A Review on the Role of Histone Deacetylase SIRT6 in High-Grade Serous Ovarian Cancer

By

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

School of Pharmacy

Brac University

March 2022

Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing my degree at Brac University.
2. The thesis 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 thesis does not contain material that 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.

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Approval

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Ethics Statement

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

Abstract

High-grade serous ovarian cancer (HGSOC) is the most common type of ovarian cancer, accounting for about 70% of all ovarian cancer patients. Despite limited advancements in its treatment over the last decade, few standard therapies are currently available, although the survival rate remains poor. Currently available treatment for HGSOC is employing PARP inhibitors, anti-angiogenic, platinum-based chemotherapy, combination therapy with carboplatin and paclitaxel, etc. Increasing knowledge about the biology and pathways of SIRT6 led to the development of a new promising therapeutic target for HGSOC. SIRT6 can be a novel approach to treat HGSOC by regulating several cellular signaling pathways, including NOTCH3 and Wnt/β-catenin. Down-regulation of SIRT6 is associated with HGSOC and thus, up-regulation of SIRT6 by therapeutic modulators can be efficiently implemented to eradicate HGSOC. This review summarizes the current knowledge about SIRT6, its association with HGSOC, and the mechanisms for targeting SIRT6 to treat high-grade serous ovarian cancer.

Keywords: High-grade serous ovarian cancer, SIRT6, NOTCH3, Wnt/β-catenin, EMT, Tumorigenesis

Dedication

Dedicated to my parents

Acknowledgement

All praise and glory be to Almighty Allah, who gave me the strength and courage to carry out this project.

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Table of Contents

List of Tables

List of Figures

List of Acronyms

HGSOC	High-Grade Ovarian Cancer
OC	Ovarian Cancer
STIC	Serous Tubal Intraepithelial Carcinoma
EMT	Epithelial–mesenchymal transition
VEGF-A	Vascular endothelial growth factor A
PARP	Poly (ADP-ribose) polymerase
CSC	Cancer stem cell
NAD	Nicotinamide Adenine Dinucleotide
TNFα	Tumour Necrosis Factor alpha
RUNX2	Runt-related transcription factor 2
USP10	Ubiquitin carboxyl-terminal hydrolase 10
Myc	Master Regulator of Cell Cycle Entry and Proliferative Metabolism
AP-1	Activator protein 1
HCC	Hepatocellular carcinoma
PKM2	Pyruvate kinase M2
DSB	Double-strand breaks
DDR	DNA damage response
NTE	NH2- terminal extensions
CTE	COOH-terminal extensions,
HIF-1α	Hypoxia-inducible factor 1-alpha
mTOR	Mechanistic Target of Rapamycin Kinase
PI3K	Phosphoinositide 3-Kinase
AKT	Nuclear factor erythroid 2-related factor 2

Chapter 1: Introduction

An overview of the Ovarian Cancer and SIRT6

1.1 Ovarian Cancer

Ovarian cancer (OC) is the fifth most common cancer in women, affecting more women than any other female reproductive system malignancy. A woman's lifetime risk of developing ovarian cancer is about 1 in 75, with a 1 in 100 chance of dying from it (Reid et al., 2017). Despite breakthroughs in ovarian cancer therapy, the survival percentage of OC patients remains low because of the late diagnosis. Symptoms of Ovarian Cancers (OCs) are usually subtle, and most cancers escape identification until they are in late stages and have migrated beyond the ovaries. The symptoms that contribute to the late diagnosis of OC include stomach discomfort, nausea, gas, bloating, urinary urgency which are incompletely elucidated, and these can be misdiagnosed as gastrointestinal problems. Therefore, identifying potential prognostic biomarkers in ovarian cancer is crucial in order to produce more effective and strong individualized treatment therapy and expand prognosis.

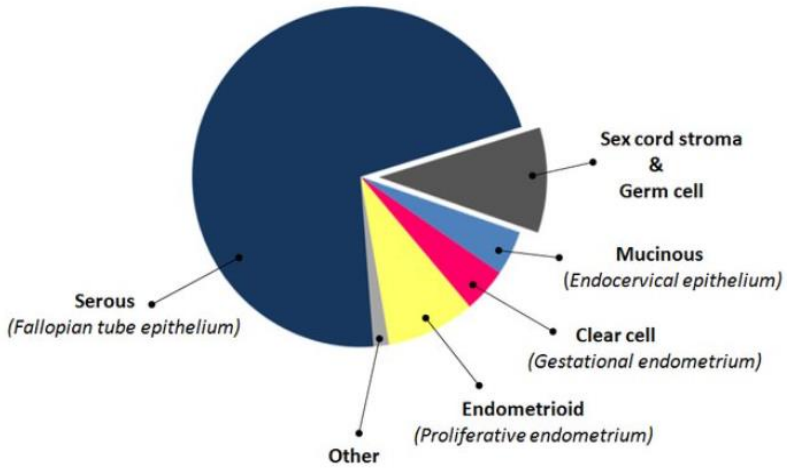

Figure 1: Types of ovarian cancers (Tone, 2010).

The lack of initial symptoms causes over 70% of patients being detected at an advanced stage while cancer cells have already started actively metastasizing (Yeung et al., 2015). Pathogenesis and the mechanism of ovarian cancer metastasis must be accurately understood to control this highly metastatic deadly disease.

1.2 Stages and Grades of ovarian cancer

To plan treatment and predict prognosis, a patient's cancer stage needs to be determined first. The stages of OC reflect the size and location of the disease as well as whether or not it has spread. Early cancer refers to Stage I, subdivided into three further stages. For instance, Stage 1a refers to cancer being confined only in one ovary or fallopian tube. In contrast, Stage 1b cancer is extended in one or both ovaries and fallopian tubes, and Stage 1c is outspread in both ovaries and fallopian tubes. In Stage 1c, there's a high possibility for cancer to disperse into the abdomen or pelvis by breaking the tissue covering the ovary or fallopian tubes (Matulonis et al., 2016).

Stage 2 ovarian cancer interprets cancer as being in both ovaries and fallopian tubes along with spreading into the surrounding regions inside the pelvis. Stage 2a and Stage 2b are the subtypes of this stage, representing cancer in the womb, fallopian tubes or ovaries, and cancer in other pelvis areas, e.g., the bladder or bowel. Stage 3 ovarian cancer implies cancer has extended outside the pelvis to the abdominal cavity lining (peritoneum). Cancer can even disseminate to the lymph nodes in the abdomen and pelvis at this phase (Hirst et al., 2018).

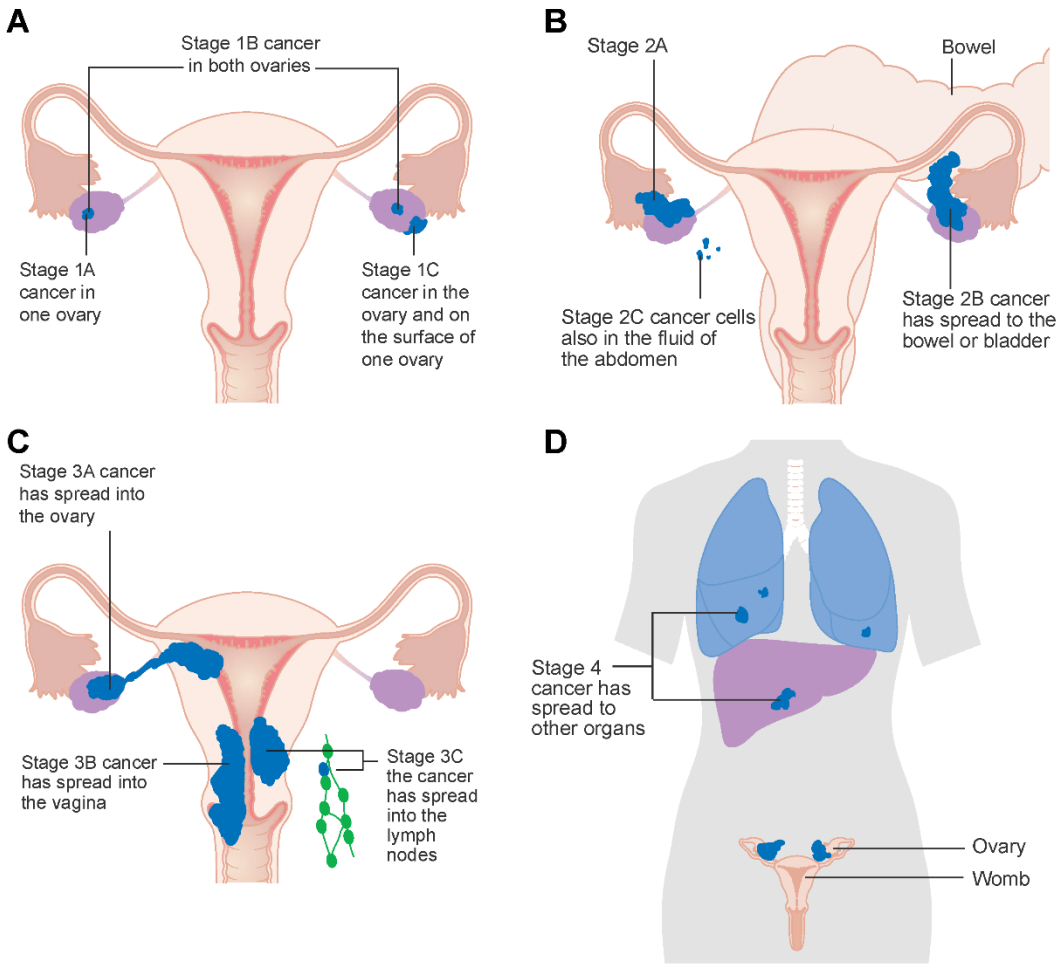

Figure 2: Stages of Ovarian Carcinoma (Colombo et al., 2006).

Stage 3a1 indicates that cancer has advanced to the lymph nodes in the abdomen, whereas Stage 3a2 implies that cancer has migrated to the peritoneum beyond the pelvis. In Stage 3b, cancer outspreads in areas less than 2 cm; on the other hand, Stage 3c covers the areas larger than 2 cm. Expansion of cancer to outside the abdomen defines Stage 4. The presence of fluid in the lining of the lungs specifies the cancer being in Stage 4a. The last stage, Stage 4b, characterizes cancer that has progressed to the interior of the liver or spleen, as well as organs exceeding the abdomen (Matulonis et al., 2016).

According to the histologic type, serous carcinoma can be divided into two grade systems: low-grade serous carcinoma and high-grade serous carcinoma. High-Grade Serous Epithelial Ovarian Carcinoma remains to be the most frequent kind of OC, accounting for roughly 70% of all ovarian malignancies (Hirst et al., 2018).

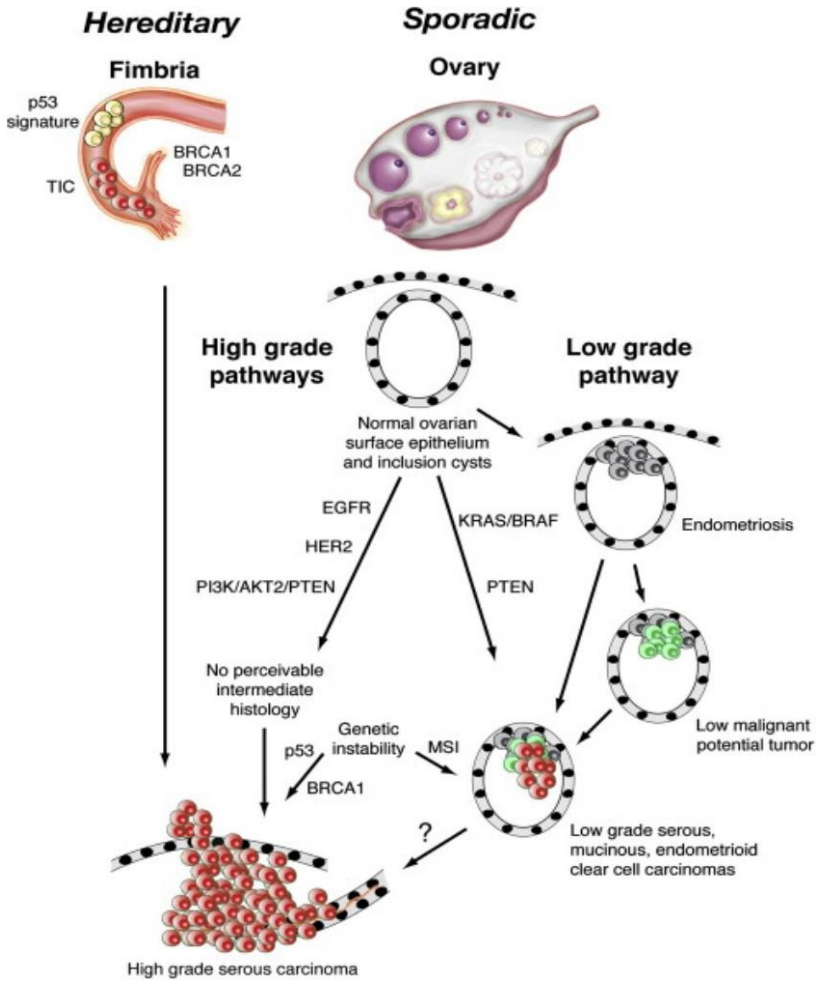

Figure 3: Pathogenesis Pathways of High-Grade Serous Ovarian Cancer (Hirst et al., 2018).

The cell of origin for each kind of OC differs and depends on tumor type. Initially, it was considered that ovarian epithelial tumors arose from the ovary's surface epithelium, which resulted from the metaplastic transformation of the mesothelium. Still, recent developments have led to a

different understanding of this theory. They can arise from the peritoneum and the fallopian tube as well as the ovary (Kim et al., 2018).

1.3 High-Grade Serous Ovarian Carcinoma

High-grade serous ovarian carcinomas (HGSOCs) are identical no matter where they arise from. However, most of them arise from tubal epithelial cells in the fimbriae of the fallopian tubes, where the precursor region is Serous Tubal Intraepithelial Carcinoma (STIC) (Kim et al., 2012). Most HGSOC has a TP53 gene mutation, while BRCA1 and BRCA2 germline mutations are present in 50% of cases. Unfortunately, HGSOC of the ovary tends to present late, with 80% of patients showing at stage III/IV (Matulonis et al., 2016).

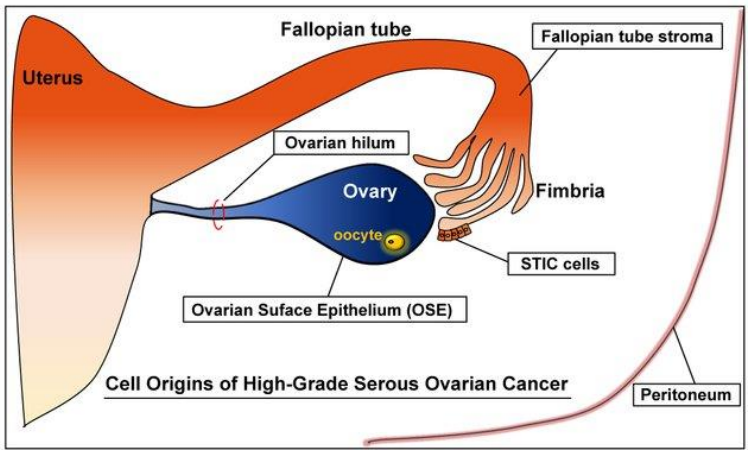

Figure 4: Origins of High-Grade Serous Ovarian Cancer cells (Kim et al., 2018).

A pathogenetic model incorporating the progression steps of HGSOC is required for useful screening and therapy that considers biomarkers of early tumorigenesis. Bowtell's concept predicts early p53 loss and BRCA loss as significant events, resulting in a lack of homologous recombination repair of double-strand break, which causes chromosomal fluctuation and extensive copy-number alterations (Bowtell, 2010). Secondary and tertiary events then alter gene expression, as well as mutations, allowing tumors to evolve (Prat, 2012).

The most recurrent molecular abnormality in HGSOC is TP53 mutation, making it nonfunctional at the genomic level. P53 is our body's most crucial tumor suppressor gene, and it protects our cells from becoming cancerous. Since p53 is very frequently mutated in OC patients, accounting for around 60% of all cases, the protein is not just a dead protein but also an active tumor-promoting protein whose continued production is required by the tumor. The depletion of this protein is highly effective in reducing the progression and invasiveness of cancer (Berns & Bowtell, 2012).

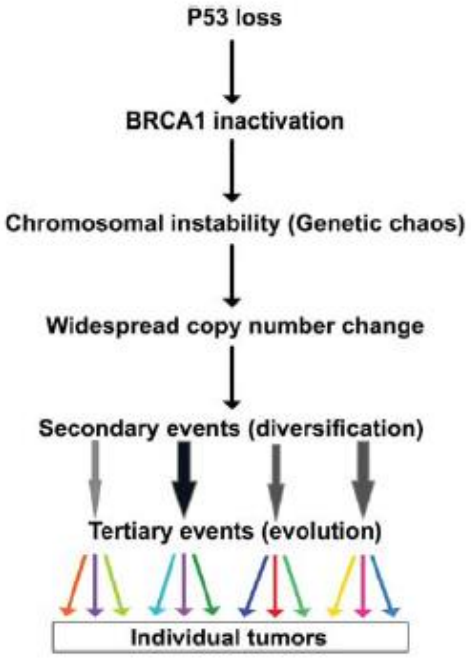

Figure 5: Initiation and progression of high-grade serous ovarian cancer (Bowtell, 2010).

About 2/3rd of those mutations is a missense mutation, where most of the protein is still made and still has some function. That function is more oncogenic than the normal function, which is tumor suppressor. About 1/3rd of ovarian cancers has a null mutation, which means the P53 gene has not been made or expressed in these tumors. So, for missense mutation, the mutations change the structures of the protein, and the protein behaves abnormally and, in many cases, helps cancer

grow more efficiently. There are small molecule inhibitors that have been explicitly developed against some of those mutations, and it helps change the way the proteins are folded. The way it is folded dictates how well it functions. Even though the p53 is mutated, the protein can change it to perform normally. Those have given some favorable results in preclinical studies in mice and cell lines (Hollis & Gourley, 2016).

Tumors lacking TP53 mutations show symptoms of p53 malfunction via MDM2 or MDM4 copy number increase, which are essential in controlling and degrading p53. Defective homologous recombination is linked to somatic and germline BRCA mutations and changes in other DNA repair pathway genes (Zhang et al., 2016). BRCA1 is required for DNA repair, transcriptional regulation, mitosis, chromatin remodeling, and cell cycle checkpoint control, whereas BRCA2 is needed for homologous recombination along with DNA repair. The BRCA1 promoter can also be found to be hypermethylated in a significant fraction of HGSOCs (Tuna et al., 2019). However, this does not affect overall survival and prognosis.

Another study shows that recurrent molecular alteration can also cause HGSOC prognosis, including the Notch pathway (Takebe et al., 2014). Notch has been associated with different stem cell maintenance events and EMT in the ovary. Notch pathway dysregulation is common in HGSOC and linked to poor survival, advanced stages, and lymph node involvement. The amount of molecular diversity defines the genomic complexity of this ovarian cancer during diagnosis, its development and transition over time, the existence of few druggable ovarian cancer driver gene mutations, and the high rate of copy number variations in genes of various signaling pathways (Inder et al., 2017). Indeed, this molecular complexity helps explain why discovering effective therapeutics for HGSOC has been difficult.

1.4 Treatment options for High-Grade Serous Ovarian Cancer

The rate of survival with traditional treatments, including chemotherapy and cytoreductive surgery, is about 90% when ovarian cancer is identified early, restricting the tumor tissues to one or both ovaries only. Whereas, when cancer has migrated to the uterus, bladder, and other pelvic organs in stage 2 the cure rate drops significantly. In addition, once the cancer is in stage 3 or the abdomen and outside the peritoneal cavity in stage 4, the survival rate decreases greatly due to the limited efficiency of tumor management and surgical removal of the tumor (Gadducci et al., 2019).

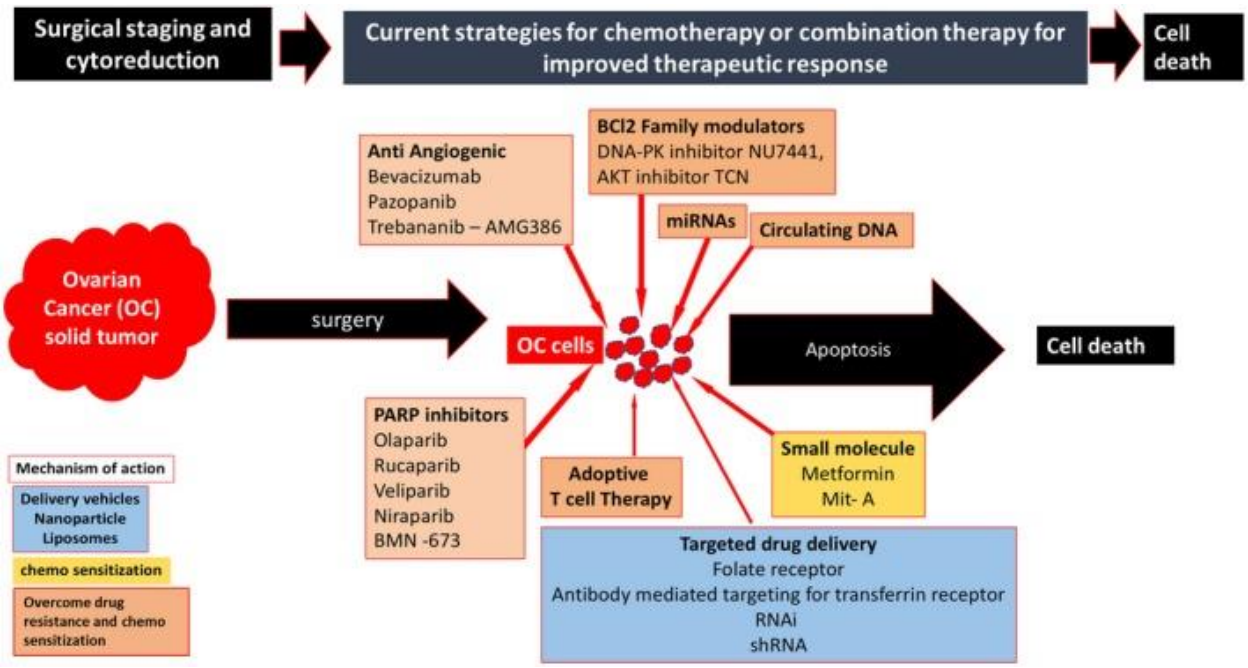

Figure 6: Current treatment options for high-grade ovarian cancer (Chandra et al., 2019).

Although there are no good screening strategies for HGSOC, individuals susceptible to developing the disease can be identified, for instance, those with BRCA1 or BRCA2 germline mutations. Risk-reduction surgery, namely - bilateral salpingo-oophorectomy, has been employed to reduce the risk of HGSOC in these patients. Screening strategies in women with an average risk of developing

HGSOC have primarily centered on the biomarker CA125 (also known as mucin 16) along with the use of transvaginal ultrasonography (Chandra et al., 2019).

Debulking surgery is now used as the first-line therapy for HGSOC, pursued by a mixture of chemotherapy drugs that includes carboplatin or cisplatin with a taxane, e.g., paclitaxel. However, HGSOC is quite susceptible to chemotherapy medications, especially platinum. Even though some individuals will achieve remission following the initial therapy, the majority will eventually relapse. Platinum-based combination or neoadjuvant chemotherapy regimens are used when patients have disease recurrence about 6-12 months, followed by the completion of first-line therapy (Gadducci et al., 2019).

In contrast, subsequent single cytotoxic drugs are used for disease recurrence less than 6 months after initial chemotherapy. Regardless, cancer recurrence after first platinum-based chemotherapy is highly prevalent in women with HGSOC; the most challenging issue in treating this disease is the outcome of platinum resistance. Only a small percentage of people with recurrent ovarian cancer are cured. Second-line therapy aims to increase survival, reduce symptomatic disease progression, and enhance the overall quality of life (Coleman et al., 2013). The number of metastases, tumor size, and BRCA mutations are independent predictors of second-line treatment response. It is crucial to know whether to undertake second-line therapy in relapsing individuals. According to evidence, initiation of second-line treatment early due to biochemical recurrence (for example, elevated cancer antigen [CA] 125) is not beneficial (Coleman et al., 2013).

There are a few second-line choices for platinum-resistant patients, but the development of targeted treatments has made a more significant impact on this. Recently, the FDA has authorized Bevacizumab, an antibody for vascular endothelial growth factor A (VEGF-A), along with carboplatin and paclitaxel (Coleman et al., 2013).

Emerging medicines for recurrent HGSOC therapy include poly (ADP-ribose) polymerase (PARP) inhibitors, angiogenesis inhibitors, and immunotherapy agents. PARP inhibitors are being used as first-line therapy, and combinations of these medications are being developed to treat newly diagnosed as well as recurrent HGSOC (Fiorentino et al., 2021). For women with newly diagnosed advanced HGSOC, standard first-line therapy often includes surgery and chemotherapy, occasionally followed by maintenance therapy which refers to an extra treatment designed to help prevent the disease from returning. However, cancer returns within three years following initial therapy for most individuals. Using a PARP inhibitor as first-line treatment, maintenance therapy, or both significantly reduced the time it took for participants' cancers to recur or worsen. PARP inhibitors are used to treat those women who have developed advanced ovarian cancer that has reappeared following previous therapy (Coleman et al., 2013). Findings of some research suggest that the medications may also assist women who have just been diagnosed with HGSOC.

Although gene sequencing and targeted treatments have enhanced OC patient survival, the 5-year survival rate remains low due to the complicated tumor processes and a lack of more specific target biomarkers (Sun et al., 2019). Several studies also demonstrate that the increased percentage of treatment failure is primarily because of medication resistance, directly connected to cancer stem cells (CSCs). Aside from the numerous genes associated with DNA damage repair and drug efflux contributing to CSC resistance to traditional chemotherapy, the distinctive role of the NOTCH3 signaling pathway in the regulation of ovarian cancer stem cell behavior and platinum chemoresistance has been well established (Perez-Fidalgo et al., 2020). NOTCH3 expression is elevated at both the mRNA and protein levels in most ovarian cancers. Therefore, improving therapy necessitates the development of novel biomarkers for diagnosis and individualized treatment of HGSOC (Ceccarelli et al., 2019).

1.5 Sirtuin 6 (SIRT6)

Sirtuins are considered a family because mammals have seven different sirtuins, conveniently called SIRT1-SIRT7. These sirtuins share an enzymatic domain, but they have differences at either end of their protein sequence that recruits them to different parts of a cell and influences what other proteins and molecules they interact with (Kratz et al., 2021). Therefore, the different sirtuins are structurally related, but they can perform various functions within a cell. Although for some of them, those activities do overlap slightly. Sirtuins are NAD+ dependent enzymes, therefore, their enzymatic activity depends on the availability of NAD+ (Nicotinamide Adenine Dinucleotide). NAD+ is an essential cofactor that is found within cells. With the availability of NAD+, the sirtuins can carry out protein deacetylation removing acyl groups from different proteins. They also possess activities of mono-ADP-ribosotransferase (a protein that can add one ADP-ribose group to another protein to modify it reversibly). SIRT6 is one of the seven mammalian sirtuins that function as an NAD+-dependent histone deacetylase as well as a mono-ADP-ribosyltransferase. The cellular localization can also influence their activities as they'll only interact with proteins localized in the same area. SIRT1, SIRT6, and SIRT7 are primarily present in the cell's nucleus, where DNA is formed. Although SIRT3, SIRT4, and SIRT5 are in the mitochondria, SIRT2 is mainly found in the cytoplasm (Tasselli et al., 2017).

SIRT6 is well-known for its roles in genome maintenance and chromatin signaling. SIRT6 defends mice from diseases linked to staging, such as - cancer and metabolic disease, which can shorten their lives. According to recent research, SIRT6 is found to be a complicated enzyme having various substrates and catalytic activities, and other SIRT6 roles preserving the biological health span have been discovered (Kratz et al., 2021).

The first homolog of the Sirtuin protein was found in yeast and abbreviated as Sir (Silent information regulator). Since then, sirtuins have been known to silence genes by deacetylating the lysine on histones causing histones to bind more tightly to specific genes and decrease their expression (Teixeira et al., 2019). The deacetylation of acetyl-lysine is coupled to the cleavage of NAD+. NAD+ is, therefore, a necessary substrate for this reaction, but most sirtuins can only bind NAD+ after binding to the acetylated substrate. In fact, SIRT6 is the only sirtuin that can bind NAD+ without first binding an acetylated substrate (You & Steegborn, 2020). Sirt6 expression declines along with age; however, overexpression of the enzyme inhibits the evolution of various cancers and increases the longevity of the male mice.

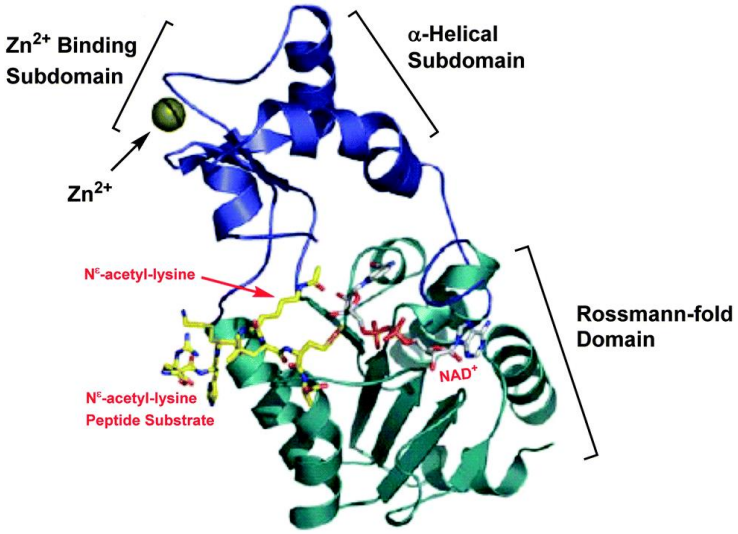

Figure 7: Overall structure of SIRT6 (Chen et al., 2015).

An extended α/β Rossman-fold part along with a short, structure-stabilizing zinc-binding part make up the whole sirtuin's catalytic domain. Numerous loops connect these two domains, forming a functional site cleft where the acylated polypeptide and NAD+ co-substrate are bound. Sirt6 is the only mammalian sirtuin with a vast acyl channel. This channel allows fatty-acetyl groups to be hydrolyzed more efficiently *in vitro* than acetyl groups themselves. *In vivo*, the demyristoylation

function of SIRT6 enhances TNFα release, and SIRT6 has potent deacetylation action against histones in chromatin and nucleosomes. The prevalence of SIRT6's physiological processes has been linked to its deacetylase activity, particularly against histone H3K9ac and H3K56ac (Teixeira et al., 2019).

Research performed by Zhang et al. suggested that SIRT6 might be a new therapeutic target for HGSOC treatment. According to the researchers, SIRT6 expression (at both the protein and mRNA levels) was significantly lower in human OC tissues compared to normal tissues (Zhang et al., 2015). Furthermore, they discovered that overexpression of SIRT6 suppressed the growth of SKOV3 and OVCAR3 ovarian cancer cells. Downregulation of SIRT6, on the other hand, promotes ovarian cancer cell proliferation. They also discovered that SIRT6 upregulation suppressed the development of OC cells, e.g., SKOV3 and OVCAR3. Downregulation of SIRT6, on the other hand, promotes ovarian cancer cell proliferation. Furthermore, they also uncovered that the level of SIRT6 is inversely proportional with NOTCH3 expression at protein and mRNA levels (Tasselli et al., 2017).

1.6 Rationale of the study

Although women with HGSOC respond early to platinum-based chemotherapy treatment, combination therapy with carboplatin and paclitaxel, PARP inhibitors, anti-angiogenic, most become chemo resistant. One of the human sirtuins, SIRT6, has been linked with HGSOC, and thus, its modifications can be targeted to create a better therapy to overcome this chemoresistance. Researchers have studied the role of SIRT6 on various ovarian cancer cells, and those experiments figured out the pathways by which SIRT6 is involved in this disease.

Therefore, this review attempts to assemble all the possible activities or mechanisms of SIRT6 in treating HGSOC.

1.7 Aim and objectives of the study

Aim:

This review aims to summarize the diverse roles of SIRT6 on HGSOC involving multiple mechanisms and pathways, which will ultimately help predict the future therapeutic target to treat this deadly metastatic disease.

Objectives:

The main purpose of this review is to gather insights on down-regulating the risk of HGSOC among women and create better predictive biomarkers for the future. This compiled information will also make references for researchers to conduct further investigation.

Chapter 2: Methodology

This review is accomplished based on the recent publications and research on PubMed, Google Scholar electronic database. More than 30 articles have been studied to complete this review paper. Compiling all the necessary recent investigations on female models and various cell lines, this paper focuses mainly on the action of SIRT6 in high-grade serous ovarian cancer. The following keywords were used to find the needed information: “SIRT6” and “ovarian cancer” along with “sirtuins,” “metastasize,” “high grade serous ovarian cancer,” “histone deacetylase,” “cell adhesion,” “Molecular Processes in Cancer Metastasis.” Additionally, references from the articles found earlier were explored to specify potentially overlooked investigations to make this review more informative.

Chapter 3: SIRT6 and Cancer

3.1 Role of SIRT6 in Cancer

Cancer therapy is based on various techniques targeted at triggering cancer cell death by various mechanisms, such as – apoptosis and DNA damage induction. Targeting SIRT6 is one pathway that functions in cancer initiation and progression. Depending on the biological environment, it can function as both a tumor suppressor and a tumor promoter, regardless of the cancer type (Fiorentino et al., 2021).

Extensive research on cancer-related dysregulated pathways has focused on characterizing several molecular targets on DNA repair and cell death. The epigenetic enzyme SIRT6 performs a vital function in carcinogenesis by maintaining telomere integrity, controlling metabolic homeostasis, preventing genome instability, and ultimately enabling DNA repair. A growing number of studies have found that SIRT6 expression is altered in cancer, both at the protein and mRNA levels (Fiorentino et al., 2021).

The molecular pathways connecting SIRT6, and cancer are glycolysis, inflammation, apoptosis, etc. Tumor cells alter their metabolic route and transform glucose to lactate using aerobic situations, referring to a process known as aerobic glycolysis (Warburg effect). SIRT6 is essential for glucose homeostasis, particularly inhibiting the glycolytic process, the desired energy resource for tumor cells, consequently limiting carcinogenesis (Vitiello et al., 2017). SIRT6 deficiency increases glucose uptake and decreases mitochondrial respiration via hypoxia-inducible factor-1 (HIF-1α).

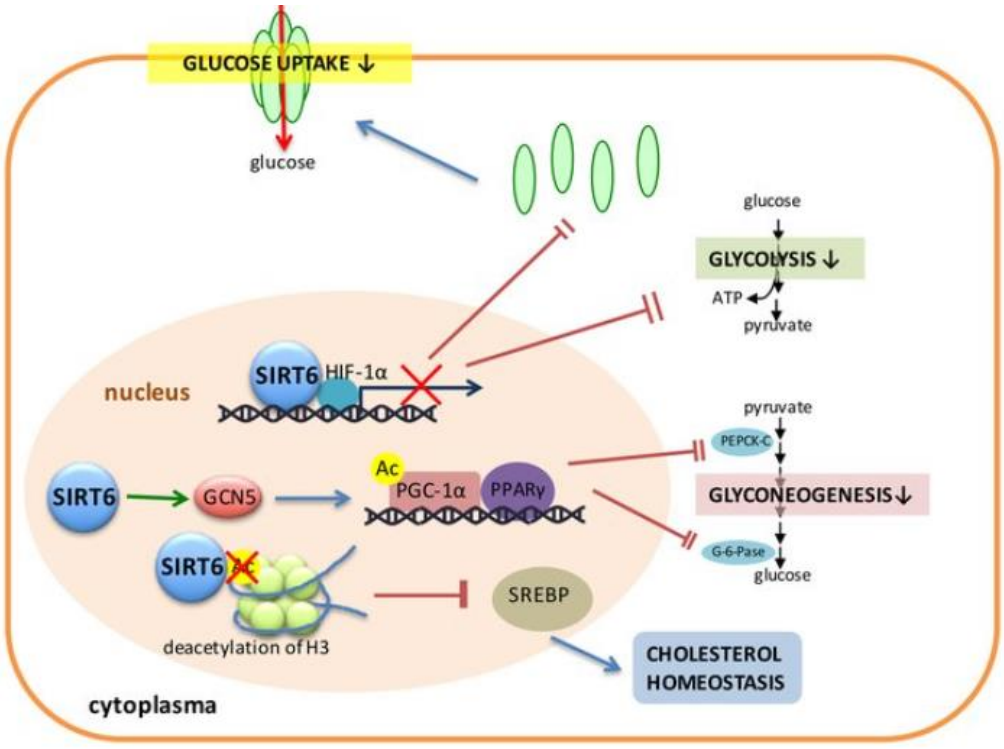

Figure 8: SIRT6 in the metabolism of cancer cells (Ye et al., 2017).

The deacetylase activity of SIRT6 has been observed to be affected by impaired mutations. Suppression of SIRT6 transcription in the early stages of liver cancer is mediated via the c-Jun/c-Fos pathway. Still, in breast cancer cells, repression of SIRT6 transcription is mediated by runt-related transcription factor 2 (RUNX2) through its regulatory action on metabolic pathways. Furthermore, in bladder and prostate cancer cell lines, E2F-1 has also been reported to decrease the transcription of SIRT6 (Vitiello et al., 2017). Reduced amounts of deubiquitinase protein USP10 in colon cancer cause SIRT6 protein instability and, as a result, increased transcriptional initiation of c-Myc, a crucial mark in cancer therapy (Desantis et al., 2018).

3.2 Regulation of SIRT6 expression and activity

SIRT6 expression and activity are regulated by several factors at the transcriptional and post-transcriptional levels, impacting tumor initiation and development function. AP-1 promotes SIRT6 transcription via its c-Fos subunit, which binds directly to the SIRT6 promoter. This connection

was established in hepatocellular carcinoma (HCC), where c-Fos-mediated SIRT6 transcriptional activation begins a tumor-suppressor pathway. The interaction of the transcription factor E2F1 with the SIRT6 promoter area, instead, inhibits SIRT6 production in both normoxia and hypoxia. Likewise, PARP1 appears to downregulate SIRT6 expression since treatment with its inhibitor PJ-34 increases SIRT6 mRNA levels (Chang et al., 2020).

The microRNA system also influences SIRT6 expression and function. For instance, MiR-33a, miR-33b, and miR-34a have been demonstrated to reduce SIRT6 mRNA and protein levels in many cell types. Furthermore, SIRT6 and miR-122 both adversely control their expression. The most prevalent hepatic miRNA, miR-122, binds to the 30-UTR of SIRT6, lowering its levels, whereas SIRT6 downregulates miR-122 via H3K56 deacetylation at its promoter. SIRT6 and miR-122 influence the transcription of the same genes implicated in metabolism and fatty acid oxidation in different directions. Similarly, SIRT6 and miR-125b decrease each other's expression, and miR-125b has been found to interact with SIRT6's 30-UTR, directly reducing its expression. However, the modifications and interactions with other proteins also influence SIRT6 functions a post-transitional level (Teixeira et al., 2019).

3.3 SIRT6 as a tumor suppressor

Several studies demonstrate that SIRT6 expression is lowered in multiple forms of cancer, indicating that this enzyme has a tumor suppressor role. Furthermore, several SIRT6 point mutations were detected in malignancies, altering, and frequently impairing its biological and enzymatic capabilities, eventually leading to metabolic alterations and transformation (Fiorentino et al., 2021). Evidence showed that SIRT6 has a tumor suppressor function in different forms of cancer, including hepatocellular carcinoma, lung cancer, and nasopharyngeal carcinoma, where

SIRT6 has been discovered to be downregulated at the gene level when compared to normal tissues (Tasselli et al., 2017).

SIRT6 overexpression inhibits cancer development in HepG2 cells by inhibiting ERK1/2 signaling and enhances apoptosis by increasing cleaved caspase-3 levels. A similar effect was reported in ovarian cancer tissues where SIRT6 expression levels are also lower than in non-transformed tissues. Upregulation of SIRT6 lowers proliferation of cancers and NOTCH3 expression in ovarian cancer cells, which is a prognostic indicator for serous ovarian carcinoma. SIRT6 also inhibits tumor cell growth in glioma cells by reducing poly(C)-binding protein 2 production via H3K9 deacetylation at its promoter. Reduced levels of SIRT6 were found in hepatocellular cancer cells, however, the levels of acetylated PKM2 at residue K433 were high (Fiorentino et al., 2021). In human colon cancer, a reduction in USP10 (a deubiquitinase protein) and SIRT6 expression is discovered. Bhardwaj et al. discovered that SIRT6 performs as a tumor repressor in the case of hepatocellular carcinoma (HCC) by deacetylating nuclear pyruvate kinase M2 (PKM2), limiting the proliferation of the cancer cells along with tumorigenesis (Desantis et al., 2018).

3.4 Role of SIRT6 in DNA Damage by Chromatin Signaling and DNA repair mechanism

The most destructive kind of DNA damage is double-strand breaks (DSB). SIRT6 uses a tunnel-like structure, providing an excellent affinity for DSB to detect DNA damage directly. In the absence of signals or recognized sensors, it migrates to damaged areas. SIRT6 stimulates DSB to repair downstream signaling by increasing H2AX phosphorylation, protein recruitment, and ATM recruitment from the homologous recombination as well as nonhomologous end-joining pathways. SIRT6 plays an elusive role as a DNA damage detector, which is crucial for activating the DNA damage response (DDR). SIRT6 activates the DDR before deciding on a repair route, preventing

genomic instability, even though several sirtuins have DSB-binding and DDR activation potential (Onn et al., 2020).

SIRT6 comes fast in the sites of damage bringing a chromatin to moderator sniff to age, allowing chromatin opening and the recruitment of downstream proteins. During DNA damage response, while double strand breaks occur, the first proteins to arrive within seconds are sensors that can belong to the MRE11, KU8o, and PARP1. Then these proteins can amplify the signals through kinases and many other proteins and give the cellular response that will go from replication, cell cycle checkpoints, repair, as well as senescence and apoptosis (Onn et al., 2020).

However, experiments have shown that SIRT6's core domain is insufficient for controlling the protein's proper placement and catalytic performance in cells. This discovery emphasizes the importance of additional SIRT6 components, including the NH2- and COOH-terminal extensions, called NTE and CTE, respectively. SIRT6 chromatin association and its H3K9 and H3K56 deacetylase activity *in vitro* need this NTE. According to several experiments, NTE is required for nucleosome binding and chromatin interaction in cells *in vitro*. Concurrently, the CTE has a diametrically opposite function of the NTE. It is more engaged with nuclear localization rather than deacetylation (Onn et al., 2020).

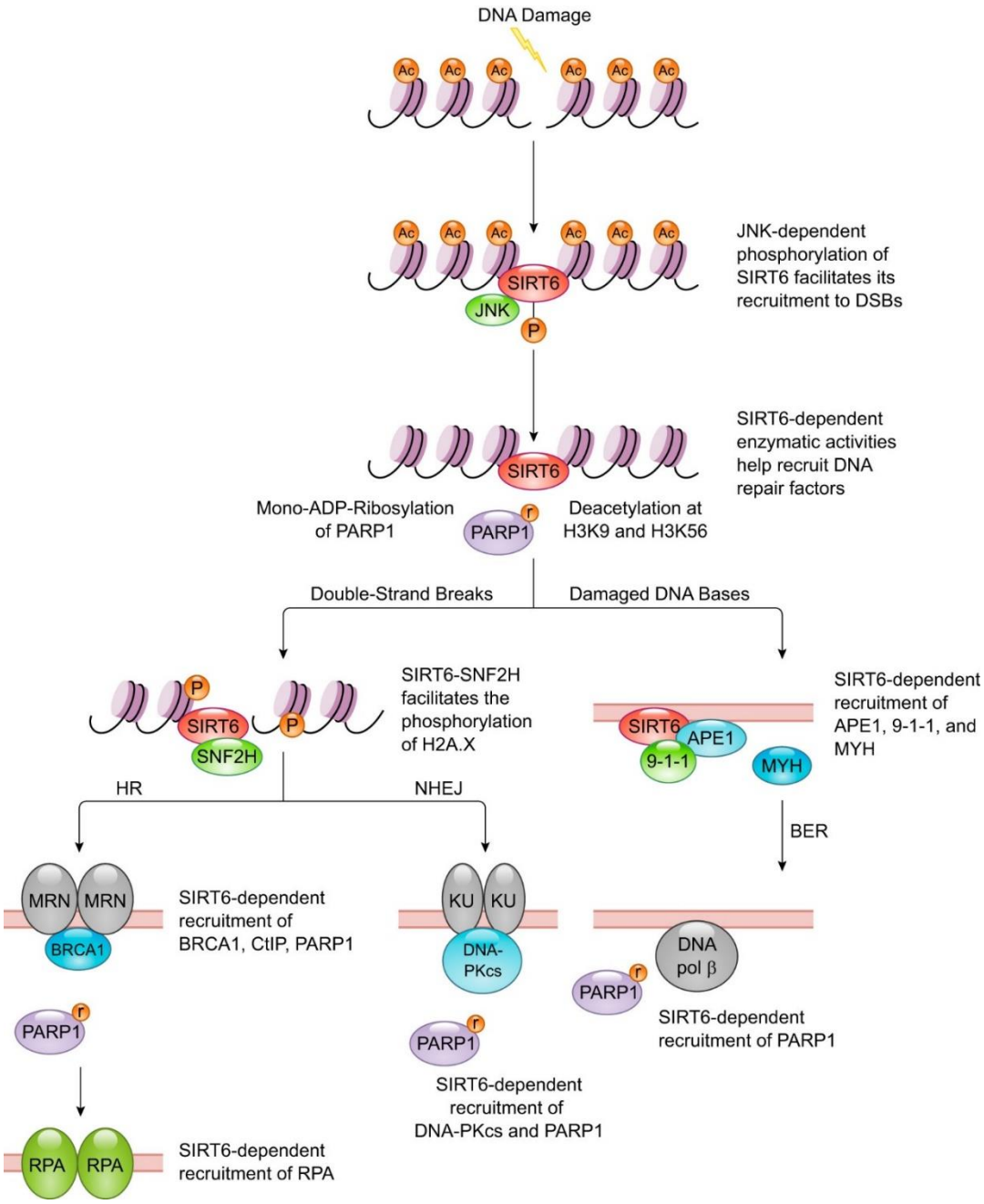

Figure 9: SIRT6 in DNA damage response (Chang et al., 2020).

SIRT6 has been shown to have various effects on genome integrity and DNA repair. It can bind directly to DNA. Studies from different research showed that SIRT6 cannot bind to closed DNA, which is not open-ended, but it can bind to various substrates such as – single strand DNA, sticky strand DNA etc. However, SIRT6 has the highest affinity for single strand DNA. SIRT6

recruitment to double-strand breaks is aided by JNK-dependent phosphorylation concerning genotoxic stress related to DNA damage (DSBs). H3K9 and H3K56 deacetylation are dependent on SIRT6, which works on damaged sites. Moreover, PARP1, ADP-ribosylation help recruit the DNA repair components (Tasselli et al., 2017). SIRT6 also employs numerous downstream repair components, depending on the kind of damage aiming to stimulate homologous recombination (HR), non-homologous end-joining (NHEJ), or base excision repair (BER) (Chang et al., 2020).

Chapter 4: SIRT6 in High Grade Serous Ovarian Cancer Suppression

SIRT6 has been shown in recent research to have an essential function in preventing HGSOC. A comparative analysis was performed using real-time PCR and western blot to compare SIRT6 protein and mRNA levels in HGSOC cells and normal tissues. The portion of SIRT6 was lower in the cancer tissues than the normal ones. However, to understand the molecular mechanism underlying the inhibitory effects of SIRT6 on HGSOC cells, SKOV3 cells are examined (Xiu et al., 2021).

4.1 SIRT6 inhibits the proliferation of ovarian cancer cells by suppressing regulation of NOTCH3

NOTCH dysregulation is a common occurrence in HGSOC. NOTCH3 overexpression was prominent among all other NOTCH receptors in HGSOC. This enzyme is expressed dramatically in more than half of HGSOCs among women (Chen et al., 2010). However, the deregulated NOTCH3 expression affects tumor recurrence, resistance to chemotherapeutic treatments, and poor prognosis (Perez-Fidalgo et al., 2020).

NOTCH3 is critical for the control of CSCs and platinum resistance. Researchers discovered that upregulating NOTCH3 in HGSOC cells leads to the maturation of CSCs and increased platinum chemoresistance. Furthermore, inhibiting NOTCH3 using GSI or siRNA transfection removes CSCs and enhances cancer susceptibility to platinum (Takebe et al., 2014). However, cisplatin and GSI all together made the only treatment strategy efficiently depleting both CSCs and tumor cells.

As a result, researchers figured out that inhibiting the NOTCH3 signaling system might be a valuable method for conquering platinum resistance and sensitizing resistant cells to chemotherapeutic drugs in HGSOC (Inder et al., 2017).

NOTCH3 activity decreases survival rate, upregulates adhesion gene expression, and increases tumor cell affinity. Several studies suggest that gene amplification and epigenetic activation of the NOTCH3 promoter in response to environmental signals may contribute to NOTCH overexpression in those cancers (Chen et al., 2010). The primary NOTCH3 ligand associated with HGSOC is Jagged1, which is also upregulated in HGSOC (Chen et al., 2010).

The most often utilized treatment strategy for suppressing NOTCH signaling in cancer is GSIs, which disrupt the S3 cleavage of the NOTCH receptor. The lack of selectivity among the GSIs disrupts the processes related to other transmembrane proteins. Regardless of their usage in suppressing the NOTCH signaling pathway, severe adverse effects can be associated with these GSIs, revealed in different preclinical studies (Ceccarelli et al., 2019).

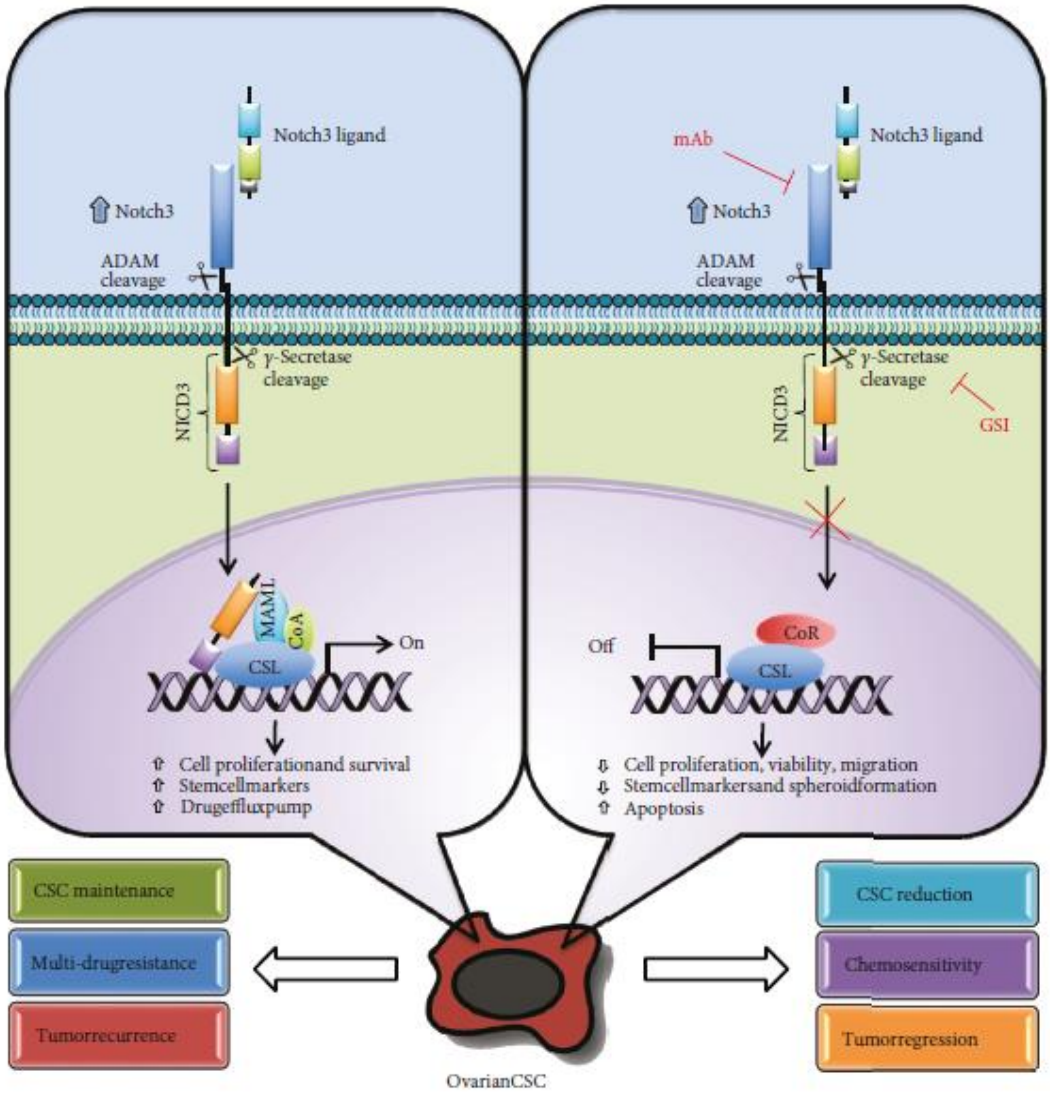

Figure 10: NOTCH3 signaling pathway in HGSOC cells (Ceccarelli et al., 2019)

The effect of SIRT6 overexpression on the NOTCH3 system is investigated to see whether NOTCH3 plays a role in SIRT6 function. Consequently, PCR and western blot investigations in SKOV3 cells exhibited higher SIRT6 expression decreased NOTCH3 expression (Ceccarelli et al., 2019).

On the contrary, downregulation of SIRT6 showed elevated results for NOTCH3 at protein and mRNA levels (Rahman et al., 2012). These results concluded that SIRT6 is inversely proportional to the NOTCH3 levels in SKVO3 cells. Along with NOTCH3, studies have shown that activation of the Wnt/β-catenin signaling pathway has been linked to the lower chemotherapeutic response of CSCs (Takebe et al., 2014).

4.2 SIRT6 inhibits the Wnt/β-catenin signaling pathway

The signaling pathway allocated for controlling cell proliferation, stem cell fate, and survival is the Wnt/β-catenin pathway. The Wnt pathway is dormant in most normal cells. The protein β-catenin, GSK3, axin, APC, CK1α, and others form the destruction complex in the cytosol. βTrCP is a ubiquitin ligase that binds to β-catenin and transfers short ubiquitin peptides. The proteasome is a collection of proteases that binds and destroys ubiquitinated β-catenin. As a result, only a small amount of cellular β-catenin is produced. A transcription factor from the TCF-LEF family and other proteins like Groucho attaches to DNA in the nucleus and suppresses gene expression (Bae et al., 2018).

The extracellular factor Wnt binds to the membrane receptor frizzled or FZD and its co-receptor LRP to activate the Wnt pathway in a normal cell. Binding activates the cytosolic protein DVL, which causes the destruction complex to dissociate. Since ubiquitin peptides no longer modify β-catenin, they cannot destroy it. The amount of β-catenin increases as the supply of β-catenin continues to rise in cytosol and nucleus. β-catenin, along with other proteins, attaches to the TCF-LEF transcription factor in the nucleus, causing it to switch from a transcriptional repressor to an activator. TCF-LEF induces gene transcription by activating an RNA polymerase. Genes coding for MYC and cyclin D1 are two examples of crucial genes triggered by the active Wnt pathway (Nguyen et al., 2019).

The Wnt pathway can be activated in tumor cells even without a Wnt signal. A mutation has been found in a gene that codes for a protein of the destruction complex. As a result, the complex disintegrates, and β-catenin can no longer be targeted for destruction. Consequently, the amount of β-catenin rises. This condition is similar to when a Wnt factor activates the pathway in a normal cell. TCF-LEF transcription factor is activated by β-catenin in the nucleus. Like a normal cell, TCF-LEF initiates an RNA polymerase that starts the transcription of many genes after being triggered by a Wnt factor (Nguyen et al., 2019). A Wnt signal can potentially activate the Wnt pathway in a tumor cell in an unintended manner. Because aberrant activation of this Wnt pathway can lead to β-catenin hyperactivity (Axin, APC mutations) in HGSOCs, targeting this pathway while developing new therapeutics is important (Chen et al., 2010).

The Wnt/β-catenin pathway has been proven to promote cancer growth in HGSOC in studies. Downregulation of SIRT6 inhibits this process and, as a result, slows the growth of OC. The mechanism behind SIRT6's downregulation of β-catenin activity is not entirely understood. According to several studies, the downregulation of SIRT6 in ovarian cancer cells lowered the production of Wnt/β-catenin signaling, which suppressed the formation of HGSOC (Nguyen et al., 2019).

4.3 SIRT6 involves in the invasiveness of HGSOC cells activating EMT-related signaling pathway

The epithelial-to-mesenchymal transition (EMT) is vital in cancer development and among the most critical mechanisms in tumor metastasis. During this phase, cells stop expressing epithelial markers and start expressing mesenchymal markers, reducing cell-cell binding, and enhancing migratory, invasive, and stem cell characteristics. Tumor invasion, metastasis, and embryonic development require EMT (Loret et al., 2019).

EMT has a role in HGSOC treatment resistance and stemness, making it a promising therapeutic target. Furthermore, the activity of EMT signaling molecules, for example - β-catenin, snail, vimentin, E-cadherin, and N-cadherin, is associated with the invasiveness of HGSOC cells mediated by SIRT6. E-cadherin, a transmembrane protein that interacts with other E-cadherin molecules, keeps HGSOC cells connected to the epithelium (Sawada et al., 2008).

Although SIRT6 expression had no effect on MMP2/MMP9 expression, it did reduce E-cadherin expression while increasing the expression of other transcription factors like - active β-catenin, N-cadherin, vimentin, and snail (Liu et al., 2021). As a result, some findings revealed the involvement of SIRT6 through EMT in HGSOC.

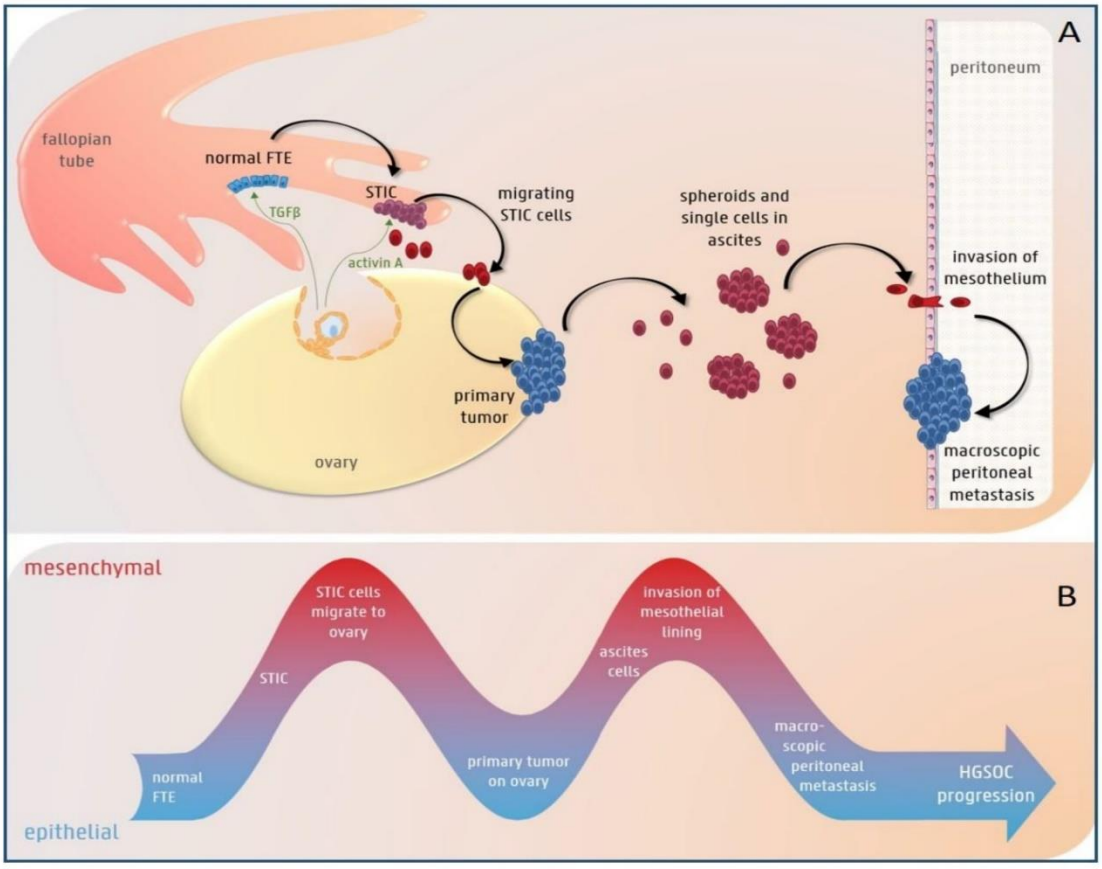

Figure 11: Epithelial-to-mesenchymal (EMT) role in HGSOC (Loret et al., 2019).

In cancer development, EMT is highly related because of the molecular characteristics. Therefore, SIRT6 and active β-catenin expression via EMT might be involved in HGSOC. Regardless of their involvement, a controversial hypothesis claims SIRT6 is a suppressor of EMT in other forms of cancers. As a result, more research is required to determine the precise connection of SIRT6 in HGSOC invasiveness (Loret et al., 2019).

4.4 SIRT6 controls aerobic glycolysis in high-grade serous ovarian cancer cells

Cancer cells, unlike normal cells, may conduct glycolysis and transform glucose to lactate in the absence of oxygen, a process known as the 'Warburg effect' or 'aerobic glycolysis.' Glucose transporter 1 (GLUT1), pyruvate kinase-M2 splice isoform (PKM2), hexokinase 2 (HK2) and lactate dehydrogenase (LDH-A) are only a few of the glycolytic proteins implicated in cancer.

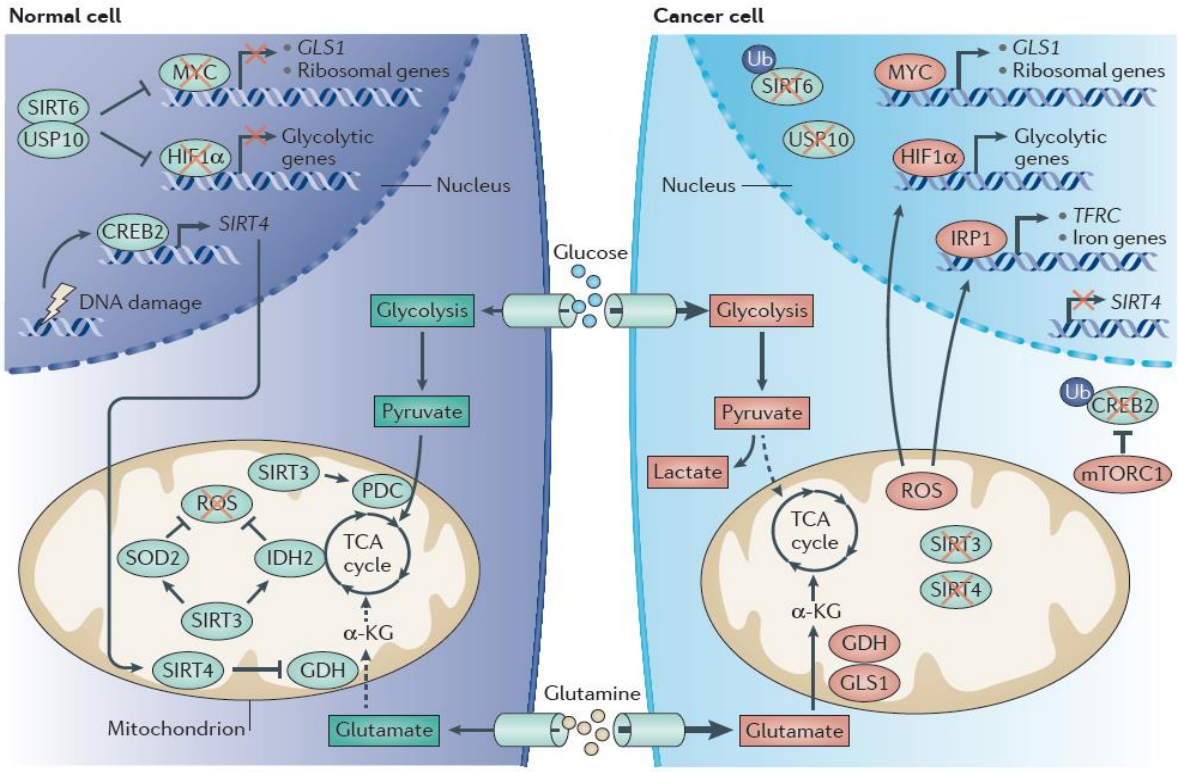

Figure 12: The role of SIRT6 in the regulation of cancer Metabolism (Chalkiadaki & Guarente, 2015).

SIRT6 regulates several glycolytic genes, which contributes to the Warburg effect. Loss of this epigenetic regulator SIRT6 promotes ovarian cancer cell growth by increasing glycolysis (Chalkiadaki & Guarente, 2015).

An *in vivo* study found that SIRT6 deficiency enhanced glucose fermentation as well as HGSOCs development. Inhibiting glycolysis reduced cancer development in SIRT6-deficient cells, suggesting glycolysis upregulation is involved in SIRT6 loss-induced carcinogenesis. Higher HIF-1α activity, increased glucose absorption, overexpression of essential glycolytic genes such as GLUT1, PFK1, and LDH, and reduced mitochondrial respiration were all seen in SIRT6 deficient cells (Sebastián et al., 2012).

One of the potential treatment options for HGSOC control is Warburg effect suppression. The main disadvantage of this method is that along with limiting glycolysis in cancer cells, it will affect normal cells as well that generate energy via this process. Thus, a powerful technique or method to limit glycolysis only in cancer cells without impacting normal cells can guide to more appropriate treatment.

Chapter 5: SIRT6 as a potential target for high-grade serous ovarian cancer treatment

SIRT6 is becoming a viable target for developing small-molecule activators and inhibitors that might be useful for treating cancer. Furthermore, particular modulators allow for a closer examination of the molecular complexities of SIRT6 function, further validating the enzyme as a pharmaceutical target in order to treat HGSOC. However, many aspects between SIRT6 biochemical activity and observable phenotypes in both healthy and pathological situations remain unknown; hence, future research should focus on discovering novel SIRT6 substrates and understanding their molecular interactions (Lisio et al., 2019).

5.1 Pharmacological modulation of SIRT6 to treat high-grade serous ovarian cancer

The structures of SIRT6 showed unique properties, involving a splayed zinc-binding domain and the lack of a helix bundle, which links the zinc-binding motif and Rossmann fold domain in other six sirtuin structures. SIRT6, on the other hand, contains a single steady helix rather than a highly flexible NAD+ binding loop. These differences made the researchers settle that SIRT6 can bind NAD without an acetylated substrate, unlike all other sirtuins. Their studies also showed that SIRT6 restrains NAD with a relatively high affinity without an acetylated substrate. This feature along with the presence of a helix instead of a cofactor binding loop explains the ordered structure even when the acylated peptide is absent (Pan et al., 2011).

SIRT6 activators have been developed in response to the significance of SIRT6 as a tumor suppressor in many malignancies, while SIRT6 inhibitors have also been developed in consideration of SIRT6's double involvement in cancers. Furthermore, effective and particular SIRT6 modulators (activators and inhibitors) permit a closer examination of the enzyme's

molecular features and further validation of the enzyme as a pharmaceutical target (Fiorentino et al., 2021).

5.1.1 SIRT6 Activators

An anticancer drug, Icariin, has been found to suppress the growth of HGSOC cells by increasing SIRT6 expression via many signaling pathways, including PI3K/AKT, Wnt/β-catenin, STAT3, mTOR/PI3K/AKT, and PTEN/AKT/HIF-1α. Icariin is mainly a plant-derived flavonoid glycoside that has been found to inhibit the proliferation of ovarian cancer in A2780 cells. According to several studies, the overexpression of miR-21 enhances the proliferation of HGSOC cells by negatively regulating PTEN and RECK cascades while favorably handling Bcl-2 expression (Li et al., 2015).

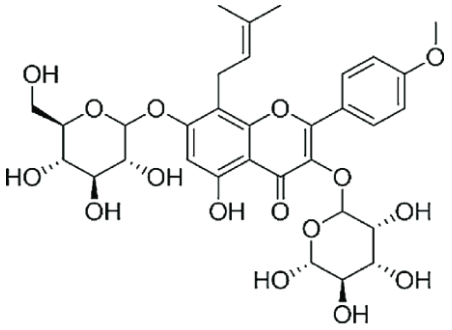

Figure 13: Structure of Icariin (Li et al., 2015)

These results indicate that icariin may have a curative anticancer effect in A2780 cells by reducing cell explosion and promoting cell death via miR-21 along with the target genes, including - PTEN, Bcl-2, and RECK. However, further investigation is needed for understanding the molecular mechanisms of Icariin in HGSOC (Fiorentino et al., 2021).

The deacetylase activity enhanced by free fatty acids (FFA) containing 14 to 18 carbons shows SIRT6 activity. Studies have shown that myristic acid (1a), oleic acid (1b), and linoleic acid (1c)

can boost SIRT6's affinity for acetylated substrates. Flavonoids with a benzene ring (A) linked to a heterocyclic pyran ring (C), on the other hand, have a phenyl group (ring B) in position 2. The hydroxyl groups present on the 5th and 7th carbon in ring A are present in these combinations (3a, 3b, 3c). Quercetin (3b) and Luteolin (3a) both show inhibitory activity at low concentration but increase the deacetylase activity at higher concentrations.

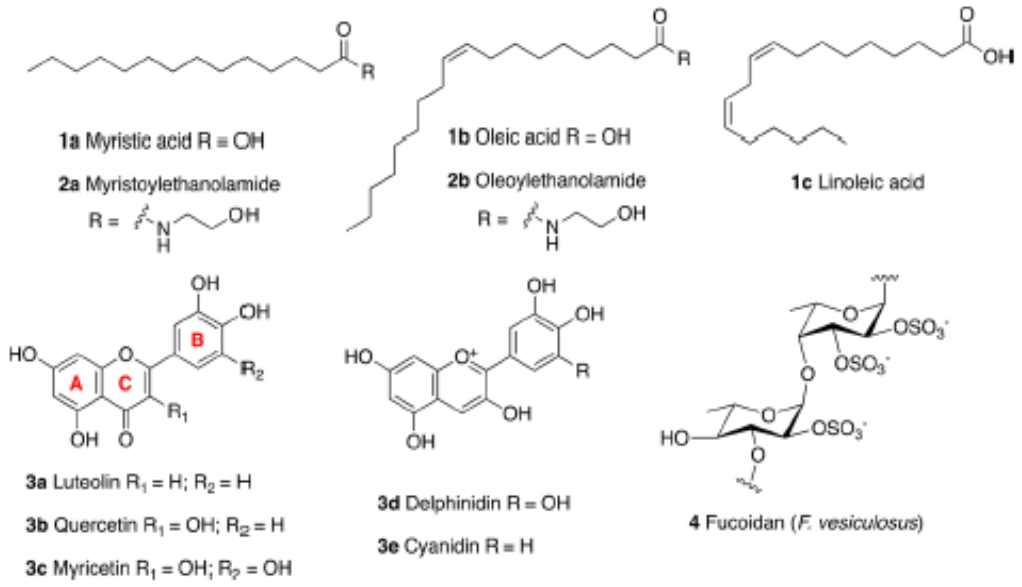

Figure 14: SIRT6 activators based on natural products (Li et al., 2015)

Small compounds may engage with an inhibitory site at small doses while creating favorable structural changes that trigger SIRT6 at higher doses, according to the findings of the study. Bearing the same structure as 3b, flavonol myricetin (3c) also shows a higher affinity for SIRT6 activation containing an additional hydroxyl group in position 3'. Because of the absence of a carbonyl group in the 4th position of B Ring, flavonoid Anthocyanidin has lower stimulating potential. The deletion of the 3' hydroxyl group, on the other hand, can increase the activation efficiency of 3e.

Table 1: Most Relevant and Specific activators of SIRT6

Compound	***In vivo* effects from different studies**	**Reference**
Icariin	In A2780 cells: miR-21 and its target genes inhibited cell growth while increasing apoptosis.	(Li et al., 2015)
1a. Myristic acid		(Feldman et al., 2013)
3e. Cyanidin	In Caco-2 cells: SIRT6 upregulation, FoxO3α upregulation, Twist1 and GLUT1 downregulation.	(Rahnasto-Rilla et al., 2018)
5 UBCS039	In colon, NSCLC and epithelial cervix malignancies: SIRT6 activation, H3K9 and H3K56 acetylation decreased.	(You et al., 2017)
7c MDL-811	In CRC cell lines: Reduction of H3K9Ac, H3K56Ac, H3K18Ac, CRC growth suppressed.	(Shang et al., 2020)

Though these 3a-e compounds showed results of SIRT6 activation, they can also interfere with several bioassays, for which, more biological studies must be needed for these compounds. Nonetheless, flavonoids can be powerful hit compounds for developing SIRT6 activators as shown with compound 3b and 3e. Both these compounds showed higher affinity for the activation of SIRT6 since the catechol component is placed into the acyl binding pocket. The 4′-hydroxyl group forms a hydrogen link with the Pro62 backbone oxygen and a conserved water molecule. The linking of these molecules creates a hydrogen connection with the Ala53 and Ile61 backbone oxygens. Compared to 3b, the carbonyl group associated with Met136/157 is missing in 3e, making it more potent. Overall, these approaches may allow to produce compounds with higher selectivity

and potency. Fucoidan (4), a brown algae-derived heterogeneous sulfated polysaccharide, is another naturally occurring compound that activates SIRT6. Researchers showed that sulfate esters of this compound play a central role while interacting with SIRT6 and results in SIRT6 activation. Further research is needed to collect more kinetic data to explain the SAR (structure-activity relationship) (Fiorentino et al., 2021).

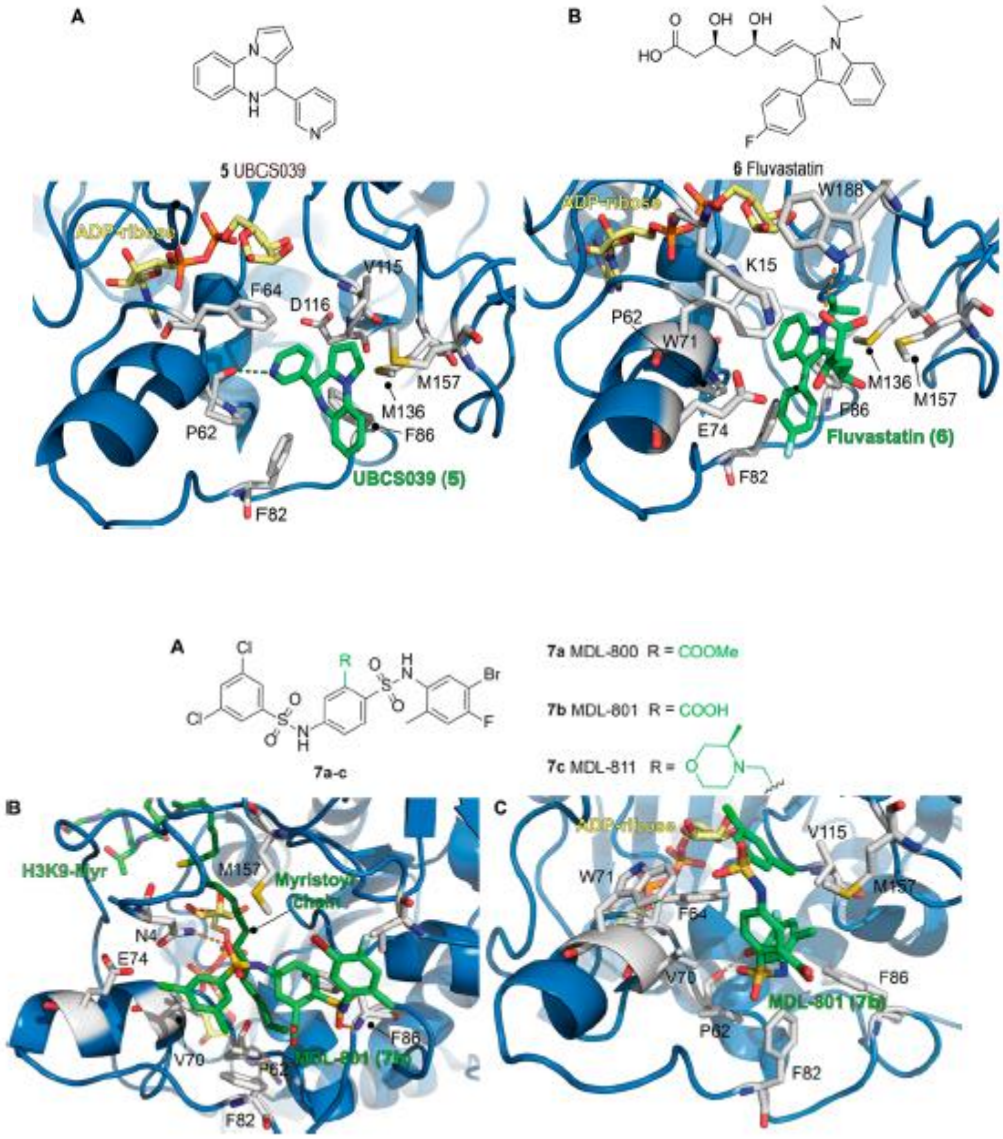

Figure 15: More SIRT6 activators (Fiorentino et al., 2021).

Besides these naturally occurring activators, there are some synthetic SIRT6 activators, among which UBCS039 was initially being developed by deriving pyrrolo[1,2-a]quinoxaline. UBCS039 showed specific binding on SIRT6, exhibiting a greater activation of the enzyme. The binding mechanism of this compound was comparable to that of 3b and 3e. One exception is that 3b contains a carbonyl group positioned near Met136/157 that could interfere with the aromatic ring's ideal hydrophobic interactions with the methionine residues. Compared to 3b, the 5 tricyclic structure (UBCS039) is better positioned for aromatic and hydrophobic interactions with Met136/157, which justifies its higher potency. In addition, studies have shown that UBCS039 activates SIRT6 in several cancer cell lines, suggesting that it might be used to target HGSOC cells (Fiorentino et al., 2021).

New SIRT6 activators, MDL-800 (7a) and MDL-801 (7b) showed enhancing the activity of SIRT6 in a study. These compounds are derived from N-phenyl-4-(phenylsulfonamido)-benzenesulfonamide. Intriguingly, 7b connects with SIRT6 through a distinctive pocket, with a carboxylic group replacing the central benzenesulfonamide. Compound MDL-811 (7c) is found by substituting an N-methyl-3-methylmorpholine in the benzene ring of 7a, which demonstrated an increased activity and bioavailability in mice, showing the specificity for SIRT6 (Fiorentino et al., 2021).

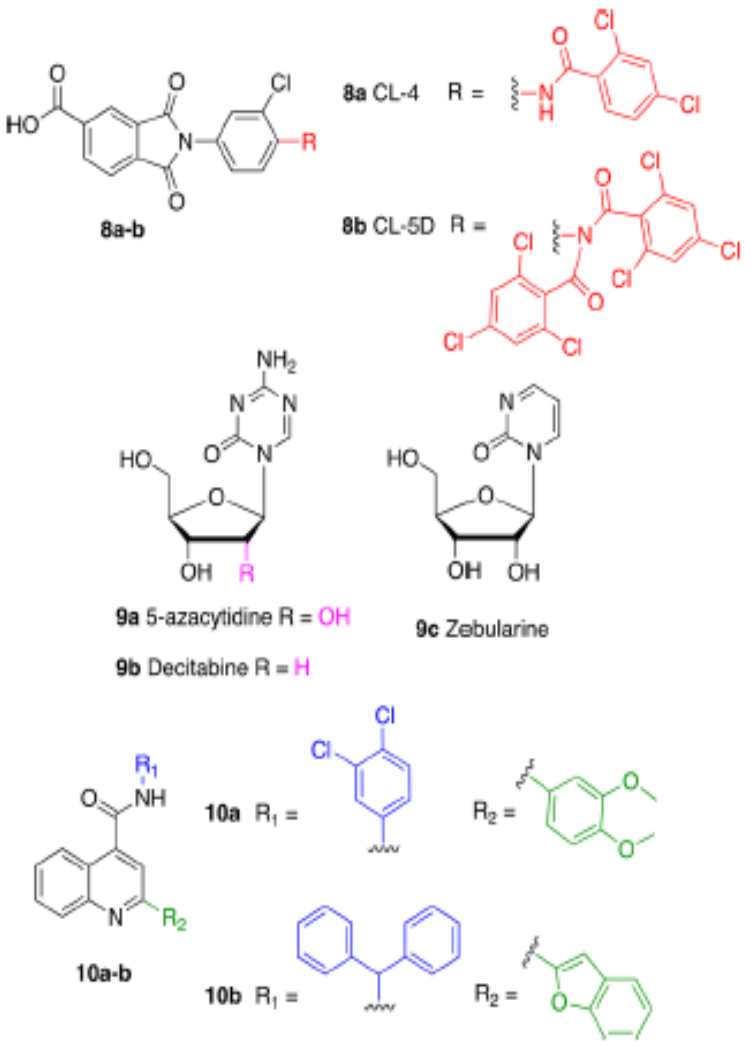

Figure 16: Synthetic SIRT6 activators (Fiorentino et al., 2021)

Lipid-like molecules also showed activation of SIRT6 deacetylase activity, e.g., 8a representing an idea lead compound for developing selective SIRT6 activators. Moreover, 8b has shown more potency over 8a due to the lack of chlorine atoms in the aniline nitrogen. These chlorine atoms can be released from 2,5-dichlorophenyl moiety or even the trichlorobenzoyl group. 9a, 9b, 10a, 10b are some of the further SIRT6 activators which need more specific data along with functional and target engagement assays to clarify the activities of these compounds.

5.1.2 SIRT6 Inhibitors

The dual involvement of SIRT6 in HGSOC indicates that inhibition of SIRT6 in specific conditions may help to build a beneficial cancer control technique. Indeed, SIRT6 inhibitors may target a variety of SIRT6-mediated processes contributing to ovarian cancer growth.

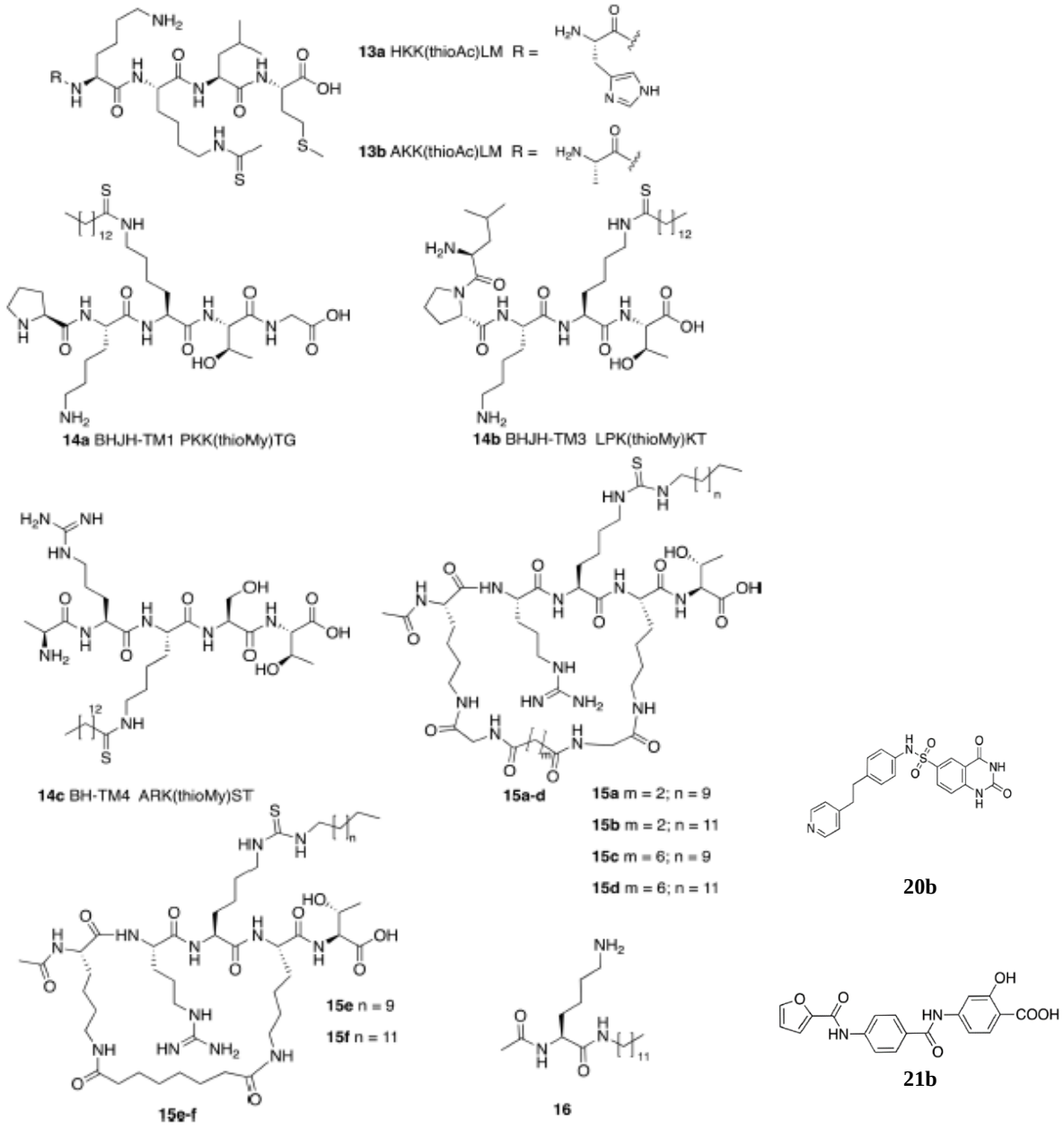

Figure 17: SIRT6 inhibitors (peptide-based) (Fiorentino et al., 2021)

Research shows that replacing a histidine (His) residue enhances SIRT6 inhibitory action. An Ala residue can replace it to achieve this activity. Although these studies have shown higher inhibitory activity and become the first synthetic SIRT6 inhibitors (13a-b), they are not selective towards SIRT6.

Table 2: Most Relevant and Specific inhibitors of SIRT6

Compound	***In vivo* effects from different studies**	**Reference**
14b BHJH-TM3	In HEK293T cells: SIRT6 inhibition, TNF-α fatty acylation increased.	(He et al., 2014)
15f	No *in vivo* effects due to poor permeability but showed high selectivity towards SIRT6.	(Liu & Zheng, 2016)
20b	In BxPC3 cells, H3K9 acetylation increased, augmented glucose uptake in L6 rat myoblasts.	(Sociali et al., 2015)
21b	TNF-α levels reduced.	(Damonte et al., 2017)

Among the peptide compounds (14a, 14b, 14c), 14b being the potent one showing inhibitory activity as well as increasing of TNF-α fatty acylation. Despite being highly selective towards SIRT6, 15f could not show inhibitory activity in human pancreatic cancer cells lines due to their poor cellular permeability resulting from their peptide character and increased molecular weight. Regardless of the shortcomings of the peptide inhibitors, these compounds can be used as lead compounds for developing further peptidomimetics, which will inhibit SIRT6 (Liu & Zheng, 2016).

When oxygen was removed from the sulphonamide side chain, and the aliphatic spacer between the aromatic groups was extended, the quinazolinedione derivative 20b showed improved inhibitory efficiency (Liu & Zheng, 2016). In addition, Furan-2-carboxamide being present in the para position of the compound 21b, dramatically boosts SIRT6's inhibitory action. Recent studies explained another series of 1-phenylpiperazine derivatives to be a SIRT inhibitor. Ultimately, these inhibitors must be selective against the SIRT6 to perform accurately to become lead compounds for HGSOC (Liu et al., 2021).

5.2 Therapeutic activation of SIRT6 to treat high-grade serous ovarian cancer

Even though increased understanding of SIRT6 activity has revealed multiple functions in human diseases, identifying effective and promising SIRT6 modulators is still in progress. SIRT6 is prominently expressed in human ovarian tissue, and it promotes chemotherapeutic resistance while suppressing proliferation and differentiation, as earlier mentioned. As a result, SIRT6 may be an appealing target for developing novel anticancer drugs for HGSOC to be employed alone or in combination with chemotherapy or radiation (Chandra et al., 2019).

Recent studies indicating the regulatory mechanism and experimental verifications of Icarrin in the treatment of HGSOC explained that Icariin can inhibit the P13K/AKT signaling pathway and ultimately suppress the cancer progression (Li et al., 2015). Though this natural product showed its activity in SKOV3 cells as well as A2780 cells, a deeper understanding is required to offer Icariin an efficient method for treating HGSOC (Wang et al., 2020).

Several investigations also revealed the existence of a SIRT6 activator-bound allosteric surface site at the outflow of the huge hydrophobic SIRT6-channel pocket. The only known activator of SIRT6 deacetylase was UBCS039. However, free fatty acids, along with this activator, did not

involve in reversing the binding of MDL801. This research proves that SIRT6 activities can be modulated by different mechanisms (Sociali et al., 2019).

Chapter 6: Conclusion & Future Prospects

Conclusion

Increasing evidence demonstrates the function of SIRT6 as a prognosis biomarker in targeting HGSOC. Although there are controversial outcomes on the role of SIRT6 in HGSOCs, this review summarizes all the possible involvements of SIRT6 in ovarian cancer cells. Taken together, investigations from different studies suggest that SIRT6 acts as a tumor suppressor by repressing NOTCH3 and Wnt/β-catenin pathways, indicating that SIRT6 depicts a potential therapeutic target for the treatment of HGSOC. However, the involvement of SIRT6 with EMT is conversely reported in some studies that SIRT6 is related to tumor progression in some cases via enhancing EMT-related invasiveness of HGSOC cells. Therefore, these findings imply that SIRT6 is involved in the development of HGSOCs and that activating or inhibiting it in some cases might be a novel therapeutic stratagem for HGSOC treatment.

Future Prospects

Over the past few years, even though rising understanding about SIRT6 biology has revealed multiple activities in human diseases, identifying effective and selective SIRT6 modulators is still in its early stages. Future studies will provide novel insights for additional targets of SIRT6 and their relevance to HGSOC. SIRT6 has an essential role in developing and progressing many gynecological conditions. Its involvement is incredibly crucial and extensively documented in cancer development in female reproductive organs; nevertheless, it is also intensively explored in terms of alterations detected in the ovary and oocyte. Furthermore, SIRT6 plays a role in various gynecological diseases as regulatory pathways linked to insulin resistance and glucose and lipid metabolic concerns.

Given its critical role in homeostasis, it is evident that SIRT6's activity governs cancer initiation and progression. Since the p53 gene is usually mutated in most cancer cells, targeting p53-dependent pathways can be suggested to target first. Several natural products derived from chromenone, such as - quercetin and vitexin, can be used as SIRT6 modulators. Besides these chromone-derived compounds, several polyphenols along with flavonoids, chalcones etc., have been demonstrated to have stimulatory or inhibitory effects on SIRT6.

Studies indicate that these natural products have undergone various clinical trials to validate SIRT6 as a tumor suppressor towards drug development to treat HGSOC. Considering these, it can be predicted that SIRT6 will become a primary target for treating such deadly metastatic cancers soon.

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