The dual roles of SIRT1: a systematic review.

Lê Nguyễn Tường Minh[1]

1 – Le Hong Phong High School for the Gifted.

Abstract

- ***Background:*** Sirtuins belong to the class III histone deacetylases (HDACs) and are NAD+-dependent enzymes. They are responsible for various cellular processes and pathways, namely cell survival, development, inflammation, aging, metabolic control, and apoptosis. SIRT1, specifically, has been reported to act as a tumor suppressor gene and an oncogene in cancer progression.
- ***Objective:*** This paper aims to understand the driving factor(s) that contribute to SIRT1's oncogenic and tumor-suppressing activities.
- ***Method:*** A systematic literature search is performed utilizing the PRISMA guidelines. This search is conducted separately for SIRT1 as an oncogene, using keywords "SIRT1," "cancer," and "oncogene," and SIRT1 as a tumor suppressor gene, with the keywords "SIRT1," "cancer," and "tumor suppressor." After screening and assessing eligibility with various criteria, 44 papers are included in this review.
- ***Results:*** Out of the 44 papers, 30 supported the oncogenic role of SIRT1. Most of these papers showed that the natural upregulation of SIRT1 in cancer cell lines has a positive correlation with tumorigenesis. Additionally, this upregulation can cooperate with other family proteins, such as FOXO or signaling pathways, like the NF-κB signaling pathway, stimulating the aforementioned transformation. 14 out of the 44 papers suggested a tumor suppressor function for SIRT1. Specifically, its anti-cancer properties seem to be enhanced in the presence of an activator; in this cellular context, SIRT1 frequently induces cell apoptosis. Alongside the induction of autophagy, SIRT1 can also inhibit tumorigenesis. In general, SIRT1 often displays contradictory roles in common solid tumors such as colorectal, breast, and liver. In liquid tumors such as leukemia, SIRT1 seems to be an oncogene.
- ***Conclusion:*** In this review, it was found that overexpression of SIRT1 has an oncogenic effect on cancer cell lines unless activated via an activator. Therefore, SIRT1 activators are prospective agents in targeting SIRT1 as a potential therapeutic treatment against cancer.

Keywords: SIRT1, oncogene, tumor suppressor gene, cancer, signaling pathway.

Introduction

Cancer is the second leading cause of death globally and is a major health enigma in every nation (Weiss, 2021). It not only concerns the lives of the affected and their families, but also the economy of those individuals, and, on a larger scale, a nation. According to The Cancer Atlas – American Cancer Society, cancer puts major burdens on the economics of not only the nation, with the budget spent on healthcare systems and research, accounting for $200 billion annually, but also the people; an average person will spend an estimate of $150,000 on cancer treatment (Head et al., 2023; *The Cost Of Cancer | SERO*, 2022). Cancer cases are projected to surpass 35 million cases by 2050, approximately twice the number predicted in 2022 (*Cancer Cases to Rise Steeply by 2050*, 2024). Some of the most common treatments of cancer are surgical removal of tumors, radiotherapy, and chemotherapy; however, every treatment has detrimental health complications for patients, some of which are nausea, weight loss, appetite loss, nerve damage, unexplained bruising, muscle, and joint paint, and even death (Devlin et al., 2017; *Types of Cancer Treatment - NCI*, 2017). Additionally, the rise of radioresistant cancer types has rendered treatments such as radiotherapy, immune checkpoint therapy, and chemotherapy ineffective. This necessitates the development of treatments capable of overcoming radioresistant cancer (de Mey et al., 2021). Researchers are interested in understanding tumorigenesis through natural compounds or enzymes. They believe this understanding will facilitate more robust and effective treatments against cancer.

Recently, SIRT1 has been rising in popularity and has been noticed as a potential target and agent for therapeutic treatment for cancer. However, its dual roles have posed a challenge for researchers: the overexpression or repression of SIRT1 can either display oncogenic or tumor-suppressing properties on cancer cell lines. Additionally, SIRT1 can interact or cooperate with several protein families and signaling pathways to facilitate initiation or inhibition of tumorigenesis. For example, the deacetylase activities of SIRT1 can suppress the expression levels of known tumor suppressors such as p53, p73, and HIC1 and take on an oncogenic role. On the other hand, the upregulation of SIRT1 may suppress the activity of the NF-κB signaling pathway, pivotal to the initiation of tumorigenesis and induction of malignancy, hence, act as a tumor suppressor: inhibiting cell proliferation, migration, and invasion, and even induces cell apoptosis (Kiernan et al., 2003).

Sirtuins belong to the class III histone deacetylases (HDACs) and are NAD+-dependent enzymes (Li, 2014). There are seven isoforms of Sirtuin, from Sirtuin-1 to Sirtuin-7, in mammals (Turkmen, 2021). They are responsible for a variety of biological as well as pathological processes and pathways, namely inflammation, aging, metabolic control, and apoptosis. From a cellular standpoint, SIRT1 is crucial to cell survival through the regulation of transcriptional activities of p53, a non-histone substrate identified for the enzyme (X. Li, 2014). To that end, the function and dysfunction of the SIRT1-p53 axis are associated with the initiation or repression of a variety of cancers, such as breast, gastric, and colorectal cancer (Dilmac et al., 2022; Wang et al., 2021; Yao et al., 2022). The

overexpression of SIRT1 has been found to promote the proliferation and migration of cancer cells; on the other hand, inhibition of SIRT1 expression represses the apoptosis of cancer cells, and further tumorigenesis (Shin et al., 2023; Yarahmadi et al., 2019). Hence, SIRT1's dual role in tumor progression is unequivocal; however, its mechanism as a tumor suppressor gene or oncogene in different cellular contexts remains unclear.

- *SIRT1 – p53 axis.*

p53 is a non-histone substrate identified for SIRT1 in which SIRT1 targets p53 for deacetylation, hence, reducing p53 transcriptional activities (Luo et al., 2001; Vaziri et al., 2001). This interaction is dependent on the deacetylase activity of SIRT1 (Vaziri et al., 2001). Similar to SIRT1, the activation of p53 also plays dual roles in tumor progression: p53 activation can have apoptotic effects or, more rarely, induce tumorigenesis (van Leeuwen & Lain, 2009). p53 frequently has anti-cancer effects on cancer cell lines via the silencing of SIRT1 expression (Langley et al., 2002). On the other hand, the upregulation of SIRT1, which silences the activity of p53, has oncogenic effects (Z. Lin et al., 2012; Luo et al., 2001).

- *SIRT1 and cell apoptosis.*

SIRT1 interaction with various autophagy proteins corroborates the autophagosome formations: these proteins include Atg5, Atg7, and Atg8 (Geng & Klionsky, 2008; Lee et al., 2008; Salminen & Kaarniranta, 2009). SIRT1 induction of cell apoptosis is deacetylase dependent, so the silencing of SIRT1 expression increases the acetylation of the aforementioned autophagy proteins, resulting in the apoptosis of cancer cell lines (Lee et al., 2008).

- *SIRT1 in NF-κB signaling pathway.*

The NF-κB signaling pathway is pivotal to the regulation of immune responses and tumorigenesis; if dysfunctional, can result in cancer cell proliferation, migration, and invasion (L. Chen et al., 2002; Kiernan et al., 2003). The presence of SIRT1 in this signaling pathway promotes RelA/p65, a REL-association protein involved in the NF-κB signaling pathway, and Set9, a histone, interaction to enhance the methylation of p65, ultimately reducing transcriptional activities of the NF-κB signaling pathway (X.-D. Yang et al., 2010; Yeung et al., 2004).

- *SIRT1 and FOXO*

FOXO family proteins regulate cell differentiation, proliferation, and survival; if dysregulated, these proteins can promote carcinogenesis. SIRT1 is believed to regulate several cellular processes via the deacetylation of FOXO family transcription factors such as FOXO1, FOXO4, and FOXO3a (Motta et al., 2004; Nemoto et al., 2005; van der Horst et al., 2004; Yang et al., 2005). However, the mechanism of such interaction is still unclear as the activation of FOXO proteins via deacetylation by SIRT1 can either result in the initiation or inhibition of the proteins' transcriptional activities. Depending on the type of

cancer and cellular context, SIRT1 can cooperate with FOXO proteins to induce metastasis (Dilmac et al., 2022).

- *SIRT1 and TGF-β.*

TGF-β is a growth factor that regulates multiple cellular events such as cell migration, differentiation, growth, and apoptosis; it is also pivotal for a variety of physiological processes (Massagué, 1998; Massagué et al., 2000). The involvement of SIRT1 in this significant pathway takes place by deacetylating Smad3 and Smad 7, which are modulators of the TGF-β family signaling (Kume et al., 2007; J. Li et al., 2010). Specifically, the upregulation of SIRT1 will repress protein levels of Smad7 as well as attenuate induction of cell apoptosis via TGF-β: SIRT1 promotes Smad7 ubiquitination by deacetylation while enhancing Smurf1-mediated degradation (J. Li et al., 2010). However, in this cellular context, TGF-β controls the expression of SIRT1, inducing the aforementioned effects on various cell lines, such as lung fibroblast cells and parenchymal cells (B. Xu et al., 2012).

To further explore the specifics of these interactions, SIRT1 activators and inhibitors are employed to observe the role of the upregulation that SIRT1 may play in cancer progression. Most notably, resveratrol is a popular SIRT1 activator centered in a variety of studies on the roles of SIRT1 in cancer progression. It is a polyphenol known to have anti-cancer properties on cancer cells and a potent activator of SIRT1 (Baur & Sinclair, 2006; Pervaiz & Holme, 2009). The upregulation of SIRT1 via resveratrol is most commonly associated with significant cell apoptosis: several studies have shown that this interaction can induce autophagy up to 4-fold (Eroglu et al., 2020). In addition, a variety of novel activators of SIRT1 is being researched to have a more multi-faceted and comprehensive approach to therapeutic treatment against cancer, such as SRT1720 or CAY10602. Similarly, SIRT1 inhibitors can also be an agent beneficial for cancer treatment. This treatment often concentrates on the SIRT1/p53 axis; specifically, SIRT1 is downregulated to increase the acetylation of p53, a tumor suppressor protein, in order to induce anti-cancer properties on cancer cell lines: cell apoptosis, in particular (Langley et al., 2002). Various inhibitors including the tenovins and EX527 are implemented to induce the aforementioned anti-cancer effects (Lain et al., 2008; Solomon et al., 2006).

SIRT1 plays contradictory roles in cancer, evident in several findings (Deng, 2009; Lin & Fang, 2013; Sun et al., 2019; Bosch-Presegué & Vaquero, 2011). Until now, the specifics of these interactions require clarification as the mechanism of SIRT1 in tumor progression is ambiguous. Hence, this paper aims to define the margins that define the oncogenic and tumor-suppressing properties displayed by SIRT1 in cancerous cellular contexts.

Methodology

- PRISMA workflow.

The data of this paper is based on past research and the data is obtained on PubMed.com. The PRISMA workflow is utilized to identify the most optimal papers to be discussed in this study. Initially, this paper aims to be based on research that concerns both oncogenic and tumor suppression effects of SIRT1; however, identification of such papers on PubMed.com with key words: “SIRT1”, “cancer”, “oncogene”, and “tumor suppressor gene” harbored no results. Hence, the identification of data must be done separately.

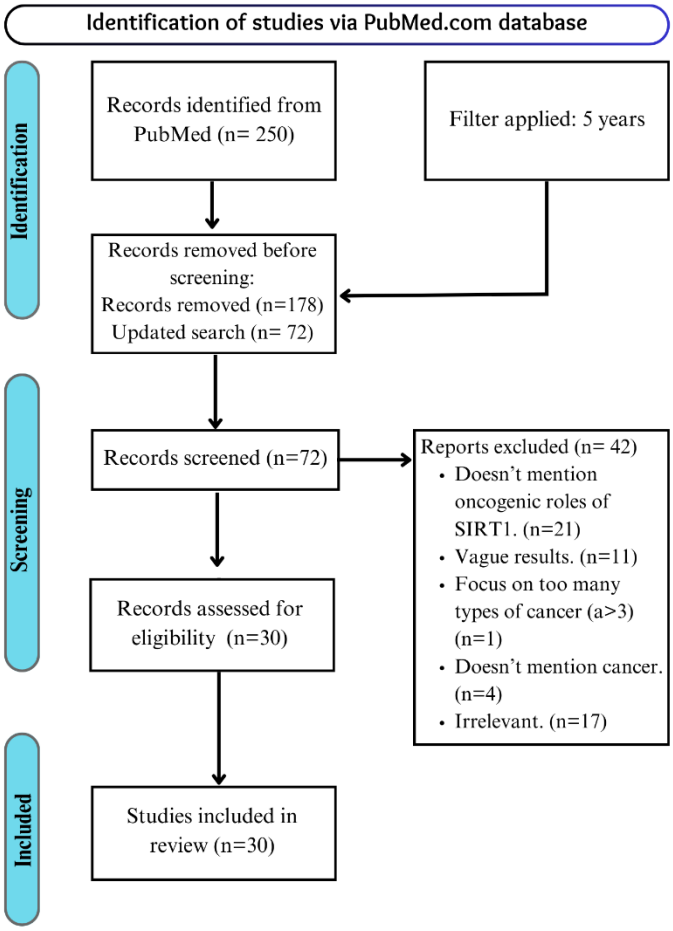

Figure 1. PRISMA workflow for SIRT1 oncogenic-related papers.

- SIRT1 as an oncogene:
  + *Identification:* The following keywords are included on the search on PubMed.com: “SIRT1”, and “cancer”, and “oncogene” which gives back 250 results. However, an initial scan of the results revealed many papers that are irrelevant to the margins of this paper or concern the topic but only to an extent,

so it was deemed unusable. Hence, filters and physical assessments of the papers are implemented in the Screening process.

+ *Screening:* First, a filter on the website was used to narrow down the resources. Confining the database to the most recent papers in the past 5 years, the database is now reduced to 72 papers. Next, the papers were assessed based on its abstract or full-text-article, if needed or accessible, the role of SIRT1 mentioned in the papers and the key details were noted down in a reference sheet. Criteria for assessment are SIRT1 must be involved in oncogenic effects (with detailed effects), the research paper must be specific with its results, the paper should focus on one specific type of cancer (if more are mentioned, it must not exceed three), and the paper must mention SIRT1, if not, it will be deemed irrelevant.

+ *Included:* After the screening process, 30 papers are included in this review.

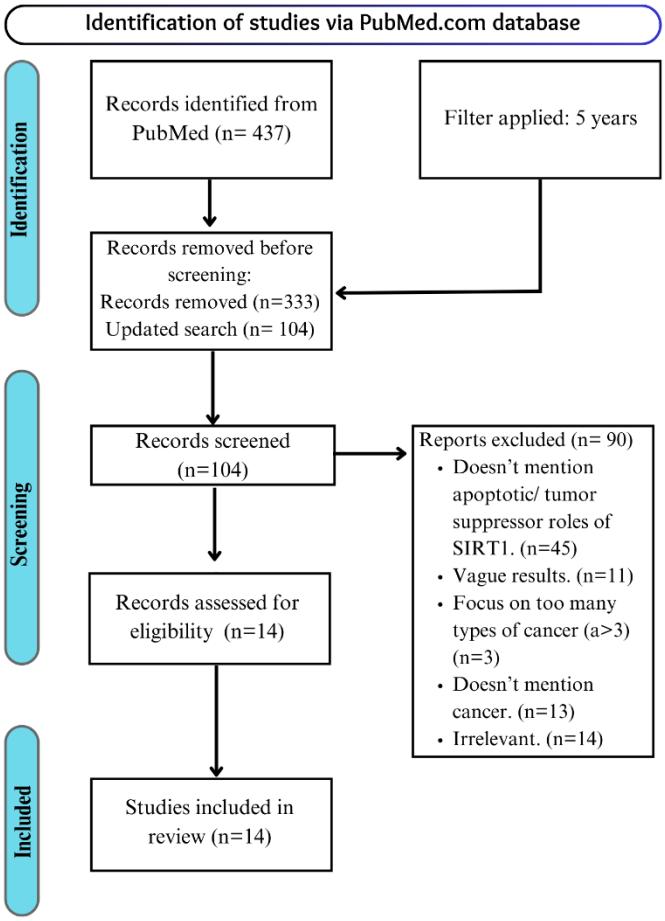

Figure 2. PRISMA workflow for SIRT1 anti-cancer related papers.

- SIRT1 as a tumor suppressor gene:

  + *Identification:* The following keywords are included on the search on PubMed.com: "SIRT1", and "cancer", and "oncogene" which gives back 437

results. An initial scan of the results revealed many papers that are irrelevant to the margins of this paper, giving opposite results (such as presenting papers on oncogenic role of SIRT1), or concern the topic but only to an extent, so it was deemed unusable. Hence, filters and physical assessments of the papers are implemented in the Screening process.
+ *Screening:* First, a filter on the website was used to narrow down the resources. Confining the database to the most recent papers in the past 5 years, the database is now reduced to 104 papers. Next, the papers were assessed based on its abstract or full-text-article, if needed or accessible, the role of SIRT1 mentioned in the papers and the key details were noted down in a reference sheet. Criteria for assessment are SIRT1 must be involved in tumor suppression effects (with detailed effects), the research paper must be specific with its results, the paper should focus on one specific type of cancer (if more are mentioned, it must not exceed three), and the paper must mention SIRT1, if not, it is irrelevant.
+ *Included:* After the screening process, 14 papers are included in this review.

- Additional data: This data is used to explain certain concepts necessary to the clarification of the material and the topic being discussed such as methodology or the function of specific chemicals and proteins not yet introduced in the introduction. These resources are screened from multiple sources using Google and PubMed.com.

Results

1. SIRT1 as an oncogene.
   - *The overexpression of SIRT1 has oncogenic effects on cancer cell lines.*

After screening and full-text analysis, 29 out of 44 papers suggested an oncogenic role for SIRT1 in many cancer types. Results are summarized in Table 1.

Table 1.

The effects of SIRT1 in multiple types of cancer as an oncogene.

Type of cancer	Effects of SIRT1	Reference
Colorectal cancer	Promotes tumor progression, metastasis, colony formation, EMT transformation	[1],[6],[11],[16],[18],[29]
Breast cancer	Tumor progression and metastasis; deficiency of SIRT1 inhibits tumor development.	[2], [5], [12],[26]

Hepatocellular carcinoma	Tumor progression, cell migration and invasion.	[3], [10], [21],[23],[27],[28]
Gastric cancer	Cancer cell cycle arrest, metastasis, and tumor progression.	[4], [14], [19]
Non-small cell lung cancer	Tumor progression.	[7], [17], [30]
Ovarian cancer	Cancer progression, promotes cell proliferation, migration, and invasion.	[15], [20], [22]
Oral cancer	Tumor development and malignancy.	[8]
T-cell acute lymphoblastic leukemia	Cell proliferation and colony formation.	[9]
HPV-driven cancer	Oncogenesis	[13]
Renal cell carcinoma	Tumor development.	[24]
Osteosarcoma	Promotes metastasis, migration, and invasion of osteosarcoma cells.	[25]

In colorectal cancer, the overexpression of SIRT1 has been associated with increased proliferation, migration, and invasion of cancer cells. The SIRT1 levels in CRC tissues have a positive correlation between the upregulation of SIRT, the depth of tumor invasion, and malignant phenotypes of this cancer. SIRT1 expression in five CRC cell lines reveals that the depletion of the gene in HCT116 and HT29 cells, specifically, inhibited cell migration and invasion. To this end, HCT116 cells, which were treated with Tenovin-6 (an inhibitor of SIRT1 and an activator of the p53 signaling pathway), showed diminished expression of SIRT1 and a significant increase in p53 expression levels, which downregulated malignant-promoting proteins such as vimentin, N-cadherin, and fibronectin, resulting in the repressed cell migration and invasion of CRC cells. The p53/miR-101 axis, which was recognized to have tumor-suppressing characteristics, was downregulated due to the silencing of p53, which further the aforementioned effects on CRC cells (X.-W. Wang et al., 2023; Yao et al., 2022). Additionally, SIRT1 plays an oncogenic role in the c-MYC/NAMPT/DBC1/SIRT1 feedback loop. The overexpression of SIRT1 contributes to the development of adenocarcinomas in $Braf^{4637E}$and $K-ras^{G12Dint}$/ Ink4a/ $Arf^{-/-}$mouse models. In another respect, SIRT1 inhibition by NOC4L promotes cell apoptosis and inhibits tumorigenesis of CRC. When NOC4L is incubated with SIRT1 in CRC tissues, it inhibits the interaction between SIRT1 and p53, since P53 has tumor-suppressing properties, its upregulation cooperates with NOC4L to induce autophagy in CRC tissues and inhibits carcinogenesis (*Nucleolar Protein NOC4L Inhibits Tumorigenesis and Progression by Attenuating SIRT1-Mediated P53 Deacetylation* | *Oncogene*, n.d.). Similarly, SIRT1 depletion in HCT116 cells decreases the expression of growth and migration-promoting proteins such as WNT16, SPARC, STC1, BMP4, and RHOJ, which significantly attenuates HCT116 cell proliferation,

migration, and invasion. On the contrary, restoration of SIRT1 reverses the aforementioned anti-cancer properties and promotes tumorigenesis and metastasis in HCT116 (Qiu et al., 2021).

From a different perspective, in hepatocellular carcinoma, the inhibition of SIRT1 significantly reduced cancer progression and cell proliferation. For instance, when HepG2 cells were incubated with LDC067, a CDK9 inhibitor, the interaction between SIRT1 and CDK9 was blocked, which in turn inhibited the phosphorylation and activity of SIRT1. P53, when in direct interaction with SIRT1, is deacetylated, causing instability of wt-p53 and promoting cancer progression. However, as phosphorylation and activity of SIRT1 is inhibited, p53 is upregulated and acetylated, corroborating stability and antiproliferative effects (Yao et al., 2022). Similarly, liver cells undergoing DEN treatment with SIRT1 deficiency show homogenous results. DEN treatment exerts damages on the genome, and hepatocyte injuries or cell death promotes cell proliferation and liver tumorigenesis. To this end, it has been found that SIRT1 deficiency reduces hepatic inflammation and expression levels of pro-inflammatory cytokines, namely IL-6, TNFα, and IL1β which are often associated with hepatocellular carcinogenesis progression (Qiu et al., 2021). Additionally, hepatocellular carcinogenesis is evaluated through SIRT1 and FTO, a gene that renders one susceptible to obesity (Z. Yang et al., 2022). FTO is found to have apoptotic effects on HCC cell lines; however, when SIRT1 is incubated along with FTO in HCC cells, FTO is significantly silenced, resulting in the reversal of its anti-cancer effects. To that end, overexpression of SIRT1 in HCC cells resulted in increased cell proliferation, invasion, and inhibition of autophagy (X. Liu et al., 2020). Similarly, miR-22-3p and SIRT1 are incubated together in HCC cell lines. miR-22-3p, alone, has anti-cancer effects; however, the upregulation of SIRT1 reverses miR-22-3p's tumor-suppressing effects while promoting cell proliferation, migration, colony formation, and EMT transition in HepG2 cells (Zhao et al., 2019).

Once again, in gastric cancer, the upregulation of SIRT1 induces cell proliferation and malignant phenotypes. First, circNOP10 and SIRT1 were applied to GC cell lines. A directly proportional relationship between the two was discovered; in other words, circNOP10 downregulation suppresses the expression of SIRT1 and vice versa. The upregulation of circNOP10 increases the expression of SIRT1 and increases malignant phenotypes such as vimentin and epithelial-mesenchymal transformation (EMT). Moreover, the levels of expression of apoptosis-related molecules attenuate, which increases the proportion of cells arrested in S-phase (J. Xu et al., 2021). Second, miR-12129 and SIRT1 are incubated with gastric cancer cells. miR-12129, alone, has an apoptotic effect on cancer cells. However, once SIRT1 is upregulated, miR-12129's anti-cancer effects are inhibited. Additionally, this upregulation was involved in cell proliferation and reversal of G0/G1-induced arrest via miR-12129. In a different respect, in gastric cancer, the silencing of SIRT1 via an inhibitor promotes gastric cancer cell progression (*MiRNA-12129 Suppresses Cell Proliferation and Block Cell Cycle Progression by Targeting SIRT1 in GASTRIC Cancer - Wei Zhang, Kai Liao, Dongning Liu, 2020*, n.d.). The c-Myc/NAMPT/SIRT1 signaling pathway is involved in the

tumorigenesis of gastric cancer cells, and its activity was evaluated through the application of inhibitors targeting specific counterparts of the pathway. In particular, SIRT1 was targeted by EX527, a potent and selective inhibitor of SIRT1 (Broussy et al., n.d.), in HGC-27 and BGC-823 cells. Its knockdown reduces the levels of pro-proliferative proteins such as Ki67 and Cyclin D1; and vitmentin, a mesenchymal marker, while increasing the expression of E-cadherin, an epithelial marker. This reduces EMT transformation and inhibits tumor progression (H. Liu et al., 2019).

In breast cancer, the upregulation of SIRT1 along with FOXO proteins has oncogenic effects on metastatic tissues. In highly metastatic breast cancer tissues, the expression of SIRT1 is significantly expressed along with FOXO1, FOXO3a, and FoxO4 - these proteins from the FOXO family are determined to promote invasion of metastatic breast cancer to distant organs such as the lungs or liver. In particular, the upregulation of SIRT1 cooperated with the aforementioned FOXO proteins to promote cell migration and invasion by silencing p53 and p21, which inhibits their apoptotic effects on cancer cells (Dilmac et al., 2022). From a different perspective, the inhibition of SIRT1 by miR-211-5p effectively induces cancer cell apoptosis. Particularly, miR-211-5p increases the acetylation of p53 and upregulates it while silencing the expression of SIRT1 in MCF-7 cells (Yarahmadi et al., 2019).

In ovarian cancer, the upregulation of SIRT1 renders the deacetylation of cortactin and promotes cell migration. SIRT1 and cortactin, an actin-binding protein (Cavaliere et al., 2021), are incubated together in OV2008 cells. It was found that SIRT1 deacetylates cortactin resulting in the promotion of migratory properties of sir2α / MEFs; hence, increasing cancer cell migration (Y. Zhang et al., 2009). Additionally, the relationship between KRAS and SIRT1 expression has oncogenic effects on ovarian cancer cells. Activation of KRAS mandates epithelial-mesenchymal transition (EMT) as well as cell migration mediated by SIRT1. It was found that in ovarian cancer cells, SIRT1 is significantly more expressed, enhancing cell migration and EMT; therefore, activating KRAS (Teasley et al., 2020). From a different perspective, the inhibition of SIRT1 in SiHa cervical cancer cells reduces cancer cell migration and induces autophagy. SiHa cells are incubated with HPV E7, a cancer-promoting virus (McLaughlin-Drubin & Münger, 2009), and SIRT1. When SIRT1 is silenced, HPV E7 shows a lowered rate of survival in SiHa cells; meanwhile, SiHa cells display an increased rate of cell apoptosis (Allison et al., 2009).

In the context of non-small cell lung cancer (NSCLC), the inhibition of SIRT1 suppresses cell proliferation, migration, and invasion while promoting cell apoptosis. SIRT1 and hsa-miR-217 expression levels were evaluated in PC-14/B cells. Initially, the overexpression of SIRT1 increases cell invasion and proliferation; however, when SIRT1 is silenced by hsa-miR-217, PC-14/B cell proliferation, invasion, and migration attenuates (W. Jiang et al., 2020). Similarly, the silencing of SIRT1 increases apoptosis in $KRAS^{Mut}$-driven lung cancer. SIR1 K/D suppresses activities along with pERK and pAkt – an endoplasmic reticulum protein that regulates unfolded protein response and a biomarker

for human cancer, respectively (Y. Chen et al., 2020; *PERKs of Plasma Membrane–ER Communication | Nature Reviews Molecular Cell Biology*, n.d.). Next, the effect of SIRT1 silencing on NSCLC cells, including H358, A427, and H727, is evaluated: the results show that proliferation rates, colony formations, and numbers decrease significantly following the deletion of SIRT1 (Shin et al., 2023).

In T-cell acute lymphoblastic leukemia, SIRT1 overexpression promotes cell proliferation of all cancerous T-ALL cells. First, two ShRNAs, ShSIRT1-1 and ShSIRT1-2, inhibit SIRT1 expression in MOLT-4 and CCRF-CEM cells. This reduces the rate and amount of cell proliferation in MOLT-4 and CCRF-CEM cells and inhibits their colony formation. On the other hand, the restoration of SIRT1 attenuates ShSIRT1 apoptotic properties while promoting cell proliferation and colony formation (*SIRT1 Regulates the Phosphorylation and Degradation of P27 by Deacetylating CDK2 to Promote T-Cell Acute Lymphoblastic Leukemia Progression | Journal of Experimental & Clinical Cancer Research | Full Text*, n.d.).

In HPV-associated cancer, the upregulation of SIRT1 expression levels is responsible for HPV-driven oncogenesis. A transcriptionally active Ac-p53 is crucial to the suppression of HPV-driven carcinogenesis, but the overexpression of SIRT1 in cancer cells degrades p53, terminating its apoptotic properties while promoting cell proliferation. In contrast, the silencing of SIRT1 via EX527, a potent and selective inhibitor of SIRT1 (Broussy et al., n.d.), restores the stability of Ac-p53 and inhibits the growth of cancerous cells. Specifically, the viral oncoproteins E6 and E7 in cell lines, along with the number of cells arrested in G_0/G_1 phase significantly attenuates. Additionally, colony-forming assays reveal that the number of clones is reduced by up to 85% in EX527-treated cell lines (*SIRT1 Is an Actionable Target to Restore P53 Function in HPV-Associated Cancer Therapy | British Journal of Cancer*, n.d.).

In the context of osteosarcoma, the increased expression levels of SIRT1 are positively associated with osteosarcoma metastasis. In osteosarcoma, SIRT1 is naturally upregulated, and this overexpression promotes metastasis, vindicated by PROgene V2 analysis. Additionally, the higher the expression of SIRT1 in osteosarcoma cells, the stronger the cells' migratory and invasive abilities. Transwell migration assay is performed on seven samples of osteosarcoma cells, three of which, MDOS-22, MDOS-19, and MDOS-21 cells, have lower SIRT1 protein levels and weaker migratory and invasive abilities compared to four other samples, namely MDOS-16, MDOS-26, MDOS-14, and MDOS-27 cells. From a different perspective, when SIRT1 is inhibited by shRNA, this knockdown significantly represses cell proliferation, migration, and invasion of primary osteosarcoma cell lines (Qu et al., 2015).

- *The downregulation of SIRT1 by an inhibitor displays oncogenic properties on cancer cells when it is not upregulated by an activator.*

In oral cancer, the downregulation of SIRT1 is positively correlated with several metastatic phenotypes. In this situation, SIRT1 represses the expression levels of

epithelial-cadherin (E-cadherin), contributing to the promotion of cancer cell invasion and metastasis. Additionally, SIRT1 promotes malignant transformation, invasion, and migration by enhancing transforming growth factor beta (TGF-β) expression levels (Islam et al., 2019). Furthermore, the upregulation of TGF-β increases myofibroblastic transdifferentiation, which contributes to the pathogenesis of oral submucous fibrosis (OSF) (Islam et al., 2019).

In renal cell carcinoma, the knockdown of SIRT1 reverses the anti-proliferative effects of MOF, a histone acetyltransferase, on RCC cell lines. Initially, the interaction between MOF and SIRT1 is evaluated, and the TCGA starbase reveals a positive association between MOF and SIRT1, in which the repression of MOF significantly diminishes the levels of protein and mRNA of SIRT1. Next, this interaction is placed in the cellular context of RCC cells, and a SIRT1 inhibitor, siRNA, is introduced: while MOF induces significant inhibition of cell proliferation, the knockdown of SIRT1 by siRNA reverses the attenuated cell proliferation and promotes tumor progression (Guo et al., 2022).

- *Downregulation of SIRT1 which upregulates p53 has apoptotic effects on cancer cell lines.*

In hepatocellular carcinoma, downregulation of SIRT1 contributes to the P53/miR-34a/SIRT1 proapoptotic pathway to terminate cancer progression. The silencing of SIRT1 upregulates p53 by decreasing its deacetylase activity while increasing the acetylation of p53, promoting its anti-cancer properties. In this specific pathway, the knock-in of p53 decreases the proliferation of cells in the liver in Ade-GFP mice models, verified by the increase in TUNEL-positive cells. Similarly, Isoimperatorin inhibits SIRT1 in Huh7 cells and induces cell apoptosis on cancer cell lines. P53 is upregulated when SIRT1 is silenced, triggering the overexpression of p21, which both attenuates cell growth and cancer progression by downregulating cell cycle genes, specifically (Ko et al., 2024). Additionally, SIRT1 downregulation via Dulcitol induces autophagy in HepG2. Once again, the silencing of SIRT1 upregulates p53; however, in this specific cellular context, this upregulation of p53 promotes the expression of cleaved-caspase 3, which is pivotal in the apoptotic pathway. Indeed, the increase in cleaved-caspase 3 expression levels significantly promotes the apoptosis of HepG2, evident in Western blot and morphological observations (X. lin Lin et al., 2020).

In the context of colorectal cancer, the repression of SIRT1 by concentrated resveratrol reduces cancer cell plasticity and proliferation. Concentrated resveratrol is incubated in CRC cells along with SIRT1; and the effects of their interaction are evaluated through the rate of cell proliferation, migration, and invasion in CRC cells. In particular, nuclear SIRT1 is downregulated due to the concentrated phenol, which, in turn, upregulates p53 acetylation. Since the acetylation of p53 due to the knockdown of deacetylase activity of SIRT1 has tumor-suppressing effects, and such anti-tumor activities are concentration-dependent, the concentrated resveratrol amplified this behavior. Increased expression of cleaved caspase-3 is the result of this upregulation. Since they are apoptosis activators,

the increased rate of apoptosis in CRC cells is not only precedented but also evident in Western blotting or TUNEL assay (Brockmueller et al., 2023).

In non-small cell lung cancer (NSCLC), downregulation of SIRT1 by tenovin-6 increases apoptosis in A549 cancer cells. First, A549 lung cancer cells are incubated with SIRT1 and tenovin-6, where the latter is a potent inhibitor of SIRT1 (Jin et al., 2015). Silencing of SIRT1 leads to the activation of tumor suppressor p53 in cancerous cells. P53's anti-cancer properties significantly induce apoptosis; specifically, the apoptosis rate in p53 upregulated A549 cells increases by 2.5-fold (Eroglu et al., 2020).

2. SIRT1 as a tumor suppressor gene.
   - *Upregulation of SIRT1 via an activator has anti-cancer effects on cancer cell lines.*

After screening and full-text analysis, 15 out of 44 papers suggested a tumor suppressing role for SIRT1 in many cancer types. Results are summarized in Table 2.

Table 2.

The effects of SIRT1 on several types of cancer as a tumor suppressor gene.

Type of cancer	Effects of SIRT1	References
Oral cancer	Facilitate apoptosis.	[31],[34]
Ovarian cancer	Inhibits cell migration and invasion.	[32], [35]
Chondrosarcoma	Attenuates cell proliferation and induces autophagy.	[33]
Bladder cancer	Inhibits tumorigenesis and tumor growth.	[37]
Breast cancer	Inhibits EMT, cell proliferation, migration, and invasion.	[36], [38], [44]
Glioblastoma cells	Inhibits cell proliferation, migration, and invasion.	[40]
Non-small cell lung cancer	Induces cell apoptosis	[39], [41], [42]
Colorectal cancer	Inhibits cell proliferation, migration, invasion, and induces cell apoptosis.	[43]

In the context of ovarian cancer, the levels of expression of SIRT1 are positively associated with cell apoptosis. COV434 cells are incubated with SIRT1, resveratrol, an activator, Ex527, an inhibitor, and H2O2. It was found that both treatment with an activator and inhibitor shows that SIRT1 plays a crucial role in the regulation of cell

apoptosis, with p53 being the key factor of this regulation. To that end, P53 is exposed to H2O2, OTS514, and an inhibitor of p53, Pifithrin-μ. P53 is acetylated and upregulated, which silences SIRT1 and exhibits tumor-promoting effects (Park et al., 2020). In other words, inhibition of p53 decreased COV434 cell apoptosis significantly. Moreover, overexpression of SIRT1 has been discovered to inhibit cell migration and invasion by suppressing epithelial-mesenchymal transformation (EMT). This is done by activating the epithelial program through promoting expression levels of epithelial markers such as CK-18/19, desmoplakin, plakophilin-2/3, periplakin, epiplakin, claudin-1, JAM1, and nectin-1, and suppressing mesenchymal markers protein expression, namely vimentin and fibronectin – malignant promoting proteins (T. Yang et al., 2019).

Similar to ovarian cancer, in breast cancer, the overexpression of SIRT1 also shows a positive correlation will cell apoptosis and inhibition of cell migration. First, TNBC cells are incubated with SIRT1, resveratrol, and CAY10602, both of which are potent activators of SIRT1, to evaluate the effect of the upregulated SIRT1 expression levels. Both activators of SIRT1 are confirmed to not affect the migration and invasion of TNBC cells, and SIRT1 impairs metastatic properties of TNBC in vitro. From a different perspective, TNBC was incubated with SIRT1 and one of its inhibitors, EX-527. The results show that the downregulation of SIRT1 significantly reduces the metastatic effects of TNBC (Y. Jiang et al., 2023). Similarly, SIRT1 is knocked down by siRNA to affirm the aforementioned anti-cancer effects on breast cancer cell lines. After the downregulation of SIRT1 in MCF-7 and MDA-MB-231 cells, colony formation assay indicates that cell proliferation and the number of tumor colonies are significantly inhibited. Additionally, the survival fraction of transfected MDA-MB-231 and MCF-7 cells is significantly reduced (X. Zhang et al., 2017). In another study, the transfection of resveratrol in breast cancer cell lines along with SIRT1 renders the enzyme's upregulation and exhibits anti-cancer properties. First, resveratrol and SIRT1 are incubated in BRCA1 cells. Once SIRT1 expression levels increase, anti-cancer effects on BRCA1 cells are evident in TUNNEL assay and RT-PCR. Specifically, BRCA1 shows decreased colony formation of up to 5-fold; similar results are also indicated in mice xenograft models. Additionally, the activity of Survivin, an anti-apoptotic protein, is monitored in this cancer. The overexpression of SIRT1 significantly inhibits Survivin expression levels in mouse embryonic fibroblast cells. Therefore, increasing apoptosis in cancer cells (R.-H. Wang et al., 2008).

Once again, in bladder cancer, the upregulation of SIRT1 expression levels has anti-cancer and apoptotic effects on cancer cell lines. MIBC organoids are incubated with SIRT1 and SRT1720, an activator of SIRT1. The results suggested that overexpression SIRT1 significantly repressed cancer cell proliferation, migration, and invasion in MIBC organoids. From a different perspective, SIRT1 is mutated via CRISPR/Cas9 to see if the enzyme can still retain its tumor suppression effects. SIRT1, once effectively disrupted, promoted tumorigenesis in MIBC organoids (*SRT1720 Inhibits the Growth of Bladder Cancer in Organoids and Murine Models through the SIRT1-HIF Axis* | *Oncogene*, n.d.).

In the context of oral cancer, SIRT1 initiates cancer cell apoptosis when upregulated by an activator. Oral cancer cells are primed with doses of SIRT1 and gallic acid, a novel activator of SIRT1. This repressed mitochondrial hyperfusion, a survival mechanism against pro-apoptotic stressors, which, in this case, is the activation of SIRT1 via gallic acid. In the primed cancer cells, this is evident by the decrease in branch length. Additionally, the level of mitochondrial superoxide significantly increases. To that end, the initiation of apoptosis is successfully driven once mitochondrial hyperfusion is suppressed (Patra et al., 2023). Similarly, GAPDH is incubated with SIRT1 in OSCC tissue samples, SCC9 and SCC25, to indirectly upregulate the mRNA protein expression of SIRT1 as, naturally, SIRT1 expression in OSCC cancer cell lines is rather low. Following this upregulation of up to 5-fold, SIRT1 exhibited anti-cancer properties by significantly inhibiting cell proliferation and migration with time, evident in transwell assays and MTC cell proliferation assays (Kang et al., 2018).

In non-small cell lung cancer (NSCLC), upregulation of SIRT1 by resveratrol increases apoptosis in A549 cancer cells. First, A549 lung cancer cells are incubated with SIRT1 and resveratrol, where the latter is a potent activator of SIRT1 (Ghosh et al., 2013). The apoptosis assay shows that following the upregulation of SIRT1 via resveratrol, approximately 14.64% of cancerous cells undergo apoptosis. In a similar case, resveratrol and SIRT1 are employed in NSCLC cell lines A549 and H1299 cells. Increased levels of autophagy biomarkers Beclin 1 and LC3 II/I are observed. Additionally, upregulation of SIRT1 significantly induces autophagy in A549 cells. In order to consolidate the conclusion that SIRT1 overexpression is responsible for the observed autophagy, siRNA, a SIRT1 inhibitor, is introduced to the NSCLC cell lines. The result of SIRT1 inhibition is the reversal of anti-cancer activities of SIRT1 overexpression and the promotion of cell proliferation, migration, and invasion in NSCLC cell lines (J. Wang et al., 2018). In another study, OPN, a multifunctional protein, and SIRT1 are introduced in NSCLC cell lines, including A549, HCI-H1299, NCI-H358, and NCI-H460). In response to the protein, SIRT1 is downregulated in the cancer cell lines, promoting the acetylation of the NF-κB signaling pathway and the p65 unit, both of which are known to be involved in the initiation of tumorigenesis. Henceforth, cell EMT, proliferation, migration, and invasion decrease. On the other hand, SIRT1 overexpression reverses cancer-promoting effects; specifically, expression levels of malignant-promoting protein vimentin decrease while upregulating the mRNA levels of E-cadherin in order to inhibit cell EMT. At the same time, cell proliferation, migration, and invasion attenuate, as evident in the CCk-8 assay and transwell assay (X. Li et al., 2018). In a similar case, lentivirus is incubated with SIRT1 in A549 and Calu-3 cells in order to knock down SIRT1 expression. This rendered the decrease in EMT marker and malignant promoting protein vimentin while enhancing the expression of E-cadherin. As a whole, inhibits EMT transformation. In addition, CCK-8 analysis shows significant decline in cell proliferation and cells arrested in phase. The invasive and migratory abilities of A549 and Calu-3 lung cancer cells are inhibited asserted by Transwell assays (*miR-138 Suppresses the Proliferation, Metastasis and Autophagy of Non-Small Cell Lung Cancer by Targeting Sirt1*, n.d.).

In the context of glioblastoma cells, SIRT1 upregulation mediated by Urolithin A decreases tumor progression in U251 and U118 cells. In particular, SIRT1 cooperates with FOXO family proteins, FOXO1, in this cellular context to display inhibitory effects on glioblastoma tumorigenesis. First, SIRT1 is knocked down, terminating the apoptotic effects of Urolithin A on cell proliferation, migration, and invasion of U251 and U118 MG cells. Additionally, the downregulation of SIRT1 inhibits the expression of FOXO1. When both SIRT1 and FOXO1 are inhibited, U251 and U119 MG cells demonstrated enhanced pro-proliferative properties. On the contrary, restoration of SIRT1 solely successfully reverses the cancer-promoting effects of SIRT1 knockdown and mediates Urolithin A's apoptotic effects on glioblastoma cell lines by decreasing cell proliferation, migration, and invasion in U251 and U119 cells (C.-L. Liu et al., 2022).

In chondrosarcoma cells, the implementation of resveratrol upregulates SIRT1 expression, which results in anti-tumor activities. First, JJ012 cells are incubated with resveratrol, a potent activator of SIRT1, and SIRT1. Western blot analysis reveals that the upregulation of SIRT1 corroborates the activation of Caspase-3, a pro-apoptotic protein that is involved in several apoptotic pathways and is crucial to the initiation of cell apoptosis (Porter & Jänicke, 1999). The significant increase in the activation and activity of Caspase-3, hence, induces cell apoptosis in JJ012 cells while alleviating cell proliferation, migration, invasion, and, ultimately, tumorigenesis in chondrosarcoma cells. Additionally, the NF- κB signaling pathway activity is attenuated following the upregulation of SIRT1 via resveratrol. Since the pathway plays a role in pro-proliferative activation, its inhibition significantly represses cancer progression in JJ012 cells as well as Nu/Nu nude mice xenograft (*Induction of Sirtuin-1 Signaling by Resveratrol Induces Human Chondrosarcoma Cell Apoptosis and Exhibits Antitumor Activity | Scientific Reports*, n.d.).

In the context of colon cancer, SIRT1 is upregulated by a tetracycline-inducible lentivirus vector, which significantly suppresses tumor growth. HCT116 cells are infected with tetracycline-inducible lentivirus vector and SIRT1. Once SIRT1 expression levels increase 4-fold, cell proliferation and tumorigenesis of colon cancer are inhibited. Additionally, SIRT1 displays potential to suppress colony formation, which is confirmed by diminished colony formation after SIRT1 upregulation by colony formation assay. In a different respect, the inactivation of SIRT1 promotes cell proliferation, migration, and invasion of HCT116 cells. A SIRT1 inhibitor, EX-527, is introduced to the culture; the inactivation of SIRT1 that followed caused a 90% increase in cell number in HCT116 cell culture. Moreover, HCT116 cell proliferation, migration, and invasion are stimulated (Kabra et al., 2009).

In gastric cancer, the introduction of mock vectors induces the overexpression of SIRT1, which displays anti-cancer properties on gastric cancer cells. First, mock vectors expressing SIRT1, pcDNA-SIRT1, and pcDNA-SIRT1-H363Y, are transfected into gastric cell lines BGC-823, SGC-7901, and AGS. This upregulation of SIRT1 expression levels and deacetylase activities significantly inhibited cell proliferation, tumor growth, and colony formation by downregulating the NF-κB signaling pathway; specifically, the

expression of the tumor-promoting protein p63 is inhibited. In order to corroborate SIRT1 tumor-suppressing properties in gastric cancer, one of its inhibitors, siRNA, is employed. MTS assay indicates that SIRT1-depleted cells show a significant increase in cell proliferation and colony formation (Q. Yang et al., 2013).

Discussion

In this study, a systematic review of 44 articles from PubMed library was conducted to evaluate the mechanism behind the dual role of SIRT1 in cancer progression. It was found that SIRT1 upregulation in cancer cell lines often display oncogenic properties, and its knock down involving the activation of tumor suppressing proteins such as p53 or p21 induces apoptosis and inhibits cancer-promoting activity (Brockmueller et al., 2023; Eroglu et al., 2020; Yao et al., 2022). Elevated SIRT1 was associated with metastasis, malignancy, cell proliferation, migration, and invasion (Qiu et al., 2021; Wang et al., 2023; Zhao et al., 2019). On the contrary, SIRT1 takes on a tumor-suppressing role when upregulated by an activator. In such cellular contexts, SIRT1 inhibits cell proliferation, migration, invasion, metastasis, and tumorigenesis; in some cases, SIRT1 may induce apoptosis.

This current study indicates that in cancer cell lines where SIRT1 is moderately upregulated, or possibly, overexpressed, the enzyme often displays oncogenic effects: promoting cell proliferation, migration, and invasion, stimulates the appearance of malignant phenotypes (vimentin, N-cadherin, and fibronectin), and tumorigenesis (Qiu et al., 2021; Wang et al., 2023; Zhao et al., 2019). Moreover, if the expression levels of SIRT1 increase, it will further the migratory and invasive abilities of cancerous cells (Y. Zhang et al., 2009). On the other hand, SIRT1 inhibition significantly attenuates and restores the pro-proliferative and tumor-promoting effects of SIRT1 overexpression in cancer cell lines. Cancer cells put under cancer cell cycle arrest from the upregulation of SIRT1 are rescued and, if in the presence of tumor suppressing proteins such as has-miR-217 or p53, undergo cell apoptosis (W. Jiang et al., 2020; Allison et al., 2009). However, if SIRT1 still takes on an oncogenic role after inhibition, then it can be inferred that SIRT1 was not overexpressed by an activator but naturally upregulated in the cancer cell lines. Additionally, SIRT1 can cooperate with other family proteins, such as FOXO or signaling pathways, namely the NF-κB pathway associated with tumor progression and the c-MYC/NAMPT/DBC1/SIRT1 feedback loop, to facilitate the emergence of tumor-promoting proteins and markers responsible for carcinogenesis (Brandl et al., 2019; Dilmac et al., 2022; Kiernan et al., 2003).

In this study, a separate analysis on SIRT1 as a tumor suppressor indicates that SIRT1 can only have anti-cancer properties on cancer cell lines when activated by an activator. In several studies, resveratrol, a polyphenol and potent activator of SIRT1 stimulates the

expression levels of SIRT1 and promotes anti-tumor activities such as inhibition of cell proliferation, migration, invasion, the initiation of tumorigenesis, and cell apoptosis (Brockmueller et al., 2023; T. Yang et al., 2019; Y. Jiang et al., 2023). For instance, in non-small cell lung cancer, upregulation of SIRT1 via resveratrol can induce apoptosis of up to 4-fold (Eroglu et al., 2020). Other types of SIRT1 activators, such as SRT1720, CAY10602, or Ex527 can induce similar effects on cancer cell lines (*SRT1720 Inhibits the Growth of Bladder Cancer in Organoids and Murine Models through the SIRT1-HIF Axis | Oncogene*, n.d.; Y. Jiang et al., 2023). Still, resveratrol is the most effective in inducing tumor suppressing properties in cancer cell lines, more than any of the aforementioned activators.

There are limitations to this study, most notably, the methodology. The collection of studies may not be the most prudent due to the usage of keywords. First, the keyword SIRT1: not all studies refer to the enzyme in its abbreviated form, several studies address it as “Sirtuin 1” or “Silent information regulator 1,” so they are not detectable by the search. The same problem happened to the key word tumor suppressor. Different studies can use different keywords to indicate SIRT1 as a tumor suppressor, such as anti-proliferative or anti-cancer properties, rendering many papers not being included in this review. Second, the keyword cancer is quite general and not specific to any types or degree, so many types of cancer that SIRT1 is experimented on is left out, making this study not as comprehensive to infer a credible generalization about the overall mechanism of SIRT1 in tumor progression. Overall, a number of papers that have the information to further our understanding of SIRT1 are left out, so it is possible for this paper to only explain SIRT1’s dual roles on a surface to intermediate level. In the future, a larger database can be used to provide a more comprehensive understanding of the subject matter.

Conclusion

In conclusion, the upregulation of SIRT1 generally displays oncogenic properties on cancer cell lines: inducing tumorigenesis, increasing metastasis, invasion, and malignancy. SIRT1’s activators have influence on the effects of SIRT1 in tumor progression. Overexpression of SIRT1 by an activator, such as resveratrol or SRT1720, has tumor suppressing effects on cancer cell lines, inhibiting cell proliferation, migration, invasion, and, if in the presence of a tumor suppressor protein, induces apoptosis. Inhibition of SIRT1 without activating it by an activator will maintain SIRT1’s oncogenic properties on cancer cell lines. Hence, SIRT1 activators are potential agents to target SIRT1 as a therapeutic treatment. However, further investigation with larger databases is needed to have a comprehensive generalization of SIRT1’s mechanism in tumor progression.

References

[1] Wang, X. W., Jiang, Y. H., Ye, W., Shao, C. F., Xie, J. J., & Li, X. (2023). SIRT1 promotes the progression and chemoresistance of colorectal cancer through the p53/miR-101/KPNA3 axis. *Cancer Biology & Therapy*, *24*(1). https://doi.org/10.1080/15384047.2023.2235770

[2] Dilmac, S., Kuscu, N., Caner, A., Yildirim, S., Yoldas, B., Farooqi, A. A., & Tanriover, G. (2022). SIRT1/FOXO Signaling Pathway in Breast Cancer Progression and Metastasis. *International journal of molecular sciences*, *23*(18), 10227. https://doi.org/10.3390/ijms231810227

[3] Liu, X., Liu, J., Xiao, W., Zeng, Q., Bo, H., Zhu, Y., Gong, L., He, D., Xing, X., Li, R., Zhou, M., Xiong, W., Zhou, Y., Zhou, J., Li, X., Guo, F., Xu, C., Chen, X., Wang, X., Wang, F., … Cao, K. (2020). SIRT1 Regulates N^6 -Methyladenosine RNA Modification in Hepatocarcinogenesis by Inducing RANBP2-Dependent FTO SUMOylation. *Hepatology (Baltimore, Md.)*, *72*(6), 2029–2050. https://doi.org/10.1002/hep.31222

[4] Zhang W, Liao K, Liu D. MiRNA-12129 Suppresses Cell Proliferation and Block Cell Cycle Progression by Targeting SIRT1 in GASTRIC Cancer. Technology in Cancer Research & Treatment. 2020;19. https://doi.org/10.1177/1533033820928144

[5] HELVACI, NAZLI; SARAÇOĞLU, HATİCE; YILDIZ, OĞUZ GALİP; and KILIÇ, ESER (2021) "The study of sirtuins in breast cancer patients before and after radiotherapy," *Turkish Journal of Medical Sciences*: Vol. 51: No. 3, Article 57. https://doi.org/10.3906/sag-2012-195

[6] Jia, X., Liu, H., Ren, X. *et al.* Nucleolar protein NOC4L inhibits tumorigenesis and progression by attenuating SIRT1-mediated p53 deacetylation. *Oncogene* **41**, 4474–4484 (2022). https://doi.org/10.1038/s41388-022-02447-y

[7] Jiang, W., Hou, L., Wei, J., Du, Y., Zhao, Y., Deng, X., & Lin, X. (2020). Hsa-miR-217 Inhibits the Proliferation, Migration, and Invasion in Non-small Cell Lung Cancer Cells Via Targeting SIRT1 and P53/KAI1 Signaling. *Balkan medical journal*, *37*(1), 208–214. https://doi.org/10.4274/balkanmedj.galenos.2020.2019.9.91

[8] Islam, S., Abiko, Y., Uehara, O., & Chiba, I. (2019). Sirtuin 1 and oral cancer. *Oncology letters*, *17*(1), 729–738. https://doi.org/10.3892/ol.2018.9722

[9] Wang, F., Li, Z., Zhou, J. *et al.* SIRT1 regulates the phosphorylation and degradation of P27 by deacetylating CDK2 to promote T-cell acute lymphoblastic leukemia progression. *J Exp Clin Cancer Res* **40**, 259 (2021). https://doi.org/10.1186/s13046-021-02071-w

[10] Yao, Jy., Xu, S., Sun, Yn. *et al.* Novel CDK9 inhibitor oroxylin A promotes wild-type P53 stability and prevents hepatocellular carcinoma progression by disrupting both

MDM2 and SIRT1 signaling. *Acta Pharmacol Sin* **43**, 1033–1045 (2022). https://doi.org/10.1038/s41401-021-00708-2

[11] Brandl, L., Zhang, Y., Kirstein, N., Sendelhofert, A., Boos, S. L., Jung, P., Greten, F., Rad, R., & Menssen, A. (2019). Targeting c-MYC through Interference with NAMPT and SIRT1 and Their Association to Oncogenic Drivers in Murine Serrated Intestinal Tumorigenesis. *Neoplasia (New York, N.Y.)*, *21*(10), 974–988. https://doi.org/10.1016/j.neo.2019.07.009

[12] Yarahmadi, S., Abdolvahabi, Z., Hesari, Z., Tavakoli-Yaraki, M., Yousefi, Z., Seiri, P., Hosseinkhani, S., & Nourbakhsh, M. (2019). Inhibition of sirtuin 1 deacetylase by miR-211-5p provides a mechanism for the induction of cell death in breast cancer cells. *Gene*, *711*, 143939. https://doi.org/10.1016/j.gene.2019.06.029

[13] Lo Cigno, I., Calati, F., Girone, C. *et al.* SIRT1 is an actionable target to restore p53 function in HPV-associated cancer therapy. *Br J Cancer* **129**, 1863–1874 (2023). https://doi.org/10.1038/s41416-023-02465-x

[14] Xu, J., Wang, X., Wang, W., Zhang, L., & Huang, P. (2021). Candidate oncogene circularNOP10 mediates gastric cancer progression by regulating miR-204/SIRT1 pathway. *Journal of gastrointestinal oncology*, *12*(4), 1428–1443. https://doi.org/10.21037/jgo-21-422

[15] Teasley, H. E., Beesley, A., Kim, T. H., Risinger, J., Young, S. L., Jeong, J. W., Schammel, D. P., Lessey, B. A., Elder, J. W., & Puls, L. (2020). Differential Expression of KRAS and SIRT1 in Ovarian Cancers with and Without Endometriosis. *Reproductive sciences (Thousand Oaks, Calif.)*, *27*(1), 145–151. https://doi.org/10.1007/s43032-019-00017-4

[16] Yu, L., Dong, L., Li, H. *et al.* Ubiquitination-mediated degradation of SIRT1 by SMURF2 suppresses CRC cell proliferation and tumorigenesis. *Oncogene* **39**, 4450–4464 (2020). https://doi.org/10.1038/s41388-020-1298-0

[17] Shin, D.H., Jo, J.Y., Choi, M. *et al.* Oncogenic KRAS mutation confers chemoresistance by upregulating SIRT1 in non-small cell lung cancer. *Exp Mol Med* **55**, 2220–2237 (2023). https://doi.org/10.1038/s12276-023-01091-0

[18] Wang, X., Liu, S., Xu, B., Liu, Y., Kong, P., Li, C., & Li, B. (2021). circ-SIRT1 Promotes Colorectal Cancer Proliferation and EMT by Recruiting and Binding to eIF4A3. *Analytical cellular pathology (Amsterdam)*, *2021*, 5739769. https://doi.org/10.1155/2021/5739769

[19] Liu, H., Liu, N., Zhao, Y., Zhu, X., Wang, C., Liu, Q., Gao, C., Zhao, X., & Li, J. (2019). Oncogenic USP22 supports gastric cancer growth and metastasis by activating c-Myc/NAMPT/SIRT1-dependent FOXO1 and YAP signaling. *Aging*, *11*(21), 9643–9660. https://doi.org/10.18632/aging.102410

[20] Allison, S. J., Jiang, M., & Milner, J. (2009). Oncogenic viral protein HPV E7 up-regulates the SIRT1 longevity protein in human cervical cancer cells. *Aging*, *1*(3), 316–327. https://doi.org/10.18632/aging.100028

[21] Qiu, P., Hou, W., Wang, H. *et al.* Sirt1 deficiency upregulates glutathione metabolism to prevent hepatocellular carcinoma initiation in mice. *Oncogene* **40**, 6023–6033 (2021). https://doi.org/10.1038/s41388-021-01993-1

[22] Zhang, Y., Zhang, M., Dong, H. *et al.* Deacetylation of cortactin by SIRT1 promotes cell migration. *Oncogene* **28**, 445–460 (2009). https://doi.org/10.1038/onc.2008.388

[23] Zhao, L., Hu, K., Cao, J., Wang, P., Li, J., Zeng, K., He, X., Tu, P. F., Tong, T., & Han, L. (2019). lncRNA *miat* functions as a ceRNA to upregulate *sirt1* by sponging *miR-22-3p* in HCC cellular senescence. *Aging*, *11*(17), 7098–7122. https://doi.org/10.18632/aging.102240

[24] Guo, R., Liang, Y., Zou, B., Li, D., Wu, Z., Xie, F., Zhang, X., & Li, X. (2022). The Histone Acetyltransferase MOF Regulates SIRT1 Expression to Suppress Renal Cell Carcinoma Progression. *Frontiers in oncology*, *12*, 842967. https://doi.org/10.3389/fonc.2022.842967

[25] Qu, W., Wang, Y., Wu, Q., Liu, J., & Hao, D. (2015). Emodin inhibits HMGB1-induced tumor angiogenesis in human osteosarcoma by regulating SIRT1. *International journal of clinical and experimental medicine*, *8*(9), 15054–15064.

[26] El-Ashmawy, N. E., Khedr, E. G., Khedr, N. F., & El-Adawy, S. A. (2023). Suppression of epithelial-mesenchymal transition and SIRT1/AKT signaling pathway in breast cancer by montelukast. *International immunopharmacology*, *119*, 110148. https://doi.org/10.1016/j.intimp.2023.110148

[27] Lin, X. L., Li, K., Yang, Z., Chen, B., & Zhang, T. (2020). Dulcitol suppresses proliferation and migration of hepatocellular carcinoma via regulating SIRT1/p53 pathway. *Phytomedicine : international journal of phytotherapy and phytopharmacology*, *66*, 153112. https://doi.org/10.1016/j.phymed.2019.153112

[28] Ko, H. J., Park, S. Y., Sim, D. Y., Kim, S. H., Hur, S., Lee, J. H., & Kim, Y. (2024). Apoptotic Effect of Isoimpertorin via Inhibition of c-Myc and SIRT1 Signaling Axis. *International journal of molecular sciences*, *25*(8), 4248. https://doi.org/10.3390/ijms25084248

[29] Brockmueller, A., Buhrmann, C., Shayan, P., & Shakibaei, M. (2023). Resveratrol induces apoptosis by modulating the reciprocal crosstalk between p53 and Sirt-1 in the CRC tumor microenvironment. *Frontiers in immunology*, *14*, 1225530. https://doi.org/10.3389/fimmu.2023.1225530

[30] Eroglu, Z., Erdem, C., Oktem, G., Bozok Cetintas, V., & Duzgun, Z. (2020). Effect of SIRT1 activators and inhibitors on CD44+/CD133+-enriched non-small cell lung

cancer cells. *Molecular medicine reports*, *22*(1), 575–581. https://doi.org/10.3892/mmr.2020.11113

[31] Patra, S., Singh, A., Praharaj, P.P. *et al.* SIRT1 inhibits mitochondrial hyperfusion associated mito-bulb formation to sensitize oral cancer cells for apoptosis in a mtROS-dependent signalling pathway. *Cell Death Dis* **14**, 732 (2023). https://doi.org/10.1038/s41419-023-06232-x

[32] Yang, T., Zhou, R., Yu, S. *et al.* Cytoplasmic SIRT1 inhibits cell migration and invasion by impeding epithelial–mesenchymal transition in ovarian carcinoma. *Mol Cell Biochem* **459**, 157–169 (2019). https://doi.org/10.1007/s11010-019-03559-y

[33] Chao, SC., Chen, YJ., Huang, KH. *et al.* Induction of sirtuin-1 signaling by resveratrol induces human chondrosarcoma cell apoptosis and exhibits antitumor activity. *Sci Rep* **7**, 3180 (2017). https://doi.org/10.1038/s41598-017-03635-7

[34] Kang, Y. Y., Sun, F. L., Zhang, Y., & Wang, Z. (2018). SIRT1 acts as a potential tumor suppressor in oral squamous cell carcinoma. *Journal of the Chinese Medical Association : JCMA*, *81*(5), 416–422. https://doi.org/10.1016/j.jcma.2017.09.004

[35] Park, J. H., Park, S. A., Lee, Y. J., Joo, N. R., Shin, J., & Oh, S. M. (2020). TOPK inhibition accelerates oxidative stress-induced granulosa cell apoptosis via the p53/SIRT1 axis. *International journal of molecular medicine*, *46*(5), 1923–1937. https://doi.org/10.3892/ijmm.2020.4712

[36] Zhang, X., Li, Y., Wang, D. *et al.* miR-22 suppresses tumorigenesis and improves radiosensitivity of breast cancer cells by targeting Sirt1. *Biol Res* **50**, 27 (2017). https://doi.org/10.1186/s40659-017-0133-8

[37] Tan, P., Wang, M., Zhong, A. *et al.* SRT1720 inhibits the growth of bladder cancer in organoids and murine models through the SIRT1-HIF axis. *Oncogene* **40**, 6081–6092 (2021). https://doi.org/10.1038/s41388-021-01999-9

[38] Jiang, Y., Luo, Z., Gong, Y. *et al.* NAD^+ supplementation limits triple-negative breast cancer metastasis via SIRT1-P66Shc signaling. *Oncogene* **42**, 808–824 (2023). https://doi.org/10.1038/s41388-023-02592-y

[39] Ye, Z., Fang, B., Pan, J., Zhang, N., Huang, J., Xie, C., Lou, T., & Cao, Z. (2017). miR-138 suppresses the proliferation, metastasis and autophagy of non-small cell lung cancer by targeting Sirt1. *Oncology reports*, *37*(6), 3244–3252. https://doi.org/10.3892/or.2017.5619

[40] Liu, C. L., Zhao, D., Li, J. J., Liu, S., An, J. J., Wang, D., Hu, F. A., Qiu, C. Y., & Cui, M. H. (2022). Inhibition of glioblastoma progression by Urolithin A in vitro and in vivo by regulating Sirt1-FOXO1 axis via ERK/AKT signaling pathways. *Neoplasma*, *69*(1), 80–94. https://doi.org/10.4149/neo_2021_210623N834

[41] Li, X., Jiang, Z., Li, X., & Zhang, X. (2018). SIRT1 overexpression protects non-small cell lung cancer cells against osteopontin-induced epithelial-mesenchymal transition by suppressing NF-κB signaling. *OncoTargets and therapy*, *11*, 1157–1171. https://doi.org/10.2147/OTT.S137146

[42] Wang, J., Li, J., Cao, N., Li, Z., Han, J., & Li, L. (2018). Resveratrol, an activator of SIRT1, induces protective autophagy in non-small-cell lung cancer via inhibiting Akt/mTOR and activating p38-MAPK. *OncoTargets and therapy*, *11*, 7777–7786. https://doi.org/10.2147/OTT.S159095

[43] Kabra, N., Li, Z., Chen, L., Li, B., Zhang, X., Wang, C., Yeatman, T., Coppola, D., & Chen, J. (2009). SirT1 is an inhibitor of proliferation and tumor formation in colon cancer. *The Journal of biological chemistry*, *284*(27), 18210–18217. https://doi.org/10.1074/jbc.M109.000034

[44] Wang, R. H., Zheng, Y., Kim, H. S., Xu, X., Cao, L., Luhasen, T., Lee, M. H., Xiao, C., Vassilopoulos, A., Chen, W., Gardner, K., Man, Y. G., Hung, M. C., Finkel, T., & Deng, C. X. (2008). Interplay among BRCA1, SIRT1, and Survivin during BRCA1-associated tumorigenesis. *Molecular cell*, *32*(1), 11–20. https://doi.org/10.1016/j.molcel.2008.09.011

[45] "The Cost Of Cancer | SERO," June 23, 2022. https://treatcancer.com/blog/cost-of-cancer/.

[46] "The Rise of Cancer Cases," February 8, 2024. https://www.medicalnewstoday.com/articles/an-estimated-35-million-new-cancer-cases-to-occur-in-2050-who-warns.

[47] "Types of Cancer Treatment - NCI." cgvMiniLanding, July 31, 2017. Nciglobal,ncienterprise. https://www.cancer.gov/about-cancer/treatment/types.

[48] Devlin, E. J., Denson, L. A., & Whitford, H. S. (2017). Cancer Treatment Side Effects: A Meta-analysis of the Relationship Between Response Expectancies and Experience. *Journal of pain and symptom management*, *54*(2), 245–258.e2. https://doi.org/10.1016/j.jpainsymman.2017.03.017

[49] Weiss C. (2021). One in Four Dies of Cancer. Questions About the Epidemiology of Malignant Tumours. *Recent results in cancer research. Fortschritte der Krebsforschung. Progres dans les recherches sur le cancer*, *218*, 15–29. https://doi.org/10.1007/978-3-030-63749-1_2

[50] Vaziri, H., S. K. Dessain, E. Ng Eaton, S. I. Imai, R. A. Frye, T. K. Pandita, L. Guarente, and R. A. Weinberg. "hSIR2(SIRT1) Functions as an NAD-Dependent P53 Deacetylase." Cell 107, no. 2 (October 19, 2001): 149–59. https://doi.org/10.1016/s0092-8674(01)00527-x.

[51] Leeuwen, Ingeborg van, and Sonia Lain. "Sirtuins and P53." Advances in Cancer Research 102 (2009): 171–95. https://doi.org/10.1016/S0065-230X(09)02005-3.

[52] Langley, Emma, Mark Pearson, Mario Faretta, Uta-Maria Bauer, Roy A. Frye, Saverio Minucci, Pier Giuseppe Pelicci, and Tony Kouzarides. “Human SIR2 Deacetylates P53 and Antagonizes PML/P53-Induced Cellular Senescence.” The EMBO Journal 21, no. 10 (May 15, 2002): 2383–96. https://doi.org/10.1093/emboj/21.10.2383.

[53] Luo, J., A. Y. Nikolaev, S. Imai, D. Chen, F. Su, A. Shiloh, L. Guarente, and W. Gu. “Negative Control of P53 by Sir2alpha Promotes Cell Survival under Stress.” Cell 107, no. 2 (October 19, 2001): 137–48. https://doi.org/10.1016/s0092-8674(01)00524-4.

[54] Lin, Zhenghong, Heeyoung Yang, Qingfei Kong, Jinping Li, Sang-Myeong Lee, Beixue Gao, Hongxin Dong, et al. “USP22 Antagonizes P53 Transcriptional Activation by Deubiquitinating Sirt1 to Suppress Cell Apoptosis and Is Required for Mouse Embryonic Development.” Molecular Cell 46, no. 4 (May 25, 2012): 484–94. https://doi.org/10.1016/j.molcel.2012.03.024.

[55] Geng, Jiefei, and Daniel J. Klionsky. “The Atg8 and Atg12 Ubiquitin-like Conjugation Systems in Macroautophagy. ‘Protein Modifications: Beyond the Usual Suspects’ Review Series.” EMBO Reports 9, no. 9 (September 2008): 859–64. https://doi.org/10.1038/embor.2008.163.

[56] Lee, In Hye, Liu Cao, Raul Mostoslavsky, David B. Lombard, Jie Liu, Nicholas E. Bruns, Maria Tsokos, Frederick W. Alt, and Toren Finkel. “A Role for the NAD-Dependent Deacetylase Sirt1 in the Regulation of Autophagy.” Proceedings of the National Academy of Sciences of the United States of America 105, no. 9 (March 4, 2008): 3374–79. https://doi.org/10.1073/pnas.0712145105.

[57] Salminen, Antero, and Kai Kaarniranta. “SIRT1: Regulation of Longevity via Autophagy.” Cellular Signalling 21, no. 9 (September 2009): 1356–60. https://doi.org/10.1016/j.cellsig.2009.02.014.

[58] Chen, Lin-feng, Yajun Mu, and Warner C. Greene. “Acetylation of RelA at Discrete Sites Regulates Distinct Nuclear Functions of NF-kappaB.” The EMBO Journal 21, no. 23 (December 2, 2002): 6539–48. https://doi.org/10.1093/emboj/cdf660.

[59] Kiernan, Rosemary, Vanessa Brès, Raymond W. M. Ng, Marie-Pierre Coudart, Selma El Messaoudi, Claude Sardet, Dong-Yan Jin, Stephane Emiliani, and Monsef Benkirane. “Post-Activation Turn-off of NF-Kappa B-Dependent Transcription Is Regulated by Acetylation of P65.” The Journal of Biological Chemistry 278, no. 4 (January 24, 2003): 2758–66. https://doi.org/10.1074/jbc.M209572200.

[60] Yang, Xiao-Dong, Emad Tajkhorshid, and Lin-Feng Chen. “Functional Interplay between Acetylation and Methylation of the RelA Subunit of NF-kappaB.” Molecular and Cellular Biology 30, no. 9 (May 2010): 2170–80. https://doi.org/10.1128/MCB.01343-09.

[61] Yeung, Fan, Jamie E Hoberg, Catherine S Ramsey, Michael D Keller, David R Jones, Roy A Frye, and Marty W Mayo. “Modulation of NF-κB-Dependent Transcription

and Cell Survival by the SIRT1 Deacetylase." The EMBO Journal 23, no. 12 (June 16, 2004): 2369–80. https://doi.org/10.1038/sj.emboj.7600244.

[62] Motta, Maria Carla, Nullin Divecha, Madeleine Lemieux, Christopher Kamel, Delin Chen, Wei Gu, Yvette Bultsma, Michael McBurney, and Leonard Guarente. "Mammalian SIRT1 Represses Forkhead Transcription Factors." Cell 116, no. 4 (February 20, 2004): 551–63. https://doi.org/10.1016/s0092-8674(04)00126-6.

[63] Nemoto, Shino, Maria M. Fergusson, and Toren Finkel. "SIRT1 Functionally Interacts with the Metabolic Regulator and Transcriptional Coactivator PGC-1{alpha}." The Journal of Biological Chemistry 280, no. 16 (April 22, 2005): 16456–60. https://doi.org/10.1074/jbc.M501485200.

[64] Yang, Yonghua, Huayan Hou, Edward M. Haller, Santo V. Nicosia, and Wenlong Bai. "Suppression of FOXO1 Activity by FHL2 through SIRT1-Mediated Deacetylation." The EMBO Journal 24, no. 5 (March 9, 2005): 1021–32. https://doi.org/10.1038/sj.emboj.7600570.

[65] van der Horst, A., Tertoolen, L. G. J., de Vries-Smits, L. M. M., Frye, R. A., Medema, R. H., & Burgering, B. M. T. (2004). FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1). *The Journal of Biological Chemistry*, *279*(28), 28873–28879. https://doi.org/10.1074/jbc.M401138200

[66] Massagué, J. "TGF-Beta Signal Transduction." Annual Review of Biochemistry 67 (1998): 753–91. https://doi.org/10.1146/annurev.biochem.67.1.753.

[67] Massagué, J., S. W. Blain, and R. S. Lo. "TGFbeta Signaling in Growth Control, Cancer, and Heritable Disorders." Cell 103, no. 2 (October 13, 2000): 295–309. https://doi.org/10.1016/s0092-8674(00)00121-5.

[68] Xu, B., Chen, H., Xu, W., Zhang, W., Buckley, S., Zheng, S. G., Warburton, D., Kolb, M., Gauldie, J., & Shi, W. (2012). Molecular mechanisms of MMP9 overexpression and its role in emphysema pathogenesis of Smad3-deficient mice. *American journal of physiology. Lung cellular and molecular physiology*, *303*(2), L89–L96. https://doi.org/10.1152/ajplung.00060.2012

[69] Kume, Shinji, Masakazu Haneda, Keizo Kanasaki, Toshiro Sugimoto, Shin-ichi Araki, Keiji Isshiki, Motohide Isono, et al. "SIRT1 Inhibits Transforming Growth Factor β-Induced Apoptosis in Glomerular Mesangial Cells via Smad7 Deacetylation *." Journal of Biological Chemistry 282, no. 1 (January 5, 2007): 151–58. https://doi.org/10.1074/jbc.M605904200.

[70] Li, Jinhua, Xinli Qu, Sharon D. Ricardo, John F. Bertram, and David J. Nikolic-Paterson. "Resveratrol Inhibits Renal Fibrosis in the Obstructed Kidney." The American Journal of Pathology 177, no. 3 (September 2010): 1065–71. https://doi.org/10.2353/ajpath.2010.090923.

[71] Baur, Joseph A., and David A. Sinclair. “Therapeutic Potential of Resveratrol: The in Vivo Evidence.” Nature Reviews. Drug Discovery 5, no. 6 (June 2006): 493–506. https://doi.org/10.1038/nrd2060.

[72] Pervaiz, Shazib, and Andrea Lisa Holme. “Resveratrol: Its Biologic Targets and Functional Activity.” Antioxidants & Redox Signaling 11, no. 11 (November 2009): 2851–97. https://doi.org/10.1089/ars.2008.2412.

[73] Lain, Sonia, Jonathan J. Hollick, Johanna Campbell, Oliver D. Staples, Maureen Higgins, Mustapha Aoubala, Anna McCarthy, et al. “Discovery, In Vivo Activity, and Mechanism of Action of a Small-Molecule P53 Activator.” Cancer Cell 13, no. 5–2 (May 6, 2008): 454–63. https://doi.org/10.1016/j.ccr.2008.03.004.

[74] Solomon, Jonathan M., Rao Pasupuleti, Lei Xu, Thomas McDonagh, Rory Curtis, Peter S. DiStefano, and L. Julie Huber. “Inhibition of SIRT1 Catalytic Activity Increases P53 Acetylation but Does Not Alter Cell Survival Following DNA Damage.” Molecular and Cellular Biology 26, no. 1 (January 2006): 28–38. https://doi.org/10.1128/MCB.26.1.28-38.2006.

[75] Li, Huige. “Sirtuin 1 (SIRT1) and Oxidative Stress.” In Systems Biology of Free Radicals and Antioxidants, edited by Ismail Laher, 417–35. Berlin, Heidelberg: Springer, 2014. https://doi.org/10.1007/978-3-642-30018-9_17.

[76] Turkmen, Kultigin. “Chapter 4 - Sirtuins in Diabetes Mellitus and Diabetic Kidney Disease.” In Sirtuin Biology in Cancer and Metabolic Disease, edited by Kenneth Maiese, 61–77. Academic Press, 2021. https://doi.org/10.1016/B978-0-12-822467-0.00014-0.

[77] Lin, Z., & Fang, D. (2013). The Roles of SIRT1 in Cancer. *Genes & cancer*, *4*(3-4), 97–104. https://doi.org/10.1177/1947601912475079

[78] Sun, M., Du, M., Zhang, W., Xiong, S., Gong, X., Lei, P., Zha, J., Zhu, H., Li, H., Huang, D., & Gu, X. (2019). Survival and Clinicopathological Significance of SIRT1 Expression in Cancers: A Meta-Analysis. *Frontiers in endocrinology*, *10*, 121. https://doi.org/10.3389/fendo.2019.00121

[79] Deng C. X. (2009). SIRT1, is it a tumor promoter or tumor suppressor?. *International journal of biological sciences*, *5*(2), 147–152. https://doi.org/10.7150/ijbs.5.147

[80] Bosch-Presegué, L., & Vaquero, A. (2011). The dual role of sirtuins in cancer. *Genes & cancer*, *2*(6), 648–662. https://doi.org/10.1177/1947601911417862