-S-ASP-133 H-M-MET-458 V-S-ASN-34 V-M-ASP-133 V-S-ASP-133 V-M-GLY-135 V-S-PRO-154 V-S-PRO-156 V-S-ASP-157	-90.41	H-M-ALA-965 H-M-GLN-966 H-M-ASN-969 H-S-ASN-969 V-S-ASN-964 V-M-ALA-967 V-M-ASN-962 V-S-ASN-962 V-M-ALA-965 V-S-LEU-968 V-S-ASN-969
2	Osimer tinib	-118.99	H-S-GLN-203 H-S-HIS-388 V-M-GLN-203 V-S-GLN-203 V-S-HIS-207 V-S-LEU-294 V-S-HIS-386 V-M-TRP-387 V-S-TRP-387 V-S-TRP-387 V-S-HIS-388 V-S-VAL-447	-80.19	H-S-SER-938 V-M-ASN-934 V-S-ASN-934 V-M-ASN-937 V-M-SER-938 V-S-SER-938 V-M-GLY-941 V-S-LYS-942 V-S-GLN-944
3	Curcu min	-96.25	H-S-HIS-122 H-S-GLU-465 V-M-ARG-44	-74.27	H-S-ASN-964 H-S-GLN-974 V-M-ALA-965

V-S-ARG-44	V-M-GLN-966
V-M-GLY-45	V-S-LEU-968
V-S-ASP-125	V-S-ASN-969
V-S-LEU-152	V-S-VAL-972
	V-M-ALA-967
	V-M-LEU-971
	V-S-LEU-971

The selected molecules like Remidesvir, Osimertinib and curcumin have shown control of mutated PTGS2 proteins of lung cancer. Osimertinib and Remidesvir found best with PTGS2 proteins. No good selected compound identified for delta virus but shown controlling activity with. Remidesvir followed by Osimertinib and curcumin (Table 7; Figure 12 and 13).





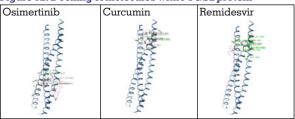
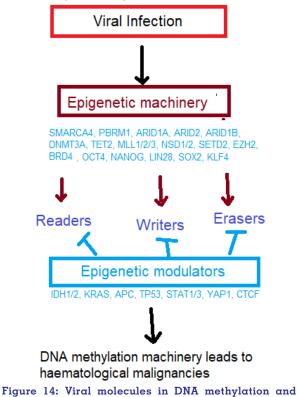


Figure 13: Docking of molecules with PTGS2 protein

Cancer is made up of abnormal cells spread (metastasize) to the lungs associated with many other types of cancer like breast or kidney cancers (Sountoulides et al., 2011). The viral infections and proteins effects epigenetic machinery leads to DNA methylation and changes in epigenetic modulator that leads to malignancies (Figure 14).



carcinogenesis

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Hence the present work shows that SARS-CoV-2 undergo mutations as important cause of disease and may further lead to lung cancer in some patients. The cancers can be controlled using medicinal compounds.

CONCLUSION

Due to PTGS2 gene mutation, Beta -catenin suppresses the Tcell responses and promotes the progression of tumors. The selected molecules like Remidesvir, Osimertinib and curcumin have shown control of mutated PTGS2 proteins. Osimertinib and Remidesvir found best with PTGS2 proteins. No good compound identified for delta virus. Self control may be the only the precautionary measures for control of Delta virus.

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