The Role of SIRT6 in Down Regulation of Colorectal Cancer

By

Rubya Tasnim Hridita
13146055

A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

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Declaration

It is hereby declared that

1. The project submitted is my own original work while completing degree at Brac University.
2. The project does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The project does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

Rubya Tasnim Hridita
13146055

Approval

The project titled “The Role of SIRT6 in Down Regulation of Colorectal Cancer” submitted by Rubya Tasnim Hridita (13146055) of Spring, 2013 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on 2 October 2019.

Examining Committee:

Supervisor:
(Member)

_____

Marzia Alam
Lecturer, Department of Pharmacy
Brac University

Program Coordinator:
(Member)

_____

Dr. Hasina Yasmin
Associate Professor, Department of Pharmacy
Brac University

Departmental Head:
(Chair)

_____

Professor Dr. Eva Rahman Kabir
Chairperson, Department of Pharmacy
Brac University

Ethics Statement

The study does not involve any kind of animal trial and human trial.

Abstract

This work is based on SIRT6 role in down regulation of colorectal cancer. Sirtuin 6 (SIRT6) is a nicotinamide adenine dinucleotide^{+} (NAD^{+}) dependent enzyme and stress response protein which can play an important role in tumorigenesis. In this paper, the relation between SIRT6 and colorectal cancer has been discussed. For this purpose, STRING and KEGG pathway were used. The link between SIRT6 and 204 different proteins were determined and the confidence score was calculated using STRING. From this study, key proteins for colorectal cancer were identified using KEGG pathway. The study showed that MYC, PTEN, PI3K and mTOR were the therapeutic targets for SIRT6. Moreover, SIRT6 inhibits PI3K/Akt signaling pathway and thus inhibits cancer cell proliferation. It also inhibits mTOR signaling pathway resulting in cancer cell apoptosis. Thus, the current study proposes that upregulating the expression of SIRT6 can be used as a therapeutic tool in combatting colorectal cancer.

Keywords: SIRT6; STRING; KEGG pathway; Colorectal cancer; Tumorigenesis.

Dedication

Dedicated to my beloved parents and all of my family members and specially dedicated to my mother-in-law

Acknowledgement

Firstly, I would like to give graces to Almighty Allah for limitless blessings to empower me the courage to complete this project work given.

I would like to convey my sincere thankfulness to my project **s**upervisor **Rubayat Islam Khan**, Senior Lecturer, Department of Pharmacy, Brac University, for his valuable direction and enthusiasm throughout this project, as well as for the support and credence he gives me from every gathering and point of interaction that happened on research purpose. I would like to convey a special thanks to **Saif Shahriar Rahman**, Senior Lecturer, Department of Pharmacy, Brac University, for his valuable direction throughout my writings and also I would like to convey my sincere thankfulness to my project supervisor **Marzia Alam**, Lecturer, Department of Pharmacy, Brac University, for her valuable direction for research purpose and my writings at the last moment. I sincerely put forward my regards and gratitude to **Dr. Eva Rahman Kabir**, Professor and Chairperson, Department of Pharmacy, Brac University for her contribution and support to the student and department.

I would like to express thankfulness towards my parents for their continuous determinations in giving me support and motivating me to pursue my visions. Without them I would not be the creature I am now.

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List of Acronyms

AKT	Nuclear factor erythroid 2-related factor 2
mTOR	Mechanistic Target Of Rapamycin Kinase
HIF-1α	Hypoxia-inducible factor 1-alpha
PI3K	Phosphoinositide 3-Kinase
RUNX2	Runt-related transcription factor 2
TGF-β1	Transforming growth factor beta 1
USP10	Ubiquitin carboxyl-terminal hydrolase 10

Chapter 1

Introduction

1.1 Background

Sirtuins are the family member of nicotinamide adenine dinucleotide (NAD^+) dependent protein deacetylases. They transferred from bacteria to eukaryotes. The structure is found in Sirtuin called active Zn^{2+} site (Pan et al., 2011). This site promotes Sirtuin to transfer acetyl group. Acetyl group basically belongs to lysine side of protein. This side is involved with water molecule to co-factor NAD^+ for activating nicotinamide 2'-*O*-acetyl-ADP ribose and a deacetylated susbtrate. Sirtuins also have the cellular metabolic state which causes backward motion of acetylation-mediated pathways by altering the cellular metabolism due to NAD^+ (Pan et al., 2011). This phenomenon also saw in cell apoptosis, cell cycle progression, organism longevity, genome maintenance and transcription. In human, Sirtuins have seven members SIRT1 to SIRT7. Cellular localization patterns and targets are the main parameters for their characterization. Among them SIRT1 and SIRT2 have been studied a lot of time. In recent days, SIRT6 have gain the prime concern because SIRT6 have a lot of benefits in human diseases such as cancer, heart diseases, neurodegenerative diseases, anti-aging and diabetics and also glucose metabolism (Pan et al., 2011). For this reason, in this paper, SIRT6 and colon cancer are the focus of attention.

STRING is mainly protein-protein association where all databases are hired from another databases like Ensembl and Swissport by their genomic factor (von Mering et al., 2005). They are basically working for select the pair of genes which are found under specific pressure and also they are functionally active (von Mering et al., 2005). It shows protein-protein interactions and their corresponding confidence score for particular organisms.

KEGG pathway is biological database of genome sequence. It shows particular biological pathway for particular disease.

1.2 Aim

The main purpose of this paper to use computational platforms such as STRING and KEGG pathway to find protein-protein interaction between SIRT6 and selected proteins and thus find out the relation between SIRT6 and colorectal cancer progressive protein to alter the progression of colorectal cancer.

1.3 SIRT6 Structure

SIRT6 consists of 355 amino acids according to length in human. It also has a putative catalytic sirtuin core with N- and C-terminal flanking extensions (Yamamoto et al., 2007). Combination of SIRT6 with NAD^+ hydrolysis produce O-acetyle-ADP, nicotinamide and a deacetylated substrate through the lysine deacetylation. SIRT6 with NAD^+ never yielded an acetylated substrate. Structural conformation of SIRT6 is build up by the presence of hydrogen bond between Zn^{2+} site and Rossman-fold site (Yamamoto et al., 2007).

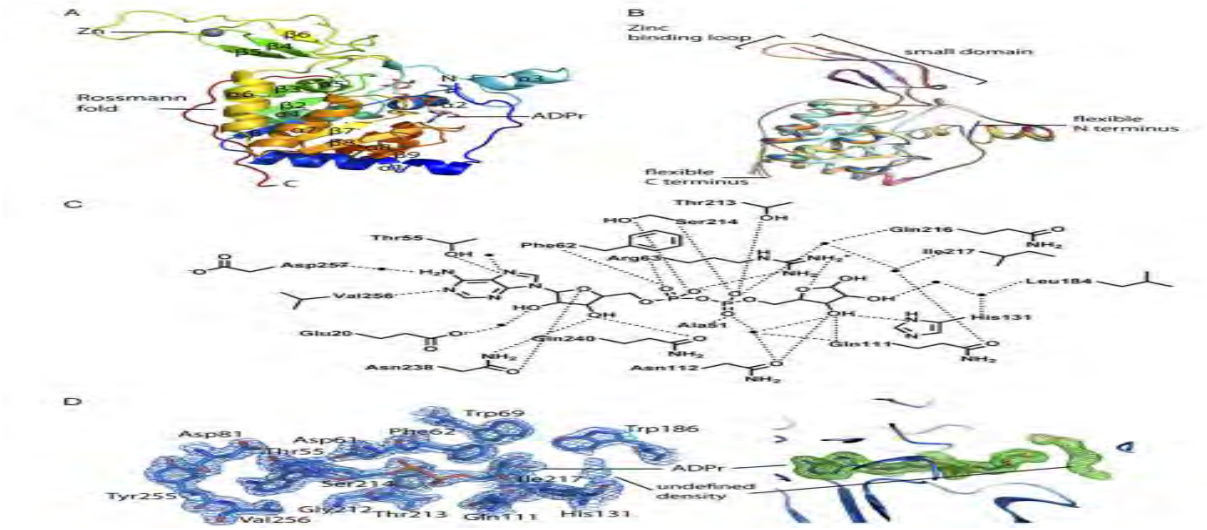

Figure 1: Human structure of SIRT6 complex with ADP-ribose (Pan et al., 2011)

1.4 Physiological Functions of SIRT6

SIRT6 is a nicotinamide adenine dinucleotide (NAD+) dependent enzyme. It is also a stress responsive protein (Pan et al., 2011). SIRT6 have concerned with regulating chromatin to maintain and have huge importance in metabolism, aging and disease (Yamamoto, Schoonjans, & Auwerx, 2007). It is functionally targeting to treat several human disease such as heart disease, some of cancers, neurodegenerative diseases and diabetes (Yamamoto et al., 2007). It has deacetylase activity towards H3K9 (Histon H3 lysin 9) and H3K56 (Histon H3 lysin 56) (Zhong& Mostoslavsky, 2010). When S-phase of cell cycle is occurred, SIRT6 modulates histone H3acetylation in telomeric chromatin. Besides, it have vital roles in DNA repairing and gene expression (Zhong & Mostoslavsky, 2010). In muscles, liver, thymus, heart and brain, SIRT6 is available in high concentration where SIRT6 affects transcriptional over expression in neurons (Yamamoto et al., 2007). SIRT6 have in vivo and in vitro deacetylase activity for free fatty acid and in addition SIRT6 with NAD^+ provide O-myristoyl-ADP ,nicotinamide and a deacetylated substrate (Zhong & Mostoslavsky, 2010). Another potential role of SIRT6 with NAD^+ yield poly polymerase substrate which provides very weak ADP-ribosylation activity. Deacetylase activity decreases by the absence of N-terminal extensions which affect the enzymatic activity of SIRT6 (Zhong & Mostoslavsky,2010).

1.5 SIRT6 and Cancer

SIRT6 is extremely important for DNA repair mechanisms as well as regulation of cell survival and cell proliferation in human (Desantis, Lamanuzzi, & Vacca, 2018). When DNA damage occurs, SIRT6 triggers the apoptotic process (Desantis et al., 2018). Some studies have shown down-regulation of SIRT6 in certain types cancers. However, up-regulation of SIRT6 have seen in other kinds of cancers in human cells (Desantis et al., 2018).

1.5.1 SIRT6 in tumor suppression

The reduction of SIRT6 expression mainly elevate tumor progression which causes poor clinical consequence to some cancers like colorectal, ovarian, lung, breast, hepatocellular and other cancers (Desantis et al., 2018).SIRT6 would promote apoptotic cell death to make sure that damaged cells would not proliferate by the DNA damage (Desantis et al., 2018).

Warburg effects are seen in SIRT6 protein. This phenomenon is important for glycolytic metabolic shift in rapid tumor growth in human. Both in vitro and in vivo, SIRT6 would promote tumor suppression through repress the hypoxia-inducible factor 1-alpha (HIF-1α). HIF-1α prohibits glycolytic metabolism in cancer cells in human (Desantis et al., 2018).

In human colon cancer, cell-cycle progression and proliferation are being regulated by USP10 and SIRT6. If USP10 do not regulate properly, it causes tumorogenesis through SIRT6 degradation, instability and also hamper c-MYC oncogene transcriptional functions (Desantis et al., 2018).

About pancreatic ductal adenocarcinoma (PDAC) in human, in Lin28 promoter SIRT6 lost its activity and MYC activation of Lin28b causes the downstream of let-7 target genes

(HMGA2, IGF2BP1) as well as IGF2BP3 which promotes PDAC progression and metastasis (Desantis et al., 2018).

About liver cancer, SIRT6 suppression regulates with the help of c-Jun/c-Fos pathway. SIRT6 transcription and repression of survivin are being induced by c-Fos. Basically, survivin repression took place by decreasing histone H3K9 acetylation as well as NF-kB activation. This situation promotes SIRT6 impairment in cancer development by selecting survivin as it has anti-apoptotic potentiality (Desantis et al., 2018). Higher level of c-Jun-survivin as well as lower level of c-FosSIRT6 identify an essential expression model in human dysplastic liver nodules (Desantis et al., 2018).

About hepatocellular carcinoma (HCC), SIRT6 deacetylates nuclear pyruvate kinase M2 (PKM2) to inhibit cell proliferation as well as tumorigenesis *by* PKM2 (Desantis et al., 2018).

About ovarian cancer, SIRT6 inhibits the proliferation of ovarian tumor cells through regulation of Notch3 by reducing the expression of neurogenic locus (Desantis et al., 2018).

About breast cancer, RUNX2 downregulates the SIRT6 expression calculated by mRNA and protein levels and also endogenous SIRT6 expression is lower in the tumor breast tissue and cell lines expressing high levels of RUNX2 regulating the metabolic pathways (Desantis et al., 2018).

About non-small cell lung cancer (NSCLC), SIRT6 prohibits Twist1 suppression which promotes tumor proliferation as well as malignant transformation in human (Desantis et al., 2018).

Finally, about bladder and prostate cancer, E2F transcription factor 1 (E2F-1) overexpression influence SIRT6 to down-regulate cancer progression as well as lower prognosis in human (Desantis et al., 2018).

1.5.2 SIRT6 in tumor promotion

On the other hand, SIRT6 have role in tumor progression by over expressing in solid and in hematologic tumors (Mcglynn et al., 2014).

Up-regulation of SIRT6 by exposure to ultraviolet B (UVB) light in human skin squamous cell carcinoma (SCC) activates AKT pathway which influence the cyclooxygenase 2 (COX-2) expression to stop AMP-activated protein kinase (AMPK) signaling. For doing this, there would need a higher rang of proliferation as well as cell survival (Desantis et al., 2018).

In HCC oncogene, SIRT6 have some part to play. SIRT6 is overexpressed which influence altering the growth factor (TGF)-β1 as well as H2O2/HOCl reactive oxygen species (ROS) that mediate tumorigenesis (Desantis et al., 2018). TGF-β1 upregulates the SIRT6 expression which induced ERK activation as well as Smad pathways and altering the effect of these proteins on cellular senescence by chromatin remodeling (Desantis et al., 2018). At molecular level, SIRT6 induces deacetylation of H3K9 that blocks Bcl-2-associated X protein (Bax) transcription by that it increases p53 as well as E2F-1 chromatin accessibility to stop apoptosis in human (Desantis et al., 2018). At basal conditions, SIRT6 and miR-122 down regulating HCC with the help of H3K56 deacetylation in the promoter site (Desantis et al., 2018). Then miR-122 combined with SIRT6 3′ UTR to decrease its altitudes (Mcglynn et al., 2014). In addition, miR-34a have vital role in the differentiation process of HCC by SIRT6 down regulation (Desantis et al., 2018).

In multiple myeloma (MM), SIRT6 is overly expressed to genomic stability which associated to proliferation and poor prognosis (Desantis et al., 2018). In vitro, human MM xenograft model explained that SIRT6 down-regulates the ERK signaling-related genes and suppresses the activity of ETS-domain transcription factor (ELK1) to uplift DNA repair level by Chk1 to resist DNA-damaging agents (Desantis et al., 2018). mRNA upregulation of SIRT6 in the acute myeloid

leukemia (AML) cells compared with low SIRT6 levels detected in normal $CD34^{+}$hematopoietic progenitors causes poor prognosis and genomic instability (Mcglynn et al., 2014). SIRT6 repairs DNA double-strand breakthrough C-terminal binding protein deacetylation, interacting protein (CtIP), poli ADP-ribosio polimerase-1 (PARP-1) as well as DNA-protein kinase (PK) complex (Desantis et al., 2018). Another way, SIRT6 expression down-regulated both *in vitro* as well as in a murine xenograft model of human AML promotes genomic instability that sensitizes AML cells to daunorubicin (DNR) and cytarabine (ARA-C) which increase sensitivity to DNA-damage agents (DDAs) (Desantis et al., 2018).

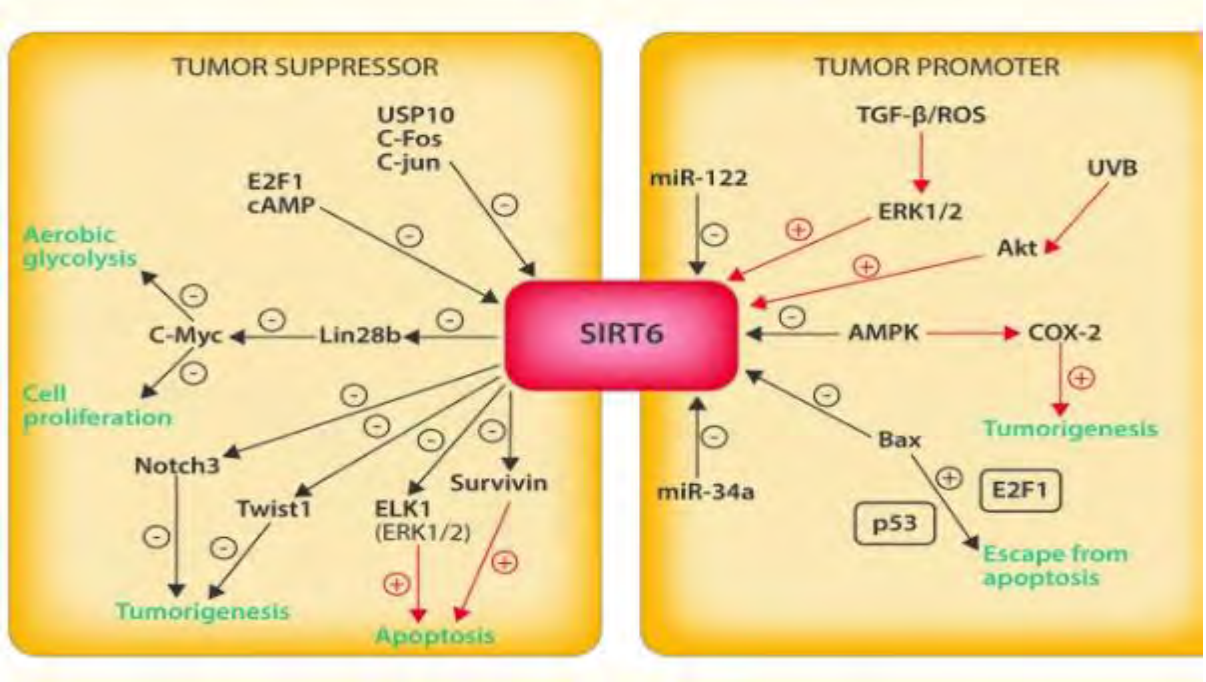

Figure 2: SIRT6 in cancer acts as tumor suppressor and tumor promoter (Desantis et al., 2018).

1.6 STRING

STRING is basically protein-protein association database for huge amount of organisms. This designs stand for simplifying the information through giving an expansive and qualitative protein-protein associations (Szklarczyk et al., 2017). STRING provides scoring scheme derived from

integrating all three types of genomic context (Szklarczyk et al., 2017). STRING recently have 730000 proteins in 180 fully sequenced organism as well as these are available in STRING database (Szklarczyk et al., 2017). STRING are pre-computed and quickly accessible for high-level network view and individual interaction record (Szklarczyk et al., 2017). STRING has several merits for three kinds of protein-protein association framework- 1) several kinds of evidence are illustrated in single, permanent proteins set with comparative analysis. 2) Known and predicted interactions often partly complement each other for increasing coverage. 3) An integrated scoring scheme. 4) Mapping as well as transferring interactions onto a huge amount of organisms facilitates evolutionary studies in protein-protein associations (Szklarczyk et al., 2017).

STRING has stocked single score for single protein-protein associations (von Mering et al., 2005). These scores have been measured between 0 to1. This scores point out the supporting evidence of protein-protein interactions which are biologically significant, special and formative (von Mering et al., 2005). On the basis of source and kind of evidence, each interaction is separated into one or more channels with help of their supporting evidence. Seven channels are being established (von Mering et al., 2005). These channels are collected, estimated and benchmarked individually. The evidence channels are contained several color when they are shown in network visualization as a web format. The user has liberty to disconnect each channel separately at any time (von Mering et al., 2005). A combined and final confidence score is calculated for per interaction on the basis of seven channels. Final sorting and filtering interactions are done by the combined score for visualizing the protein-protein interactions scores (von Mering et al., 2005). Gene-locus resolution is the main component of STRING interactions. Whereas protein-coding gene site is the main parameters of STRING interacting units (von Mering et al., 2005).

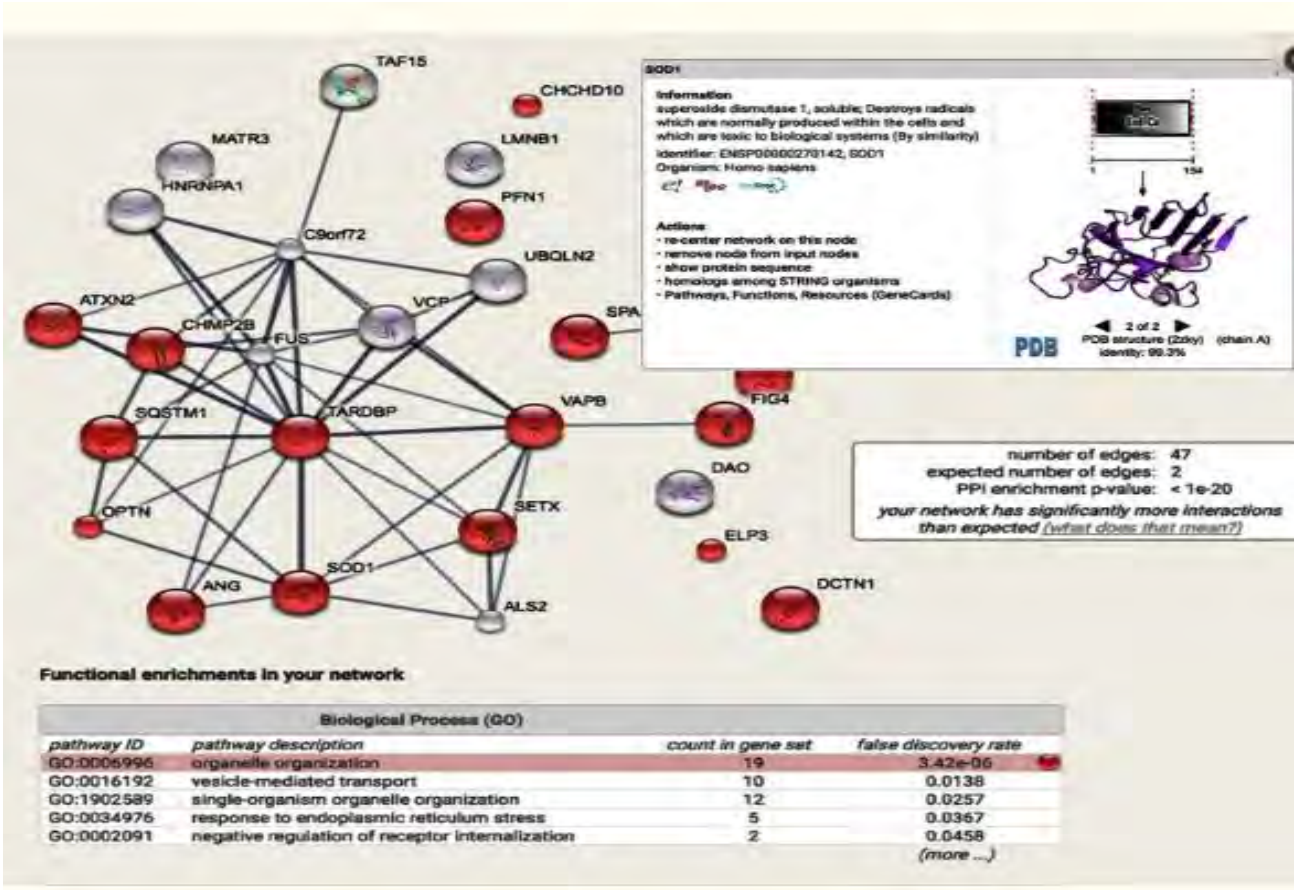

Figure 3: Network and Enrichment Analysis of STRING (Szklarczyk et al., 2017).

1.7 KEGG Pathway

Kyoto Encyclopedia of Genes and Genomes (KEGG) database system developed by Japanese Human Genome Program which are mainly biological database of genome sequence with the help previous working knowledge (Kanehisa, Sato, Furumichi, Morishima, & Tanabe, 2019). The major goal to build KEGG is to set up a bond between combined sets of genes on genome to top-level uses of the cell as well as the organism through KEGG mapping (Kanehisa, Sato, Kawashima, Furumichi, & Tanabe, 2016). In addition, KEGG have differentiated among four databases- Pathway, Enzyme, Compound lastly Genes. Firstly, KEGG pathway mapping establish on Enzyme due to its metabolic mapping then it has been expanded according to Pathway for BRITE and

MODULE, Genes for Genomes, Compound for GLYCAN and REACTION and Enzyme is modified for KO (KEGG ORTHOLOGY). Now, KEGG has become most used biological database system in the world because KEGG has different kinds of Genome annotation database (Kanehisa et al., 2019).

Several researches on KEGG has been conducted to advance the way of KEGG to develop its links about protein (Tanabe, Sato, Morishima, Furumichi, & Kanehisa, 2016). KEGG have categorized its three databases into some new way. Firstly, PATHWAY, BRITE and MODULE databases deal with KEGG pathway maps, BRITE hierarchy and table files have multiple column where the data were sequentially collected (Kanehisa et al., 2016). This data are mainly created based on manually published literature. Basically BRITE table files are needed for doing drug classification as well as to show the relationship between diseases and drugs. KEGG modules have also same function as BRITE with high level of functioning (Kanehisa et al., 2019). The genomic information group originally holds GENOME and GENES databases which are mainly the genomic information about organisms which were talked about genomes as well as its gene catalogs. This database mostly collected from RefSeq and GenBank databases of genomes (Tanabe et al., 2016). Another part of KEGG is health related where DRUG, DISEASES and ENVIOR are categorized (Tanabe et al., 2016). An advance system is added named KEGG MEDICUS for integrating with developed database with drug labels (Kanehisa et al., 2019).

In most recent years, KEGG database have introduced two more databases based on its human-specific which is mostly talk about health information (Kanehisa et al., 2016). They are- KEGG NETWORK and KEGG VARIANT.KEGG NETWORK is first revolutionary work on single species according to genome variations which is deal with human diseases and drugs (Kanehisa et al., 2019). KEGG NETWORK can be used for any species with different kind of variation

(Kanehisa et al., 2016). In human, KEGG NETWORK has developed a Cancer Network Variant, viral infections and several endrocrine and metabolic diseases. KEGG NETWORK databases also have the potentiality on drug-target relationship for targeted protein and drugs. It also helps to improve DRUG, DISEASES via drug target relationship and drug labels. KEGG VARIANT have same as KEGG NETWORK (Kanehisa et al., 2016). Although KEGG VARIANT database have outsider database information like ClinVar, dnSHP and COSMIC (Kanehisa et al., 2019).

1.8 Selection of Protein for confidence score analysis

According to the relation between SIRT6 and other proteins, all the proteins were collected on the basis of reviewing journals. Some proteins are directly related to SIRT6 and some proteins are the 2^{nd} linked protein of SIRT6.

Chapter 2

Methodology

For finding the relationship between SIRT6 and colorectal cancer, 204 different proteins with possible interactions with SIRT6 and also probable involvement in colorectal cancer tumorigenesis pathway were selected by journal review. In the study, STRING was used to calculate the confidence score for a protein interaction with SIRT6. Firstly, 'Multiple proteins' query was selected in STRING web interface. On there, protein names have to put with their respective organisms. After searching the particular proteins, STRING would show the protein-protein network. From this network, confidence score was found for particular protein against the predicted one. This network allows users to find an interaction up to 2000 proteins. For example, SIRT6 and AKT1 protein interaction is showing below:

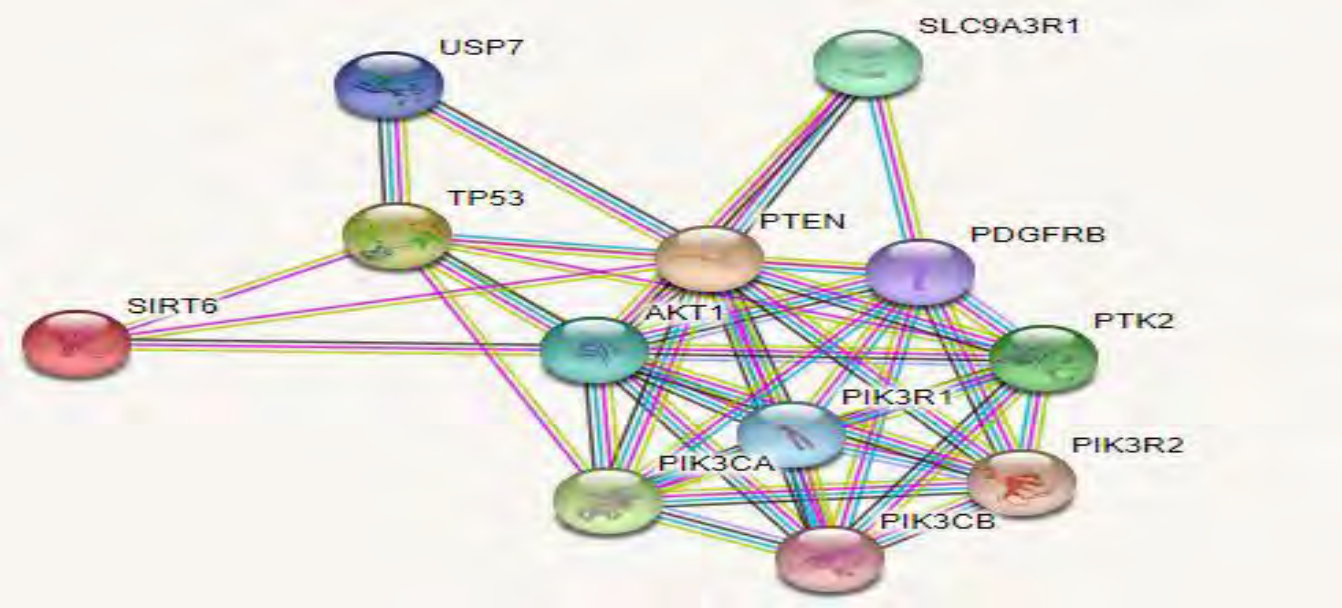

Figure 4: Interaction of SIRT6 protein with other protein (STRING, 2019).

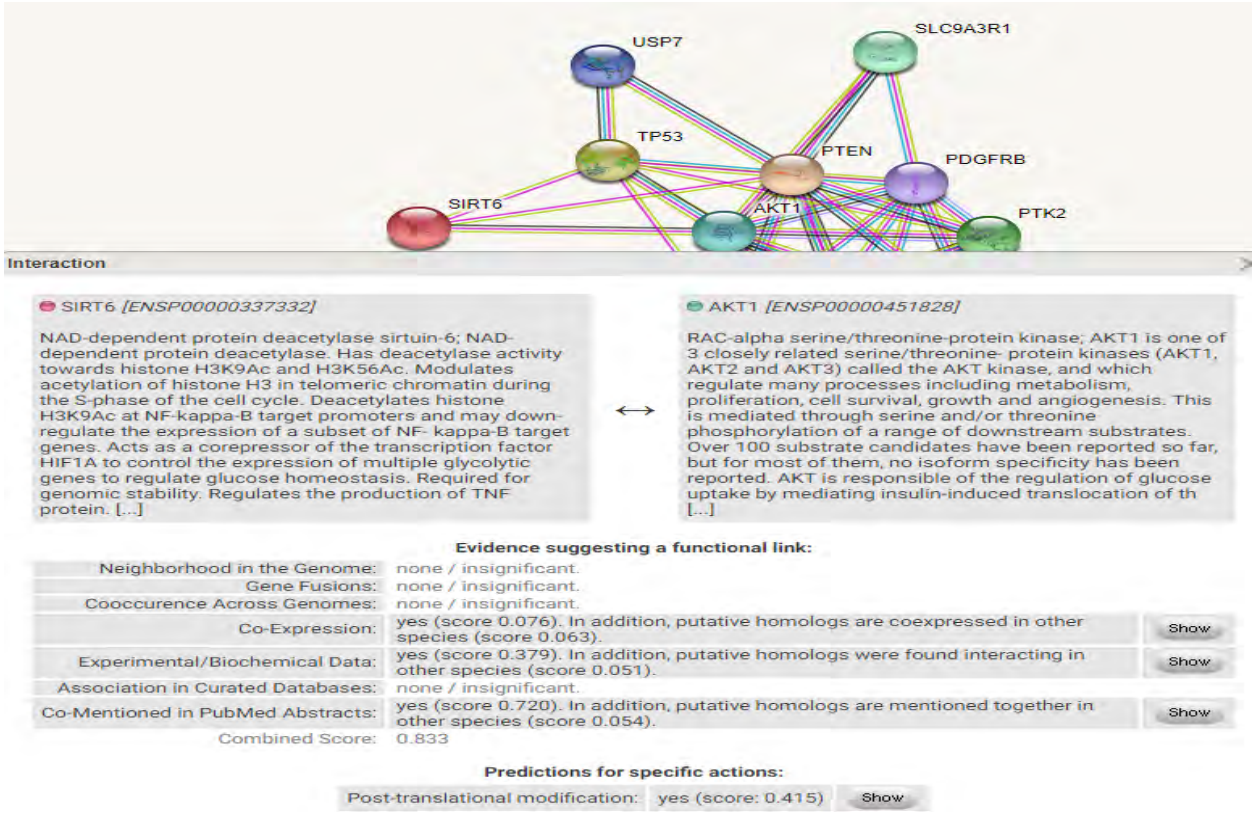

Figure 5: Protein-protein interaction score (STRING, 2019).

In our study, by using STRING, about 204 protein-protein interactions were shown in Table 1. There, SIRT6 was predicted protein where confidence scores were measured by other proteins against SIRT6. From STRING, first and second link scores were measured for SIRT6. Then the true combined scores were calculated by using summation and multiplication. After that, on the basis of tumor suppression and promotion, the therapeutic targets of SIRT6 against the first and the second link were calculated for clarifying the potential role of SIRT6 and other proteins on cancer. Finally, KEGG pathway for colon cancer was downloaded from KEGG database which is found in KEGG DISEASE database category in Human Diseases. In result and discussion section, all data and KEGG pathway were presented for more clear identification.

Chapter 3

Results

Table 1: The combined score for interaction between SIRT6 and selected proteins

Serial No.	1st link of SIRT6	1st link score	2nd link of SIRT6	2nd link score
1	**RBBP8**	0.963	**ATM**	0.990
2	**TP53**	0.683	**E2F1**	0.834
3	**HDAC1**	0.754	**SIN3A**	0.998
4	**MYC**	0.902	**MAX**	0.981
5	**JUN**	0.514	**FOXP3**	0.966
6	**HIF1A**	0.839	**EGLN1**	0.995
7	**RUNX2**	0.496	**SP7**	0.964
8	**AKT1**	0.755	**PTEN**	0.998
9	**MTOR**	0.436	**MLST8**	0.999
10	**TP53**	0.683	**ESFR**	0.996
11	**EXO1**	0.619	**BRCA1**	0.973
12	**DNA2**	0.512	**PCNA**	0.984
13	**MRE11A**	0.467	**NBN**	0.998
14	**RAD50**	0.510	**H2AFX**	0.988
15	**RAD50**	0.510	**CTBP1**	0.912
16	**MRE11A**	0.467	**PCNA**	0.981
17	**EX01**	0.619	**H2AFX**	0.637
18	**RBBP8**	0.963	**BRCA1**	0.995
19	**RBBP8**	0.963	**MRE11A**	0.990
20	**CHEK2**	0.434	**ATM**	0.999
21	**TP53BP1**	0.499	**ATM**	0.997
22	**CHEK2**	0.434	**H2AFX**	0.990

23	**CHEK2**	0.434	**MDC1**	0.989
24	**TP53BP1**	0.499	**CHEK2**	0.995
25	**HDAC1**	0.754	**MTA1**	0.998
26	**HDAC1**	0.754	**RBBP7**	0.998
27	**HDAC1**	0.754	**RBBP4**	0.998
28	**HDAC1**	0.754	**SIN3B**	0.996
29	**TP53BP1**	0.499	**H2AFX**	0.997
30	**TP53BP1**	0.499	**ATM**	0.997
31	**TP53BP1**	0.499	**MDC1**	0.998
32	**XRCC6**	0.591	**RNF168**	0.939
33	**PRKDC**	0.744	**RAD50**	0.988
34	**XRCC6**	0.591	**MDC1**	0.955
35	**XRCC6**	0.591	**H2AFX**	0.983
36	**XRCC6**	0.591	**RAD50**	0.996
37	**XRCC6**	0.591	**TP53BP1**	0.990
38	**XRCC6**	0.591	**PAXIP1**	0.911
39	**XRCC6**	0.591	**CHEK2**	0.841
40	**PRKDC**	0.744	**PAXIP1**	0.924
41	**MYC**	0.902	**RB1**	0.993
42	**MYC**	0.902	**BCL2**	0.995
43	**MYC**	0.902	**CDKN2A**	0.993
44	**MYC**	0.902	**JUN**	0.993
45	**MYC**	0.902	**TP53**	0.996
46	**MYC**	0.902	**KAT2A**	0.992
47	**MYC**	0.902	**CDKN1A**	0.992
48	**MYC**	0.902	**AKT1**	0.989
49	**MYC**	0.902	**CDK4**	0.990
50	**MYC**	0.902	**MYB**	0.991
51	**KAT2A**	0.577	**TAF10**	0.998
52	**AKT1**	0.755	**PIK3CB**	0.999

53	**KAT2A**	0.577	**TADA3**	0.998
54	**KAT2A**	0.577	**SUPT3H**	0.999
55	**KAT2A**	0.577	**TAF9**	0.998
56	**KAT2A**	0.577	**TADA2A**	0.998
57	**KAT2A**	0.577	**TRRAP**	0.997
58	**KAT2A**	0.577	**TADA2B**	0.996
59	**KAT2A**	0.577	**ATXN7L3**	0.996
60	**KAT2A**	0.577	**USP22**	0.996
61	**KAT2A**	0.577	**CCDC101**	0.997
62	**AKT1**	0.755	**PIK3CD**	0.999
63	**AKT1**	0.755	**PIK3CA**	0.999
64	**AKT1**	0.755	**MTOR**	0.999
65	**AKT1**	0.755	**PIK3CG**	0.999
66	**AKT1**	0.755	**RICTOR**	0.997
67	**AKT1**	0.755	**OGN**	0.529
68	**AKT1**	0.755	**FOXO3**	0.999
69	**AKT1**	0.755	**FOXO1**	0.999
70	**PIK3CG**	0.449	**MTOR**	0.998
71	**FOXO1**	0.782	**AKT3**	0.991
72	**FOXO3**	0.638	**SIRT1**	0.997
73	**PIK3CG**	0.449	**PTEN**	0.998
74	**PIK3CG**	0.449	**NRAS**	0.996
75	**PIK3CG**	0.449	**RRAS**	0.995
76	**PIK3CG**	0.449	**RRAS2**	0.994
77	**PRKAA1**	0.459	**PRKAB1**	0.999
78	**PIK3CG**	0.449	**AKT3**	0.989
79	**PIK3CG**	0.449	**AKT2**	0.991
80	**AKT1**	0.755	**RRAS**	0.575
81	**AKT1**	0.755	**NRAS**	0.628
82	**PIK3GC**	0.449	**PDPK1**	0.991

83	**FOXO1**	0.782	**SIRT1**	0.997
84	**FOXO1**	0.782	**G6PC**	0.987
85	**FOXO1**	0.782	**AKT2**	0.996
86	**FOXO1**	0.782	**AKT1**	0.999
87	**FOXO1**	0.782	**SGK1**	0.976
88	**FOXO1**	0.782	**SMAD3**	0.979
89	**EP300**	0.479	**MYB**	0.985
90	**FOXO1**	0.782	**CTNNB1**	0.977
91	**FOXO1**	0.782	**CDKN1A**	0.977
92	**FOXO1**	0.782	**EP300**	0.977
93	**FOXO3**	0.638	**SGK1**	0.993
94	**FOXO3**	0.638	**AKT3**	0.991
95	**FOXO3**	0.638	**AKT2**	0.992
96	**FOXO3**	0.638	**AKT1**	0.999
97	**SOD2**	0.488	**GPX3**	0.984
98	**FOXO3**	0.638	**SMAD3**	0.979
99	**FOXO3**	0.638	**IKBKB**	0.983
100	**FOXO3**	0.638	**BCL2L11**	0.989
101	**HIF1A**	0.839	**EGLN3**	0.994
102	**HIF1A**	0.839	**VEGFA**	0.996
103	**HIF1A**	0.839	**VHL**	0.998
104	**HIF1A**	0.839	**HIF1AN**	0.996
105	**HIF1A**	0.839	**EGLN2**	0.989
106	**HIF1A**	0.839	**ARNT**	0.992
107	**HIF1A**	0.839	**KDR**	0.802
108	**HIF1A**	0.839	**FLT1**	0.980
109	**MDM2**	0.432	**CDKN2A**	0.992
110	**HDAC1**	0.803	**AML**	0.969
111	**HIF1A**	0.839	**CREBBP**	0.993
112	**FOS**	0.511	**JUND**	0.999

113	**JUN**	0.514	**ATF3**	0.998
114	**JUN**	0.514	**MAPK8**	0.999
115	**JUN**	0.514	**MAPK9**	0.998
116	**JUN**	0.514	**FOS**	0.999
117	**JUN**	0.514	**FOSB**	0.998
118	**JUN**	0.514	**MAPK1**	0.997
119	**JUN**	0.514	**MAPK10**	0.998
120	**JUN**	0.514	**FOSL1**	0.997
121	**JUN**	0.514	**FOSL2**	0.992
122	**JUN**	0.514	**MAPK3**	0.996
123	**JUN**	0.514	**NFAYC2**	0.996
124	**RUNX2**	0.496	**SMAD3**	0.982
125	**RUNX2**	0.496	**RB1**	0.969
126	**RUNX2**	0.496	**BGLAP**	0.996
127	**RUNX2**	0.496	**WWTR1**	0.940
128	**RUNX2**	0.496	**SMAD4**	0.944
129	**RUNX2**	0.496	**MAPK1**	0.962
130	**CDKNB1**	0.529	**CCND1**	0.996
131	**MTOR**	0.436	**RICTOR**	0.999
132	**MTOR**	0.436	**RPS6KB1**	0.999
133	**MTOR**	0.436	**RPTOR**	0.999
134	**MTOR**	0.436	**PIK3CG**	0.998
135	**MTOR**	0.436	**FKBP1A**	0.998
136	**MTOR**	0.436	**TSC2**	0.998
137	**MTOR**	0.436	**EIF4EBP1**	0.999
138	**MTOR**	0.436	**RHEB**	0.997
139	**MTOR**	0.436	**EIF4E**	0.996
140	**KAT2B**	0.607	**CCDC101**	0.996
141	**EP300**	0.479	**CITED2**	0.994
142	**EP300**	0.479	**CREB1**	0.995

143	**EP300**	0.479	**TBP**	0.986
144	**EP300**	0.479	**HDAC1**	0.991
145	**EP300**	0.479	**NCOA3**	0.987
146	**SOD2**	0.488	**SOD1**	0.997
147	**SOD2**	0.488	**CAT**	0.997
148	**SOD2**	0.488	**GPX1**	0.991
149	**SOD2**	0.488	**SOD3**	0.990
150	**SOD2**	0.488	**GSR**	0.858
151	**TP53**	0.683	**CREBBP**	0.998
152	**TP53**	0.683	**MDM4**	0.999
153	**TP53**	0.683	**CDKN1A**	0.998
154	**TP53**	0.683	**BCL2**	0.998
155	**TP53**	0.683	**BAX**	0.998
156	**TP53**	0.683	**ATM**	0.997
157	**TP53**	0.683	**BCL2**	0.998
158	**TP53**	0.683	**CDK2**	0.997
159	**TP53**	0.683	**MDM4**	0.999
160	**TP53**	0.683	**PCNA**	0.994
161	**KAT2B**	0.607	**TADA2A**	0.997
162	**KAT2B**	0.607	**SUPT3H**	0.996
163	**KAT2B**	0.607	**TRRAP**	0.997
164	**KAT2B**	0.607	**TADA3**	0.999
165	**KAT2B**	0.607	**ATXN7L3**	0.995
166	**KAT2B**	0.607	**TAF10**	0.996
167	**HDAC3**	0.742	**RBBP4**	0.992
168	**KAT2B**	0.607	**EP300**	0.994
169	**MDM2**	0.431	**ATM**	0.991
170	**MDM2**	0.431	**MDM4**	0.992
171	**MDM2**	0.431	**RB1**	0.982
172	**MDM2**	0.431	**CHEK2**	0.980

173	**MDM2**	0.431	**RPL23**	0.949
174	**PRKDC**	0.744	**H2AFX**	0.995
175	**RPL11**	0.531	**RPL3**	0.999
176	**RPL35**	0.632	**RPL5**	0.999
177	**RPL9**	0.517	**RPL12**	0.999
178	**FOS**	0.571	**CREB1**	0.996
179	**FOS**	0.517	**MAPK1**	0.996
180	**FOS**	0.517	**JUNB**	0.999
181	**VEGFA**	0.516	**FLT1**	0.999
182	**VEGFA**	0.516	**FLT4**	0.994
183	**VEGFA**	0.516	**IGF1**	0.991
184	**HDAC3**	0.742	**NCOR1**	0.991
185	**HDAC3**	0.742	**SIN3A**	0.992
186	**PRKDC**	0.744	**XRCC5**	0.998
187	**PRKDC**	0.744	**XRCC6**	0.999
188	**GAPDH**	0.548	**PKM2**	0.969
189	**XRCC6**	0.591	**MDC1**	0.955
190	**EXO1**	0.619	**BLM**	0.996
191	**EXO1**	0.619	**MLH1**	0.997
192	**RAD51**	0.456	**PCNA**	0.993
193	**RAD51**	0.456	**MSH2**	0.932
194	**WRN**	0.544	**MLH1**	0.857
195	**G3BP1**	0.474	**USP10**	0.996
196	**DNA2**	0.512	**WDHD1**	0.989
197	**DNA2**	0.512	**RPA1**	0.997
198	**RPA1**	0.604	**MCM6**	0.998
199	**RPA1**	0.604	**RAD52**	0.998
200	**RPA1**	0.604	**PCNA**	0.998
201	**PPARGC1A**	0.580	**NRF-1**	0.980
202	**PTEN**	0.419	**PTK2**	0.993

203	**TP53**	0.842	**USP10**	0.970
204	**PARP-1**	0.821	**CASP3**	0.990

Table 2: True combined score of SIRT6 with selected proteins

1st Link to SIRT6	**1st Link Score**	**2nd Link to SIRT6**	**2nd Link Score**	**True Combined score (Sum)**	**True Combined score (Multiplication)**
RBBP8	0.963	ATM	0.990	0.953	0.999
TP53	0.683	E2F1	0.834	0.517	0.947
HDAC1	0.754	SIN3A	0.998	0.752	0.999
MYC	0.902	MAX	0.981	0.883	0.998
JUN	0.514	FOXP3	0.966	0.480	0.983
RUNX2	0.496	SP7	0.964	0.460	0.981
MTOR	0.436	MLST8	0.999	0.435	0.999
TP53	0.683	ESFR	0.996	0.679	0.998
EXO1	0.619	BRCA1	0.973	0.592	0.989
DNA2	0.512	PCNA	0.984	0.496	0.992
MRE11A	0.467	NBN	0.998	0.465	0.998
RAD50	0.510	H2AFX	0.988	0.498	0.994
RAD50	0.510	CTBP1	0.912	0.422	0.956
MRE11A	0.467	PCNA	0.981	0.448	0.989
EX01	0.619	H2AFX	0.637	0.256	0.861
RBBP8	0.963	BRCA1	0.995	0.958	0.999
RBBP8	0.963	MRE11A	0.990	0.953	0.999
CHEK2	0.434	ATM	0.999	0.433	0.999
TP53BP1	0.499	ATM	0.997	0.496	0.998
CHEK2	0.434	H2AFX	0.990	0.424	0.994
CHEK2	0.434	MDC1	0.989	0.423	0.993
TP53BP1	0.499	CHEK2	0.995	0.494	0.997
TP53BP1	0.499	MDC1	0.998	0.497	0.998

TP53BP1	0.499	H2AFX	0.997	0.496	0.998
HDAC1	0.754	MTA1	0.998	0.752	0.999
HDAC1	0.754	RBBP7	0.998	0.752	0.999
HDAC1	0.754	RBBP4	0.998	0.752	0.999
HDAC1	0.754	SIN3B	0.996	0.750	0.999
TP53BP1	0.499	H2AFX	0.997	0.496	0.998
TP53BP1	0.499	ATM	0.997	0.496	0.998
TP53BP1	0.499	MDC1	0.998	0.497	0.998
XRCC6	0.591	RNF168	0.939	0.530	0.975
PRKDC	0.744	RAD50	0.988	0.732	0.996
XRCC6	0.591	MDC1	0.955	0.546	0.981
XRCC6	0.591	H2AFX	0.983	0.574	0.993
XRCC6	0.591	RAD50	0.996	0.587	0.998
XRCC6	0.591	TP53BP1	0.990	0.581	0.995
XRCC6	0.591	PAXIP1	0.911	0.502	0.963
XRCC6	0.591	CHEK2	0.841	0.432	0.934
PRKDC	0.744	PAXIP1	0.924	0.668	0.980
MYC	0.902	RB1	0.993	0.895	0.999
MYC	0.902	BCL2	0.995	0.897	0.999
MYC	0.902	CDKN2A	0.993	0.895	0.999
MYC	0.902	JUN	0.993	0.895	0.999
MYC	0.902	TP53	0.996	0.898	0.999
MYC	0.902	KAT2A	0.992	0.894	0.999
MYC	0.902	CDKN1A	0.992	0.894	0.999
MYC	0.902	AKT1	0.989	0.891	0.998
MYC	0.902	CDK4	0.990	0.892	0.999
MYC	0.902	MYB	0.991	0.893	0.999
KAT2A	0.577	TAF10	0.998	0.575	0.999
AKT1	0.755	PIK3CB	0.999	0.754	0.999
KAT2A	0.577	TADA3	0.998	0.575	0.999

KAT2A	0.577	SUPT3H	0.999	0.576	0.999
KAT2A	0.577	TAF9	0.998	0.575	0.999
KAT2A	0.577	TADA2A	0.998	0.575	0.999
KAT2A	0.577	TRRAP	0.997	0.574	0.998
KAT2A	0.577	TADA2B	0.996	0.573	0.998
KAT2A	0.577	ATXN7L3	0.996	0.573	0.998
KAT2A	0.577	USP22	0.996	0.573	0.998
KAT2A	0.577	CCDC101	0.997	0.574	0.998
AKT1	0.755	PIK3CD	0.999	0.754	0.999
AKT1	0.755	PIK3CA	0.999	0.754	0.999
AKT1	0.755	MTOR	0.999	0.754	0.999
AKT1	0.755	PIK3CG	0.999	0.754	0.999
AKT1	0.755	RICTOR	0.997	0.752	0.999
AKT1	0.755	OGN	0.999	0.754	0.999
AKT1	0.755	FOXO3	0.999	0.754	0.999
AKT1	0.755	FOXO1	0.999	0.754	0.999
PIK3CG	0.449	MTOR	0.998	0.447	0.998
FOXO1	0.782	AKT3	0.991	0.773	0.998
FOXO3	0.638	SIRT1	0.997	0.635	0.998
PIK3CG	0.449	PTEN	0.998	0.447	0.998
PIK3CG	0.449	NRAS	0.996	0.445	0.997
PIK3CG	0.449	RRAS	0.995	0.444	0.997
PIK3CG	0.449	RRAS2	0.994	0.443	0.996
PRKAA1	0.459	PRKAB1	0.999	0.458	0.999
PIK3CG	0.449	AKT3	0.989	0.438	0.993
PIK3CG	0.449	AKT2	0.991	0.440	0.995
AKT1	0.755	RRAS	0.575	0.330	0.895
AKT1	0.755	NRAS	0.628	0.383	0.908
PIK3GC	0.449	PDPK1	0.991	0.440	0.995
FOXO1	0.782	SIRT1	0.997	0.779	0.999

FOXO1	0.782	G6PC	0.987	0.769	0.997
FOXO1	0.782	AKT2	0.996	0.778	0.999
FOXO1	0.782	AKT1	0.999	0.781	0.999
FOXO1	0.782	SGK1	0.976	0.758	0.994
FOXO1	0.782	SMAD3	0.979	0.761	0.995
FOXO1	0.782	CTNNB1	0.977	0.759	0.994
FOXO1	0.782	CDKN1A	0.977	0.759	0.994
FOXO1	0.782	EP300	0.977	0.759	0.994
FOXO3	0.638	SGK1	0.993	0.631	0.997
FOXO3	0.638	AKT3	0.991	0.629	0.996
FOXO3	0.638	AKT2	0.992	0.630	0.997
FOXO3	0.638	AKT1	0.999	0.637	0.999
FOXO3	0.638	SMAD3	0.979	0.617	0.992
FOXO3	0.638	IKBKB	0.983	0.621	0.993
FOXO3	0.638	BCL2L11	0.989	0.627	0.996
HIF1A	0.839	EGLN3	0.994	0.833	0.999
HIF1A	0.839	VEGFA	0.996	0.835	0.999
HIF1A	0.839	VHL	0.998	0.837	0.999
HIF1A	0.839	HIF1AN	0.996	0.835	0.999
HIF1A	0.839	EGLN2	0.989	0.828	0.998
HIF1A	0.839	ARNT	0.992	0.831	0.998
HIF1A	0.839	KDR	0.802	0.641	0.968
HIF1A	0.839	FLT1	0.980	0.819	0.996
MDM2	0.432	CDKN2A	0.992	0.424	0.995
HDAC1	0.803	AML	0.969	0.772	0.993
HIF1A	0.839	CREBBP	0.993	0.832	0.998
FOS	0.511	JUND	0.999	0.510	0.999
JUN	0.514	ATF3	0.998	0.512	0.999
JUN	0.514	MAPK8	0.999	0.513	0.999
JUN	0.514	MAPK9	0.998	0.512	0.999

JUN	0.514	FOS	0.999	0.513	0.999
JUN	0.514	FOSB	0.998	0.512	0.999
JUN	0.514	MAPK1	0.997	0.511	0.998
JUN	0.514	MAPK10	0.998	0.512	0.999
JUN	0.514	FOSL1	0.997	0.511	0.998
JUN	0.514	FOSL2	0.992	0.506	0.996
JUN	0.514	MAPK3	0.996	0.510	0.998
JUN	0.514	NFAYC2	0.996	0.510	0.998
RUNX2	0.496	SMAD3	0.982	0.478	0.990
RUNX2	0.496	RB1	0.969	0.465	0.984
RUNX2	0.496	BGLAP	0.996	0.492	0.997
RUNX2	0.496	WWTR1	0.940	0.436	0.969
RUNX2	0.496	SMAD4	0.944	0.440	0.971
RUNX2	0.496	MAPK1	0.962	0.458	0.980
CDKNB1	0.529	CCND1	0.996	0.525	0.998
MTOR	0.436	RICTOR	0.999	0.435	0.999
MTOR	0.436	RPS6KB1	0.999	0.435	0.999
MTOR	0.436	RPTOR	0.999	0.435	0.999
MTOR	0.436	PIK3CG	0.998	0.434	0.998
MTOR	0.436	FKBP1A	0.998	0.434	0.998
MTOR	0.436	TSC2	0.998	0.434	0.998
MTOR	0.436	EIF4EBP1	0.999	0.435	0.999
MTOR	0.436	RHEB	0.997	0.433	0.998
MTOR	0.436	EIF4E	0.996	0.432	0.997
KAT2B	0.607	CCDC101	0.996	0.603	0.998
EP300	0.479	CITED2	0.994	0.473	0.996
EP300	0.479	CREB1	0.995	0.474	0.997
EP300	0.479	TBP	0.986	0.465	0.992
EP300	0.479	HDAC1	0.991	0.470	0.995
EP300	0.479	NCOA3	0.987	0.466	0.993

SOD2	0.488	SOD1	0.997	0.485	0.998
SOD2	0.488	CAT	0.997	0.485	0.998
SOD2	0.488	GPX1	0.991	0.479	0.995
SOD2	0.488	SOD3	0.990	0.478	0.994
SOD2	0.488	GSR	0.858	0.346	0.927
TP53	0.683	CREBBP	0.998	0.681	0.999
TP53	0.683	MDM4	0.999	0.682	0.999
TP53	0.683	CDKN1A	0.998	0.681	0.999
TP53	0.683	BCL2	0.998	0.681	0.999
TP53	0.683	BAX	0.998	0.681	0.999
TP53	0.683	ATM	0.997	0.680	0.999
TP53	0.683	BCL2	0.998	0.681	0.999
TP53	0.683	CDK2	0.997	0.680	0.999
TP53	0.683	MDM4	0.999	0.682	0.999
TP53	0.683	PCNA	0.994	0.677	0.998
KAT2B	0.607	TADA2A	0.997	0.604	0.998
KAT2B	0.607	SUPT3H	0.996	0.603	0.998
KAT2B	0.607	TRRAP	0.997	0.604	0.998
KAT2B	0.607	TADA3	0.999	0.606	0.999
KAT2B	0.607	ATXN7L3	0.995	0.602	0.998
KAT2B	0.607	TAF10	0.996	0.603	0.998
HDAC3	0.742	RBBP4	0.992	0.734	0.997
KAT2B	0.607	EP300	0.994	0.601	0.997
MDM2	0.431	ATM	0.991	0.422	0.994
MDM2	0.431	MDM4	0.992	0.423	0.995
MDM2	0.431	RB1	0.982	0.413	0.989
MDM2	0.431	CHEK2	0.980	0.411	0.988
MDM2	0.431	RPL23	0.949	0.380	0.970
PRKDC	0.744	H2AFX	0.995	0.739	0.998
RPL11	0.531	RPL3	0.999	0.530	0.999

RPL35	0.632	RPL5	0.999	0.631	0.999
RPL9	0.517	RPL12	0.999	0.516	0.999
FOS	0.571	CREB1	0.996	0.567	0.998
FOS	0.517	MAPK1	0.996	0.513	0.998
FOS	0.517	JUNB	0.999	0.516	0.999
VEGFA	0.516	FLT1	0.999	0.515	0.999
VEGFA	0.516	FLT4	0.994	0.510	0.997
VEGFA	0.516	IGF1	0.991	0.507	0.995
HDAC3	0.742	NCOR1	0.991	0.733	0.997
HDAC3	0.742	SIN3A	0.992	0.734	0.997
PRKDC	0.744	XRCC5	0.998	0.742	0.999
PRKDC	0.744	XRCC6	0.999	0.743	0.999
GAPDH	0.548	PKM2	0.969	0.517	0.985
XRCC6	0.591	MDC1	0.955	0.546	0.981
EXO1	0.619	BLM	0.996	0.615	0.998
EXO1	0.619	MLH1	0.997	0.616	0.998
RAD51	0.456	PCNA	0.993	0.449	0.996
RAD51	0.456	MSH2	0.932	0.388	0.963
WRN	0.544	MLH1	0.857	0.401	0.934
G3BP1	0.474	USP10	0.996	0.470	0.997
DNA2	0.512	WDHD1	0.989	0.501	0.994
DNA2	0.512	RPA1	0.997	0.509	0.998
RPA1	0.604	MCM6	0.998	0.602	0.999
RPA1	0.604	RAD52	0.998	0.602	0.999
RPA1	0.604	PCNA	0.998	0.602	0.999
PPARGC1A	0.580	NRF-1	0.980	0.576	0.997
PTEN	0.419	PTK2	0.993	0.410	0.984
TP53	0.842	USP10	0.970	0.833	0.978
PARP-1	0.821	CASP3	0.990	0.805	0.999

Table 3: Minimum and maximum scores from calculated score

Minimum 1st Link Score with MDM2	0.432
Maximum 1st Link Score with RBBP8	0.963
Minimum 2nd Link Calculated Score with EXO1	0.256
Maximum 2nd Link Score with RBBP8	0.958

The table 3 shows the minimum and maximum 1st link score of SIRT6 with MDM2 and RBBP8 are respectively 0.432 and 0.963 and the minimum and maximum 2nd link calculated score of SIRT6 with EXO1 and RBBP are respectively 0.256 and 0.958.

3.1 Therapeutic targets of SIRT6 on Cancer

Table 4: SIRT6 on Tumor promotion

1st	1st Relationship to SIRT6 (+/-)	1st link Score	Cancer Status (U/D)	2nd link	2nd Relationship to SIRT6 (+/-)	2nd link Score	Cancer Status (U/D)
AKT1	"+"	0.755	D	PRKAB 1	"-"	0.999	U
PARP -1	"-"	0.821	U				
AKT1	"+"	0.755	D	OGN	"-"	0.999	U
TP53	-	0.683	-	BAX	"-"	0.998	U

*All linked concentration is being increased.

*All "-" means promotion of tumor

*Red indicates N/A Relationship so this will not show any cancer status.

Table 5: SIRT6 on Tumor suppression

1st link	1st Relationship to SIRT6 (+/-)	1st link Score	Cancer Status (U/D)	2nd link	2nd Relationship to SIRT6 (+/-)	2nd link Score	Cancer Status (U/D)
MYC	"+"	0.902	D	Lin28b	"-"	0.932	U
TP53	-	0.683	-	E2F1	"-"	0.834	U
RUNX2	"-"	0.496	U				
G3BP1	-	0.53	-				
KAT2A	-	0.577	-	Notch3	"-"	0.908	U
MYC	"+"	0.902	D	Twist1	"-"	0.482	U
GAPDH	-	0.466	-	PKM2	"-"	0.982	U
TP53	-	0.683	-	USP10	"+"	0.970	D
Akt1	“+”	0.755	D	PIK3CA	“-“	0.999	U
CDKNB1	-	0.529	-	CCND1	“+”	0.996	D
mTOR	“+”	0.436	D				
PTEN	“+”	0.419	D	PIK3CA	“-“	0.999	U

*All linked concentration is being increased.

*All “+” means suppression of tumor

*Red indicates N/A Relationship so this will not show any cancer status.

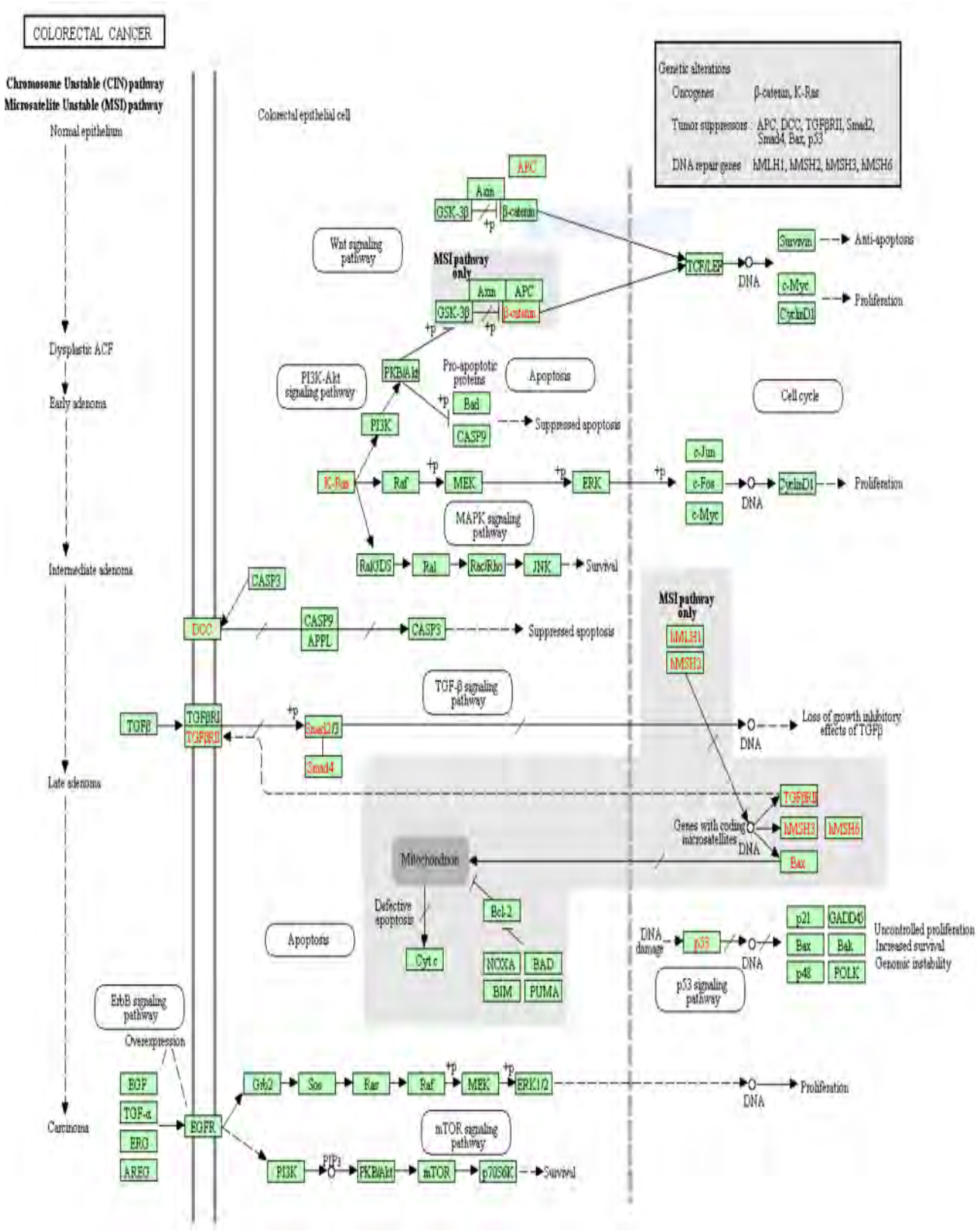

Figure 6: KEGG Pathway for Colorectal Cancer (KEGG Pathway, 2018)

Chapter 4

Discussion

The results show a direct physical binding of SIRT6 and 204 specific proteins. From that data, MYC, RUNX2,CDKNB1, TP53, GAPDH, KAT2A, G3BP2, Lin28B, Notch3, USP10, PTEN, E2F1, PKM2, Twist1, AKT1, PARP-1, PRKAB1, OGN (TGF-β1), BAX, mTOR, PI3K, CCND1 have identified therapeutic targets with SIRT6 on cancer. Some of proteins such as Lin28B, Notch3, E2F1, PKM2, Twist1 marked with negative sign (Table 3, 4) have up regulation function and also some such as MYC, USP10, AKT1 marked with positive sign (Table 3,4) have down regulation function on several cancers. Among them, MYC, USP10, TP53, AKT1 have potential effect on SIRT6 for down-regulating the colorectal cancer.

From the result section (Table 1, 2), our study has shown that SIRT6 and PTEN have interaction score 0.419. As it is a 1st linked interaction score, the score is weak. Although, the confidence score is weak, SIRT6 and PTEN have potential relationship to suppress the colorectal cancer because SIRT6 and PTEN both are tumor suppressors. In addition, SIRT6 and PI3K have interaction score is 0.999 which is very strong interaction score but SIRT6 would suppress PI3K for down-regulating colorectal cancer. SIRT6 also have interaction score with c-myc, mTOR, cyclin d1 and Akt1 protein. The score is respectably 0.902, 0.436, 0.996 and 0.755. These proteins help SIRT6 to suppress colorectal cancer. Figure 6 shows the KEGG pathway for colorectal cancer.

From reviewing journals and KEGG pathway for colorectal cancer has explained that PTEN which is a tumor suppressor gene located in human chromosomes prohibit PI3K/AKT signaling in colorectal cancer (J. Tian & Yuan, 2018). As PI3K/Akt1 signaling pathway would increase cell proliferation, cell survival as well as cell migration for cancer cells (J. Tian & Yuan, 2018) . In our

study, SIRT6 and PTEN have moderately strong interaction score which indicated that SIRT6 also would regulate PI3K/Akt1 signaling pathway. Over-expression of SIRT6 could inhibit cell proliferation, migration and survival by inhibiting PI3K protein. On that case, cyclin d1, AKT1, and c-myc protein expression would reduce by SIRT6 over-expression (J. Tian & Yuan, 2018). In mTOR signaling pathway, SIRT6 over-expression inhibit PI3K protein which lead to inhibition of mTOR. Inhibition of mTOR would cause the prohibition of cell survival (J. Tian & Yuan, 2018). SIRT6 up-regulation would increase the cell apoptosis as well as would prohibit the cell growth. This study demonstrates that SIRT6 could be the effective therapeutic targets in colorectal cancer. Moreover, SIRT6 role on Wnt pathway, p53 pathway, ErbB pathway, TGF-β1 signaling pathway and MSI pathway are not yet cleared. Further study is needed to explain the relation between SIRT6 and these pathways.

Chapter 5

Conclusion

To conclude, SIRT6 has potential role in tumorigenesis. This is just the beginning of SIRT6 era as a biological function in the treatment of most life threatening diseases in the world. Basically SIRT6 would have decent activities on gene expression in the nucleus to find chromatin factor. Among the 7 members of sirtuins, SIRT6 has the positive role in treating colon cancer. And also there is some controversy about SIRT6 functioning in cancer but our study is mostly concern about the important factor of SIRT6 in colon cancer. About colon cancer, SIRT6 expression has helped to cure the disease and alter the prognosis of colon cancer. For more accurate treatment, the thorough knowledge of the mechanistic differences in tumor types of the complex biology of SIRT6 and therapeutic approaches in cancer may need to suppress the progression of cancer in human body. Moreover, most effectively screening cancer patients for proteomic, metabolic abnormalities, dysfunctional signaling cascades and genomic would be the main alterations which would use the identification for targets on personalized cancer therapy as well as more successful cancer treatment.

Chapter 6

Future Direction

SIRT6 in various diseases are needed to be studied further. Technically, SIRT6 have shown some spectacular role colorectal cancer but the therapeutic targets are not very strong enough to introduce it in drug design for curing diseases. There are so many diseases like tumorigenesis, osteoblastogenesis, heart diseases and diabetes are not sufficiently clear yet. Another possibility of SIRT6 role in Parkinson's disease, Huntington's diseases and cerebal ischemia and more are not properly investigated. More time and resources are needed to imply so that potential therapeutic targets can be identified to cure these types of life-threatening diseases.

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