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DNA METHYLATION AND MOLECULAR THERAPY FOR COLORECTAL CANCER CAUSED DUE TO JOHN CUNNINGHAM VIRUS

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ABSTRACT

John Cunningham virus (JCV) contain small, closed circular and double-stranded 5.1 kilobase pairs DNA genome that belongs to nonenveloped and icosahedral virus. The complete sequence was 5,130 bp genomic sequence length, circular and with GC% of 40.55. There are 5 genes predicted based on FGENESV0 server: agnoprotein, VP2, VP1, large T antigen and small T antigen. Methylation frequency is above 50 is shown for CDKN2A, RASSF1, MGMT, CDH1, MLH1, DAPK1, APC and GSTP1genes that are related to colorectal cancer. Irinotecan may be the best option molecular therapy in control of colorectal cancer caused due to JCV.

Keywords: John Cunningham virus (JCV), large T antigen, small T antigen, colorectal cancer

INTRODUCTION

John Cunningham virus (JC virus) belongs to the family of Polyomavirus that plays an important role in the tumorigenesis and progressive multifocal leukoencephalopathy (PML) (Zheng et al., 2009). JCV contain small, closed circular and double-stranded 5.1 kilobase pairs DNA genome that belongs to nonenveloped and icosahedral virus (Núñez, 2016). Human polyomavirus 2, a type of human polyomavirus that is formerly known as papovavirus is commonly referred to as the John Cunningham virus or JC virus (Pietropaolo et al., 2018; Bellizzi et al., 2013).

Large T antigen and small T antigen is a large nuclear phosphoprotein produced due to alternative splicing of early region promotes viral DNA replication, binds to viral replication region, unwinding of double helix and recruitment of cell proteins for DNA synthesis, might have a possible role with concern to colorectal cancers (Hori et al., 2005; An et al., 2012). The capsid structural proteins VP1, VP2 and VP3 are essential to assemble with viral DNA to form viroids formed due to alternative splicing and the small regulatory protein known as agnoprotein by encoding of late region (Figure 1).

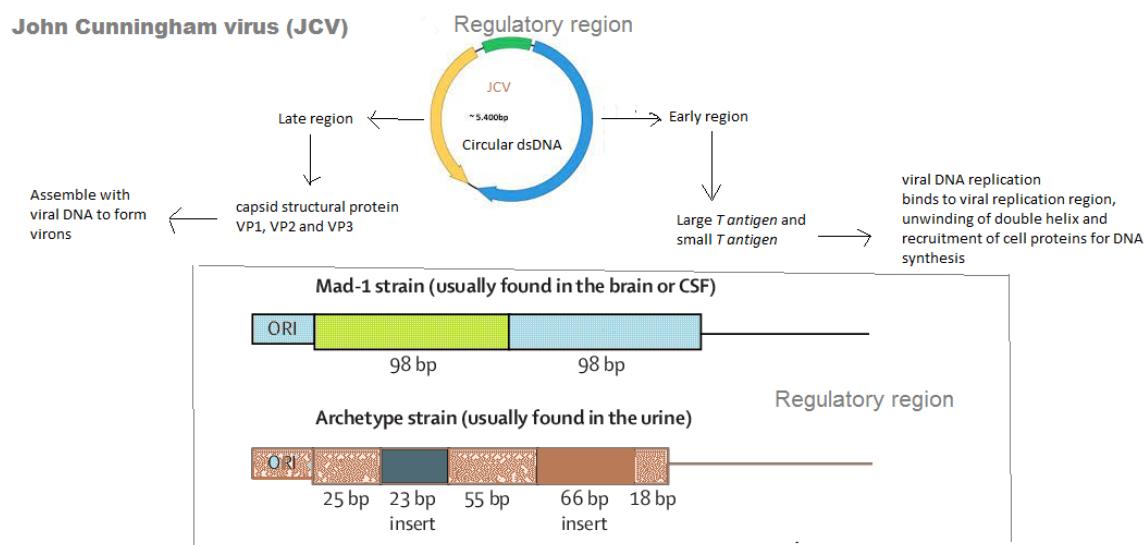


Figure 1: John Cunningham virus (JCV)

Colorectal cancer (CRC) is the third most common cancer in women and fourth

most common cancer in men worldwide and constitutes the second leading cause

of cancer mortality in humans (Herszenyi and Tulassay, 2010; Favoriti et al., 2016). Genetic changes in the germ line drive the progression from adenoma to carcinoma. Obesity, nutritional intake, water property, alcoholic beverages, smoking and physical inactivity may be the factors for colorectal cancer (Giovannucci, 1995). Promoter association of hypermethylation profiles of DAPK1, SFRP2, HIC1, MGMT and p16 genes and BRAF and KRAS mutations cause secondary epigenetic changes in colorectal cancer with JCV (Pehlivan et al., 2010; Bagci, et al., 2016). Tumor suppressor gene, Death-associated protein kinase 1 (DAPK1) is encoding to a calcium/calmodulin-dependent protein kinase induces apoptosis positively and may be used in colorectal cancer research and diagnostics (Farag and Roh, 2019).

MATERIALS AND METHODS

The complete genome of JC polyomavirus was retrieved from NCB with the accession number NC_001699. The complete sequence was 5,130 bp genomic sequence length, circular and with GC% of 40.55.

FGENESV0

(<http://www.softberry.com/berry.phtml?to>

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pic=virus0&group=programs&subgroup=gfi
ndv) is the server that predicts viral genes present in viral genome.

BLASTp

(https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) is a protein alignment server that works with local alignment and shows identification of genes.

System properties

Intel ® Core ™ i5 CPU 650 @3.20 GHz processor with 8 GB RAM and 64-bit Operating System.

Pubmeth

Pubmeth is a cancer methylation database that is designed based on annotated and reviewed process on automated text mining of literature (Chaitanya et al., 2012). The database includes reporting of genes that are methylated in several cancer types like colorectal cancer cancer. The website for search for PUBMETH is <http://pubmeth.biobix.be/search.html>.

SPDBV

SPDBV tool is used for identifying related proteins based on protein superimposition method.

String v11.0

(https://string-db.org/cgi/input?sessionId=byDwXWOC0ymQ&input_page_active_form=multiple_identities) is a database that is used to predicted protein-protein interactions.

Retrieval of ligands and proteins

The drug molecules are retrieved from Drugbank (Figure 2)

Table 1: Drugs for Colorectal Cancer from DrugBank

S.No	Name	ChEBI
1	5-Fluorouracil (5-FU)	DB00544
2	Capecitabine	DB01101
3	Irinotecan	DB00762
4	Oxaliplatin	DB00526
5	Trifluridine	DB00432
6	Tipiracil	DB09343

The mutated protein of tumor gene DAPK1 (Figure 2) is retrieved from string via PDB database (<https://www.rcsb.org/>). Large T

antigen and small T-antigen were modelled using swiss-model (Figure 2).

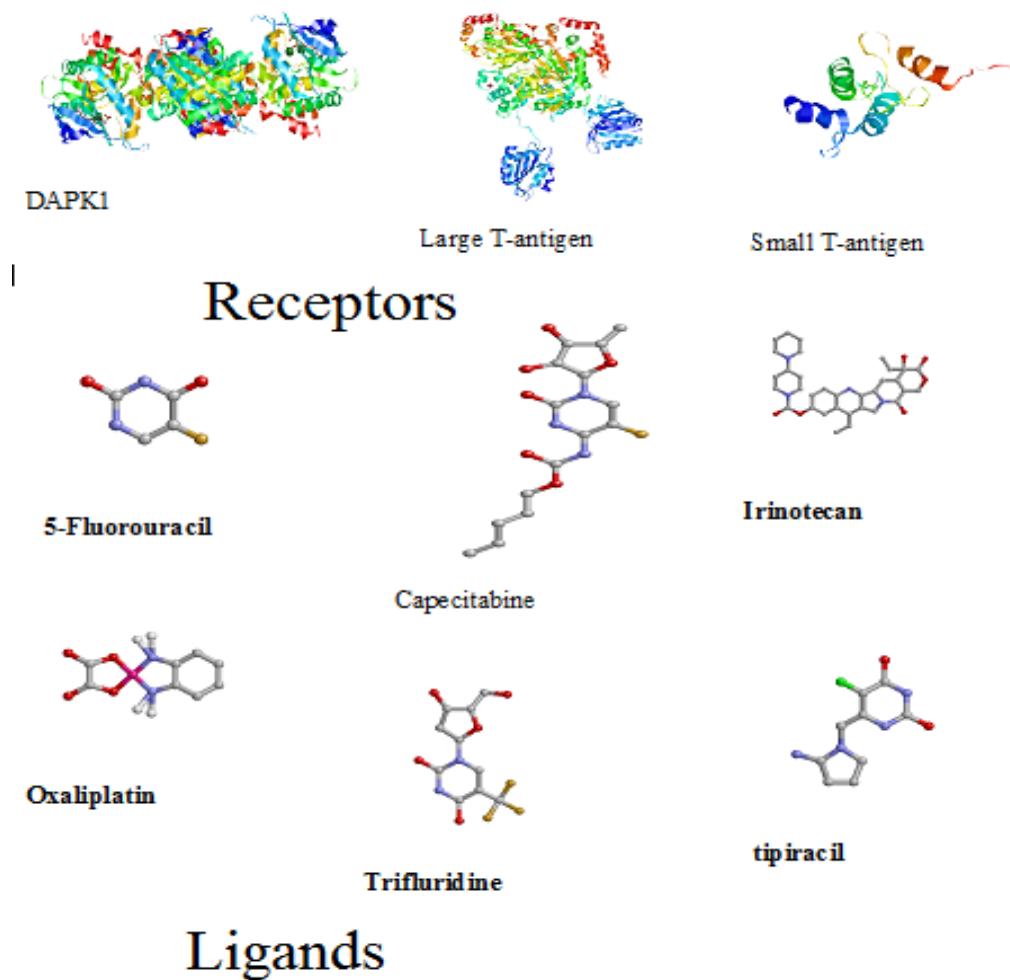


Figure 2: Molecules used in present study

iGEMDOCKv2.1

iGEMDOCK v2.1 is a docking tool used for virtual screening of ligands with selected proteins.

RESULTS

There are 5 genes predicted based on FGENESV0 server. The predicted genes are identified as agnoprotein, VP2, VP1, large T antigen and small T antigen (Table 2).

FGENESV0: Prediction of potential genes in viral genomes

Time: Tue Jan 1 00:00:00 2005

Seq name: test sequence

Length of sequence - 5130 bp

Number of predicted genes – 5

N	S	Start	End	Score
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1	+	CDS	277 -	492	216
2	+	CDS	526 -	1560	1035
3	+	CDS	1463 -	2533	1071
4	-	CDS	2603 -	4339	1737
5	-	CDS	4495 -	5013	519

Predicted protein(s):

>GENE 1 277 - 492 71 aa, chain +

MVLRQLSRKASVKVSKTWSGTKKRAQRILIFLLEFLDFCTGEDSVDGKKRQRHSGLTEQTYSLPEP
KAT

>GENE 2 526 - 1560 344 aa, chain +

MGAALALLGDLVATVSEAAAATGFSVAEIAAGEAAATIEVEIASLATVEGITSTSEAIAA
IGLTPETYAVITGAPGAAGFAALVQTVTGGSAIAQLGYRFFADWDHKVSTVGLFQQPAMALQLFNPE
DYYDILFGVNAFVNNIHYLDPRHWGPSLFSTISQAFWNLVRDDLPALTSEQEIQRRTQKLFVESLARFL
EETTWAIVNSPANLYNYISDYYSLSPVRPSMVRQVAQREGTYISFGHSYTQSIDDADSIQEVTQRLDL
KTPNVQSGEFIERSIAPGGANQRSAPQWMLPLLLGLYGTVPALAEAYEDGPNKKRKEGPRASSKTS
YKRRSRSSRS

>GENE 3 1463 - 2533 356 aa, chain +

MKMAPTKRKGERKDPVQVPKLLIRGGVEVLEVKTGVDSITEVECFLETPEMGDPDEHLRGFSKSISISDT
FESDSPNRDMLPCYSVARIPLPNLNEDLTCGNILMWEAVTLKTEIGVTSLMNVHSNGQATHDNGAG
KPVQGTSFHFSSVGEALELQGVLFNYRTKYPDGTIFPKNATVQSQVMNTEHKAYLDKNKAYPVECW
VPDPTRNENTRYFGTLTGGENVPPVLHITNTATTVLLDEFGVGPLCKGDNLYLSAVDVCGMFTNRSGS
QQWRGLSRYFKVQLRKRRVKNPYPISFLTDLINRRTPRVDGQPMYGMADAQVEEVRFEGTEELPGD
PDMMRYVDKYGQLQTKML

>GENE 4 2603 - 4339 578 aa, chain -

MFASDDENTGSQHSTPPKKKKVEDPKDFPVDLHAFLSQA VFSNRTVASFAVYTTKEKAQILYKKLM
EKYSVTFISRHGFGGHNILFFLTPHRHRSAINNYCQKLCTFSFLICKGVNKEYLFY SALCRQPYAVV
E SIQGGLKEHDFNPEEPEETKQVSWKLVTQYALETKCEDVFLLMGMYLDFQENPQQCKCEKKDQPNH
FNHHEKHYNAQIFADSKNQKSICQQAVDTVAAKQRVDSIHMTREEMLVERFNFLDKMDLIFGAHG
NAVLEQY MAGVAWIHCCLPQM DTVIYDFLKCI VLNIPKKRYWLKG PIDSGKTT LAA ALLDLCGGKSL
NVNMPLERLN FELGVGIDQFMVV FEDVKGTGAESRD LPSGHGISNLDCLRDYLDGSVKVNLERHQ
N KRTQVFPPI GIVTMNEY SV PRTLQARFVRQIDFRPKAYLRKSLSCSEYLLEKRILQSGMTLLL
LIWFRPV
ADFAAAIHERIVQWKERLDLEISMYTFSTMKANVGMGRPILDFPREEDSEAEDSGHGSSTESQSQCFSQ
VSEASGADTQENCTFHICKGFQCFKKPKTPPPK

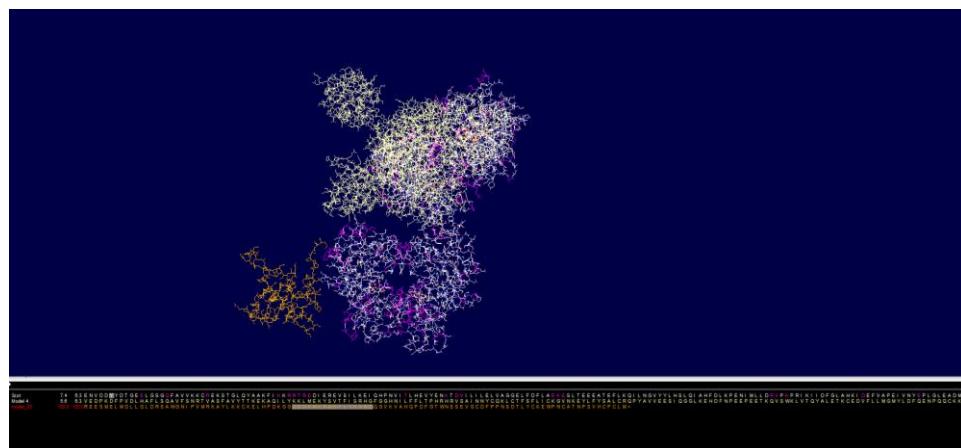
>GENE 5 4495 - 5013 172 aa, chain -

MDKVLNREESMELMDLLGLDRSAWGNIPVMRKAYLKKCKELHPDKGGDEDKMKRMNFLYKKMEQ
GVKVAHQPDFGTWNSSEVGCDFFPPNSDTLYCKEWPNATNPSVHCPCLMCMLKLRHRNRKFRLRSSPL
VWIDCYCFDCFRQWFGCDLTQEALHCWEKVLGDTPYRDLKL

Table 2: Identification of genes

Gene number	Gene name
1	agnoprotein [JC polyomavirus]
2	vp2 [JC polyomavirus]
3	vp1 [JC polyomavirus]
4	large T antigen [JC polyomavirus]
5	small T antigen [JC polyomavirus]

Figure 3 shows the superimposition of Small T antigen with large T antigen and DAPK1. The large T antigen shows 5% alignment and DAPK1 shows 7.4% alignment with small T antigen. The molecules shows non similar properties based in structural alignment.

**Figure 3 Superimposition of Small T antigen with large T antigen and DAPK1****Table 3: Methylated frequency and details in colorectal cancer genes using Pubmeth**

Gene	Number of references	Number of references in gastric cancer	Number of samples	Methylation frequency	Details for methylation In Gastric
CDKN2A	205	13	1742	19	No subtype; adenoma 3;carcinoma 2;adenocarcinoma 1
RASSF1	125	6	522	17	no subtype specified (5) adenoma (1)

MGMT	8610	10	1445	35	no subtype specified (6) adenoma (2) carcinoma (2)
CDH1	81	8	421	35	no subtype specified (3) adenocarcinoma (2) adenoma (1) sporacarcinoma (1) sporadic (1)
MLH1	69	20	2126	13	no subtype specified (12) carcinoma (3) hereditary non-polyposis (2)
DAPK1	68	3	365	47	no subtype specified (2) no subtype specified (2)
APC	65	12	684	30	no subtype specified (8) adenoma (2) carcinoma (2)
GSTP1	56	2	185	11	carcinoma (1), no subtype specified (1)

Table 3 has shown that Methylation frequency above 50 is shown for CDKN2A, RASSF1, MGMT, CDH1, MLH1, DAPK1, APC and GSTP1.

Table 4: Diseases related to Colorectal Cancer

S.No	Related Disease	Top Affiliating Genes (text searches by Pubmeth)
1	No Subtype	Cdkn2a/ RASSF1; MGMT; CDH1; Mlh1; DAPK1 ; APC; GSTP1
2	Adenoma	Cdkn2a
3	Carcinoma	APC; MGMT; CDKN2A
4	Adenocarcinoma	Cdkn2a
5	Carcinoma	GSTP1
5	Adenoma	APC; MGMT
6	Sporacarcinoma	CDH1
7	Hereditary Non-Polyposis	Mlh1

Table 4 have shown that DAPK1 is related to several diseases related to Colorectal Cancer.

Figure 4 has shown that CDKN2A, RASSF1, MGMT, CDH1, MLH1, DAPK1, APC and GSTP1 that are related to colorectal cancer are also related to several other cancer causing genes like MSH4, BLM, MSH3, MSH2, MSH3, CDK4, AXIN1, CTNNB1, CBLL1, WTAP and KIAA1429.

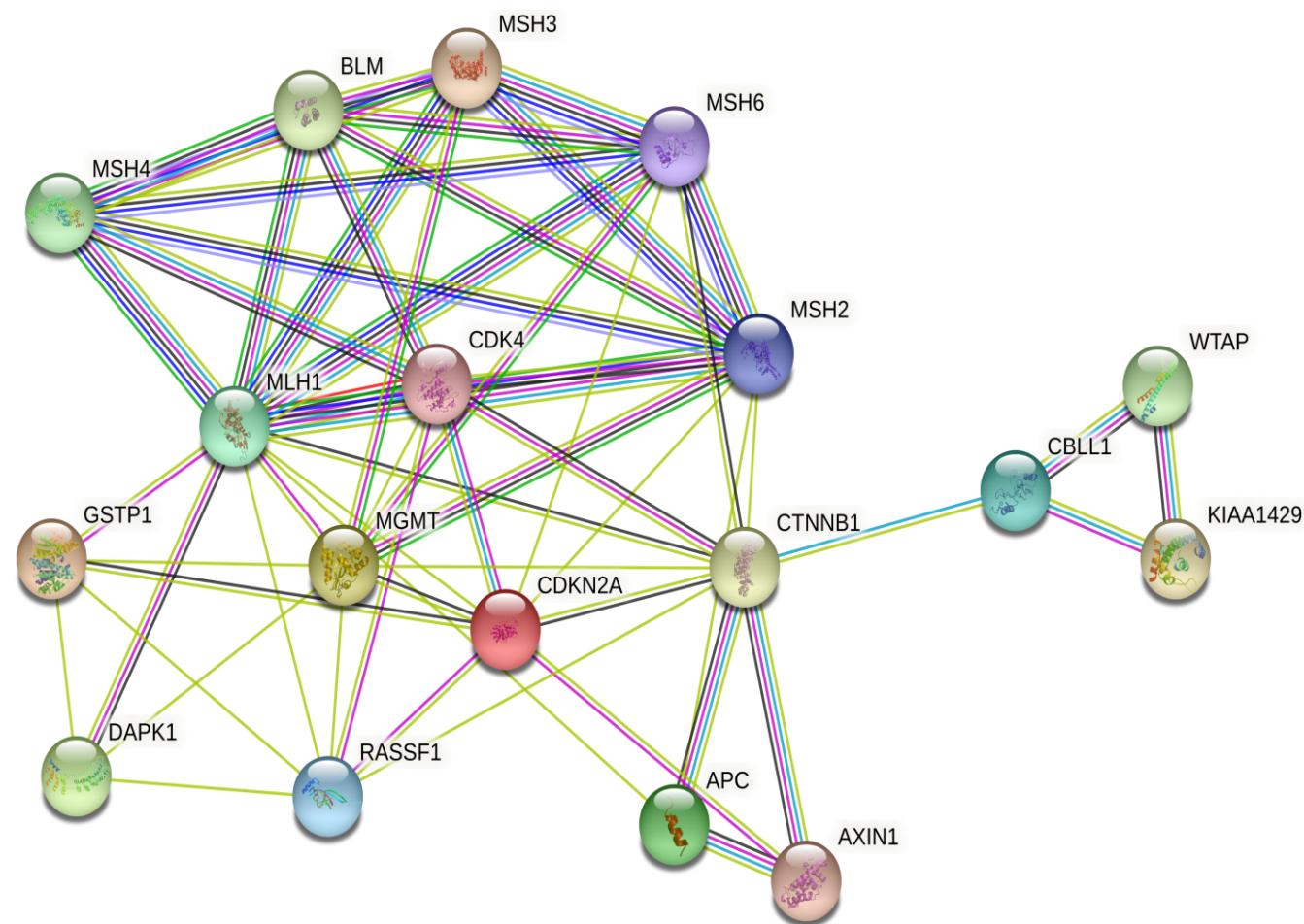


Figure 4: Protein-Protein interaction analysis

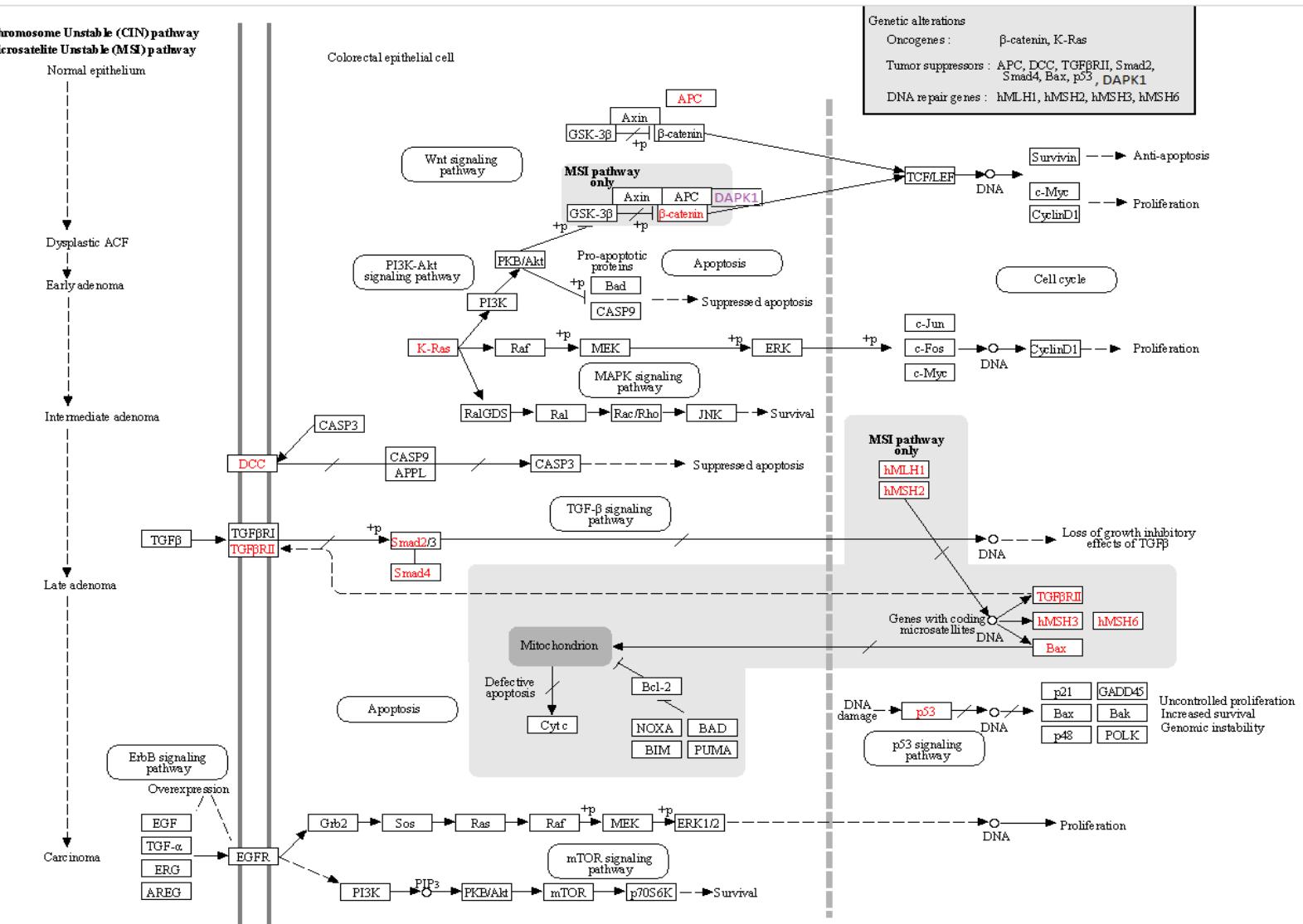


Figure 5: Mechanism of DAPK1 in Colorectal cancer

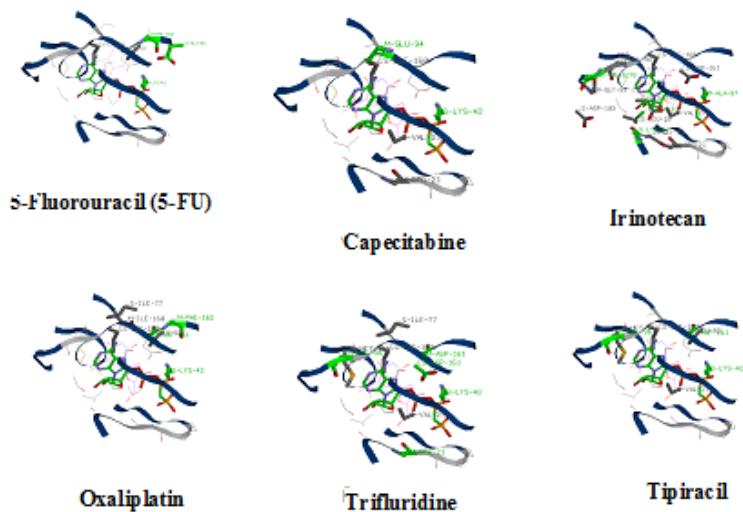
Figure 5 has shown that DAPK1 promotes the progression of tumors by down regulation and acts as tumor suppression process.

Table 5: Activity of drugs against DAPK1 gene as molecular therapy for Colorectal cancer

S.No	Name of Drug	DAPK1	
		Total energy in Kcal/mol	Active site
1	5-Fluorouracil (5-Fu)	-51.2	H-S-LYS-42/H-S-GLU-64/H-M-PHE-162/V-S-LYS-42/V-S-LEU-93/V-S-ILE-160
2	Capecitabine	-88.33	H-S-LYS-42/H-M-GLU-94/V-M-SER-21/V-S-VAL-27/V-S-LYS-42/V-S-ILE-160
3	Irinotecan	-111.54	H-M-LEU-19/H-S-LYS-42/H-M-ALA-97/H-S-MG-1278/V-S-LEU-19/V-M-GLY-20/V-M-SER-21/V-S-VAL-27/V-M-VAL-96/V-M-ALA-97/V-M-GLY-99/V-S-ASP-103/V-S-GLU-143/V-S-ILE-160/V-S-ASP-161
4	Oxaliplatin	-77.36	H-S-LYS-42/H-M-ASP-161/H-M-PHE-162/V-S-LYS-42/V-S-ILE-77/V-S-LEU-93/V-M-ILE-160/V-S-ILE-160
5	Trifluridine	-88.9	H-M-SER-21/H-S-LYS-42/H-M-VAL-96/H-M-ASP-161/H-S-ASP-161/V-S-VAL-27/V-S-LYS-42/V-S-ILE-77/V-S-MET-146/V-S-ILE-160
6	Tipiracil	-71.11	H-S-LYS-42/H-M-VAL-96/H-M-ASP-161/V-S-VAL-27/V-S-LEU-93/V-S-MET-146/V-S-ILE-160

The selected molecules like 5-Fluorouracil (5-Fu), Capecitabine, Irinotecan, Oxaliplatin, Trifluridine and Tipiracil have shown control of mutated DAPK1 proteins of colorectal cancer. The Irinotecan has shown best activity and 5-Fluorouracil has shown least activity in control of DAPK1 protein. Hence Irinotecan is the best option molecular therapy in control of colorectal cancer followed by Trifluridine, Capecitabine, Oxaliplatin, Tipiracil **and** 5-Fluorouracil (Table 5). Figure 6 has shown

the docking poses and active site of drugs against DAPK1 protein respectively.

**Figure 6: Docking reports of DAPK1 with selected compounds**

The selected molecules like 5-Fluorouracil (5-Fu), Capecitabine, Irinotecan, Oxaliplatin, Trifluridine and Tipiracil have shown control of Large and small T-

antigens of proteins of Colorectal cancer. The Irinotecan has shown best activity with Large and small T-antigens of proteins from John Cunningham Virus.

Table 6: Docking of molecules with Large and small T-antigens of proteins from John Cunningham Virus

S.No	Name of Drug	Total energy in Kcal/mol	
		Large T-antigens	Small T-antigens
1	5-Fluorouracil (5-FU)	-67.1	-63.72
2	Capecitabine	-96.42	-77.2
3	Irinotecan	-106.87	-110.36
4	Oxaliplatin	-80.81	-75.23
5	Trifluridine	-92.74	-80
6	tipiracil	-81.4	-81.8

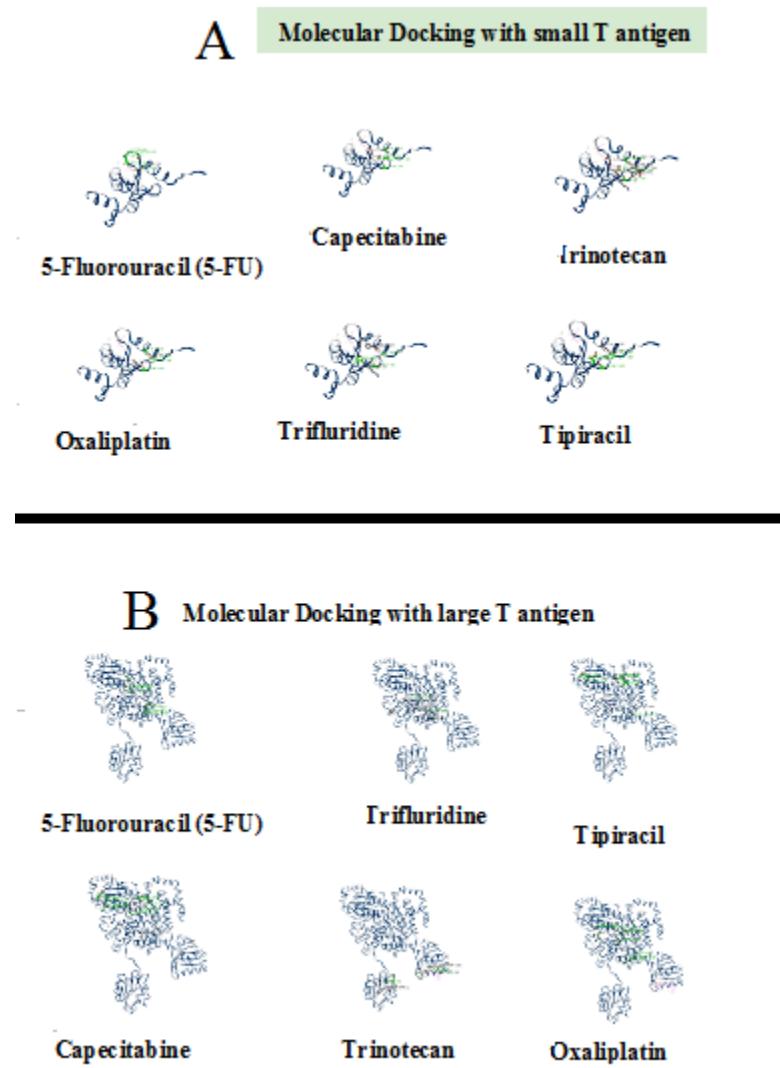


Figure 7: Docking reports of molecules with large and T antigens from John Cunningham Virus (JCV)

Human polyomavirus 2, commonly referred to as the JC virus or John Cunningham virus, is identified by electron microscopy in 1965 by Zuhrein and Chou, and Silverman and Rubinstein and later isolated in culture and named using the two initials of a patient, John Cunningham (Vivekanandan et al., 2021). Human polyomavirus 2 found in the central nervous system of PML

patients that is also linked to colorectal cancer, but these findings are still controversial. Several types are recognized Europe (a, b and c), African type—Af1, Asian- B1-a, B1-b, B1-d, B2, CY, MY and SC (Sugimoto et al., 2002).

A 143-base-pair deletion that was identified in the agnogene that codes for a 10-amino-acid truncated peptide may be believed to mediate CPN tropism (Moens et al., 2007). Previous analysis of the JCVM variant has revealed that archetype-like regulatory regions has shown no mutations in coding sequences but have DNA methylation (Miskin and Koralnik, 2015). Irinotecan is the first approved Chemopreventive drug used for treatment of metastatic colorectal cancer (CRC) obstinate to 5-fluorouracil (5-FU) in 1996 (Tsekouras, 2019). Irinotecan, is a water-soluble, semisynthetic derivative of camptothecin used alone as first-line treatment or second-line treatment of colorectal cancer.

CONCLUSION

DAPK-1–p53 interactions are linked to John Cunningham Virus infection causing colorectal cancer. The selected molecules like 5-Fluorouracil (5-Fu), Capecitabine, Irinotecan, Oxaliplatin, Trifluridine and Tipiracil have shown control of Large and small T-antigens of proteins of Colorectal cancer. The Irinotecan has shown best activity with Large and small T-antigens of proteins from John Cunningham Virus. The Irinotecan has also shown best activity with DAPK1 gene may be better compound in the control of colorectal cancer (Fuchs et al., 2006; Garcia-Carbonero and Supko, 2002; Kaladhar and Tantravahi, 2021).

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CONFLICTS OF INTEREST: There is no known conflict of interest associated with the publication.

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