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The expression of Sirtuin1 and its role in ovarian cancer

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1. Abbreviations

Aβ	Amyloid-beta
Akt	protein kinase B
AMPK	5′ AMP-activated protein kinase
AP-1	activator protein-1
ATGL	adipose triglyceride lipase
Bclaf1	Bcl-2 associated transcription factor 1
BRCA1	breast cancer type 1
CR	caloric restriction
CRC	colorectal cancer
DBC1	deleted in breast cancer 1
DDR	DNA damage response
DOR	diabetes- and obesity-regulated nuclear factor
dUTP	deoxyuridine triphosphate
eNOS	nitric oxide synthase 3
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GATA3	GATA binding protein 3
GSH-PX	glutathione peroxidase
HCC	hepatocellular carcinoma
HDACs	histone deacetylases
HIF-1α	hypoxia-inducible factor-1α
HMGB1	high mobility group box 1 protein
HO-1	heme oxygenase-1
IFNγ	interferon-gamma

iNOS	inducible nitric oxide synthase
IRE1α	inositol-requiring enzyme 1 α
KLF4	Kruppel-like factor 4
LC3	Microtubule-associated protein 1A/1B-light chain 3
MDM2	mouse double minute 2 homolog
MDSCs	myeloid-derived suppressor cells
MMP-2	matrix metalloproteinase-2
mTOR	mammalian target of rapamycin
NAD	nicotinamide adenine dinucleotide
NAMPT	nicotinamide phosphoribosyltransferase
NRFs	nuclear respiratory factor
Nrf2	nuclear factor erythroid 2-related factor 2
NSCLC	non-small cell lung cancer
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
N-Myc	basic helix-loop-helix protein 37
PARP	Poly adenosine diphosphate ribose polymerase
PBS	Phosphate-buffered saline
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PPARα	proliferator-activated receptor alpha
PPARγ	proliferator-activated receptor gama
PVDF	Polyvinylidene difluoride
RIPA buffer	Radioimmunoprecipitation assay buffer
RSV	Resveratrol, 3,5,4'-trihydroxystilbene
RXR	retinoid X receptor

SIRT1	Silent mating type information regulation 2 homolog 1
SOD	superoxide dismutase
SOX2	sex determining region Y-box transcription factor 2
TdT	terminal deoxynucleotidyl transferase
Tfam	mitochondrial transcription factor A
TNF-α	tumor necrosis factor α
TopBP1	topoisomerase binding protein 1
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
VDR	vitamin D receptor
XRCC1	X-ray repair cross-complementing protein 1
YAP	Yes-associated protein

2. Figure legends

3. Table legends

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4. Publication list

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Sirtuin1 Expression and Survival in Endometrial and Clear-Cell Uterine Cancer

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5. Introduction

5.1 SIRT1 localization

Silent mating type information regulation 2 homolog 1 (SIRT1) was discovered at yeast in the 1990s [1]. Subsequently, it was widely recognized in bacteria, yeasts, plants, and animals [2]. In 2013, the crystal structure of SIRT1 was identified [3]. SIRT1 is a member of the Sirtuin group, which belongs to the family of histone deacetylases (HDACs). This family shares a conserved catalytic core domain with nicotinamide adenine dinucleotide- dependent protein deacetylase activity. Nicotinamide adenine dinucleotide (NAD+) serves as a connection with oxidative stress, longevity, DNA damage and repair, caloric restriction (CR), exercise, inflammation, etc. And it extends the life span [4]. Few years later, Sirtuins were studied at molecular, cellular, and organismal levels. Although the members of the Sirtuin group share a relatively similar conserved catalytic core domain, their N- and C-terminal extensions vary. Therefore, their divergent biological functions such as subcellular localization, enzymatic activities and binding substrates are ascribed to the varying terminal extensions. SIRT2 is primarily a cytosolic protein; SIRT3, SIRT4, and SIRT5 are dominantly present in mitochondria, while SIRT6 and SIRT7 exist in the nucleus. The subcellular localization of SIRT1 has been believed in debate [5,6]. Most scientists esteemed that SIRT1 shuttled between nucleus and cytoplasm; however, a new study has shown that SIRT1’s reside in nuclear localization and conventional cell fractionation makes SIRT1 in nucleus leaking to the cytoplasm [7]. The function of SIRT1 as deacetylation of histones leads to DNA coiling and gene silencing [8]. Through deacetylation of histone proteins by rising positive charge on the histone proteins, it enhances DNA affinity to histone proteins. As a result, it forms a condensed chromatin suppression transcription [9].

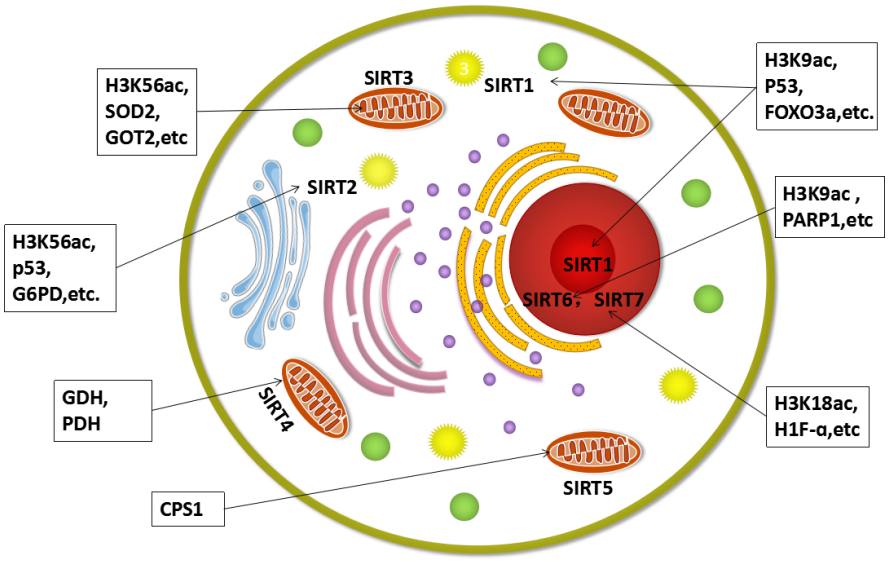


Figure 1. The subcellular localizations of the Sirtuin group and their substrates targets. SIRT1 is localized in the nucleus and cytoplasm. SIRT2 floats in the cytoplasm. SIRT3, SIRT4, and SIRT5 present in mitochondrial. SIRT6 and SIRT7 are dominantly in the nucleus. Adapted from:[10,11]

5.2 Interaction between SIRT1 and proteins

Compared to other Sirtuins, SIRT1 has been the most studied regulator during the last three decades. SIRT1 is a central pivotal interacting factor with plenty of complex networks of proteins that finally modulates plenty of biological processes [12]. Human SIRT1 interacts with different proteins and it plays an critical role in deciding cellular fate through DNA damage and repair mechanism, cell cycle regulation, energy metabolism, autophagy, apoptosis and others [13-18].

p53 and SIRT1

p53 functions as “the guardian of the genome“ by conserving genomic stability [19]. During the abundant nutrient environment, p53 represses the *SIRT1* gene promoter and decreases *SIRT1* activity [20]. p53 and Yes-associated protein (YAP) crosstalk via SIRT1 modulates cell G0/G1 arrest and apoptosis [21]. SIRT1 impedes the apoptotic progress of neurons by deacetylating and repressing p53 activity [22]. Heme oxygenase-1 (HO-1) positively regulates SIRT1, which modulates macrophage

activation through the downstream SIRT1-p53 signaling pathway and regulates hepatocellular death [23]. Besides, the SIRT1-p53 signaling pathway promotes the apoptotic death [24,25], the pathogenesis of diabetic [26], and cancer cell sensitivity [27]. The SIRT1-p53 regulatory axis has multiple roles in aging-related diseases and cellular reprogramming [28], the fancy mechanism and basic principles for SIRT1 interacting with p53 should be investigated further.

NF-κB and SIRT1

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is an essential upregulating protein that controls cell survival. Scientists found that SIRT1 closely cross-talks with NF-κB which aroused their interest, since NF-κB regulates innate immunity defense and SIRT1 modulates the oxidative respiration of cell [14,29]. Further evidence suggests that NF-κB activates glycolytic metabolism in acute inflammation progress. SIRT1 silences the NF-κB and stimulates oxidative metabolic processes that contribute to suppressing inflammation [14]. In addition, NF-κB decreases SIRT1 deacetylase activity by interferon-gamma (IFNγ) [30]. Therefore, the interaction between NF-κB and SIRT1 is quite well understood.

AMPK and SIRT1

5′ AMP-activated protein kinase (AMPK) modulates the cellular metabolism and energy expenditure through modulating the functional activity of SIRT1. More specifically, SIRT1 can activate AMPK and AMPK can activate SIRT1. So, AMPK and SIRT1 could interact with each other and share many downstream proteins and factors [31]. Allyl isothiocyanate, irisin, pinolenic acid, tilianin, resveratrol, and others are possibly related to activation of AMPK and SIRT1, influencing inflammation, apoptosis, oxidative stress, and energy metabolism [32-37]. SIRT1 interacts with resveratrol to stimulate AMPK in mitochondria [38]. Long-term exercise directly

activates lysosome biogenesis through ascending AMPK-SIRT1 signaling and altering the autophagy/lysosome system [39].

The interaction between SIRT1 and AMPK can promote endocrine β-cell recovery and progenitor cell differentiation, and enhance the insulin sensitivity of peripheral skeletal muscle [40,41]. It is also reported that AMPK can be activated independently of SIRT1 [42].

PGC-1α and SIRT1

Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is be known as regulating mitochondrial function such as biogenesis and energy metabolism [43]. SIRT1 deacetylates PGC-1α and its co-activator peroxisome proliferator-activated receptor alpha (PPARα) [44,45] finally altering the transcriptional activity of PGC-1α in the skeletal muscle and liver [46,47]. SIRT1/PGC-1α signaling is involved in metabolic control [48] and oxidative stress [49]. In addition, AMPK and SIRT1 are gatekeepers of mitochondria biosynthesis: AMPK, SIRT1, and PGC-1α form an orchestrated metabolic homeostasis network [46].

SIRT1 bonding with PGC-1α enhances tissue antioxidant capacity and increases the level of superoxide dismutase (SOD) as well as glutathione peroxidase (GSH-PX) in cellular [46,50,51]. SIRT1 rules the acetylation of PGC-1α and dominates its downstream nuclear transcription factors and proteins such as nuclear respiratory factor (NRFs), further shaping mitochondrial biogenesis and performance [52]. In addition, SIRT1 is necessary for mitochondrial biogenesis and oxidative metabolism in cells, for example, induction of PGC-1α/PPARα signaling pathway to increased adipose triglyceride lipase (ATGL)-mediated lipolysis [53].

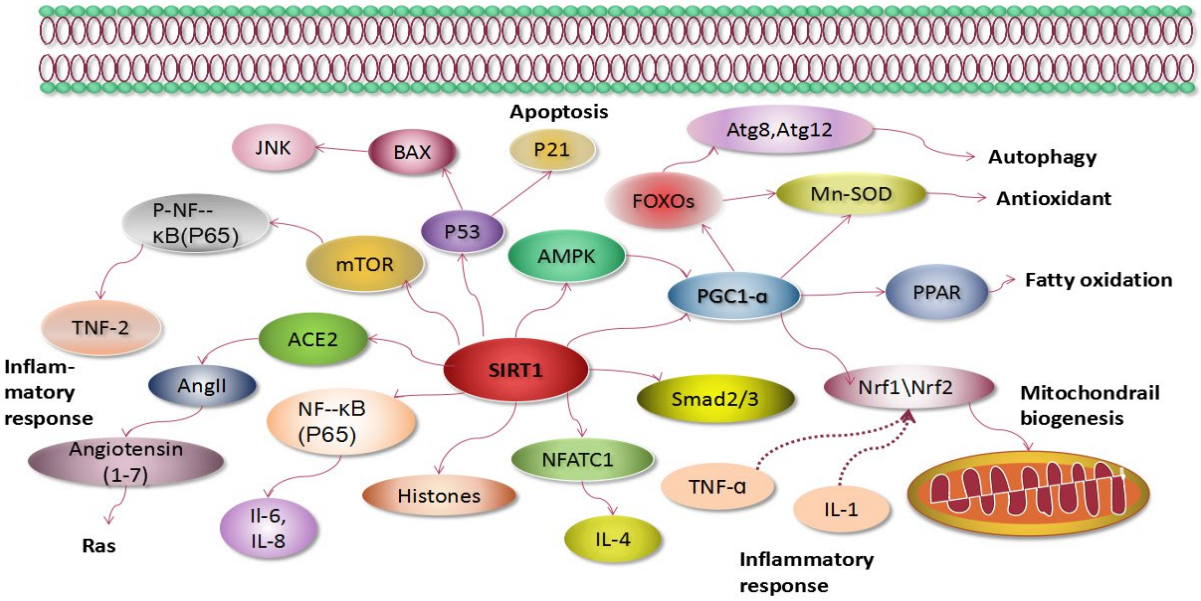


Figure 2. SIRT1 cell signaling pathways. →, positive regulation; ---ł, negative regulation. ACE2, Angiotensin-converting enzyme 2;Atg8, Autophagy-related protein 8; Atg12, Autophagy-related protein 12; AMPK, AMP-activated protein kinase; AngII, Angiotensin II; BAX, Bcl-2-associated X protein; FOXO, forkhead box O; IL-1, interleukin-1; IL-4, interleukin-4; IL-6, interleukin-6; IL-8,interleukin-8; JNK, Jun amino-terminal kinases; MnSOD, manganese superoxide dismutase; mTOR, mammalian target of rapamycin; NFATC1, Nuclear Factor Of Activated T Cells 1; Nrf2, nuclear factor erythroid 2-related factor 2; NF-κB, nuclear factor-kappa B; PPARα, peroxisome proliferator-activated receptor coactivator 1-α; PGC1-α, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; SIRT, sirtuin1; Smad2, SMAD Family Member 2 ;TNF-2, Tumor necrosis factor receptor 2. Adapted from: [54]

5.3 Activator for SIRT1: Resveratrol

One activator of SIRT1 is resveratrol (3,5,4'-trihydroxystilbene; RSV). It is a plant phenol and originates from the grapes, berries and peanuts [55]. Resveratrol is an effective agent in antimicrobial, antioxidant, anti-inflammatory and anti-cancer therapy [56,57]. It also has been demonstrated that resveratrol stimulates SIRT1 through PGC-1α-mediated mitochondrial biogenesis, and as a result, ameliorates cardiac injuries in diabetic cardiomyopathy [58]. Further evidence proved that resveratrol inhibits cell apoptosis by ischemia/reperfusion. As mechanism, resveratrol is involved in the cardioprotective effects through increasing SIRT1-p53 signaling activation [59]. For example, resveratrol upregulates SIRT1 and alleviates acute hepatotoxicity [60]. Resveratrol also has anti-inflammatory and antioxidant function by increasing the

SIRT1 level in neuroprotection [22]. A new study revealed that resveratrol activates SIRT1 to inhibit osteoarthritis disease progression [61]. Besides that, resveratrol has an anti-hyperuricemia function in mice [62]. Shatti pointed out that resveratrol protects against cadmium chloride-induced hippocampal neurotoxicity through activating SIRT1-AMPK signaling pathway [35]. Even though plenty of studies have shown the therapeutic effect of resveratrol, the weakness of this compound should be considered: Resveratrol has a high oral absorption (75%) but a low bioavailability (less than 1%) [63]. Therefore, more approaches to resveratrol's pharmaceutical formulation should be investigated [64]. In addition, this compound has been examined in phase 1 and phase 2 clinical trials [65-67]. So, the application of resveratrol in clinical practice is foreseeable within the next years. SRT1720, SRT1460, SRT2183, and SRT2104 are all derivatives of an imidazothiazole scaffold and 1,000-fold more potent than resveratrol [68].

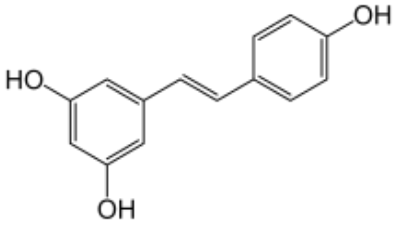


Figure 3. The structure of resveratrol. From:[69]

5.4 Inhibitor for SIRT1: EX527

EX527 has been identified as a highly selective small-molecule inhibitor against SIRT1 [70]. Treatment with EX527 leads to suppression of SIRT1 activity, which enhances p53 acetylation, while it does not change human cell line performance following DNA damage [71]. On the one hand, EX527 activated p53 and its downstream proteins contribute to apoptotic death in gliomas [72]. On the other hand, EX527 was proved harmful to the early development of vertebrate embryos, it could induce neural tube defects and other abnormalities and malformations [73]. Recently,

EX527 (SEN0014196) has been used in Huntington's disease Phase II clinical trial[74]. As a weak SIRT1 inhibitor, nicotinamide reacts with the α-1′-O-alkylamidate intermediate to produce plenty of Nε-acyl-lysine substrates [75]. Except for the above compounds, Wang summarized thoroughly the other SIRT1 inhibitor from the structures and their activities [76].

5.5 Function of SIRT1

5.5.1 SIRT1 and cellular metabolic process and mitochondrial biogenesis

In the central nervous system, SIRT1 is strongly participated in tissue and cell metabolism of vertebrates. Caloric restriction (CR) is an efficiently dietary intervention to delay aging and extending life arrange from yeasts to primates. SIRT1 is involved in caloric restriction through the functioning of the hypothalamic-pituitary axis [77]. For instance, SIRT1 deacetylates proteins and factors in the ventromedial hypothalamic nucleus [78] and pro-opiomelanocortin neurons [79]. As a result, it retains mammal energy homeostasis maintenance on the neuroendocrine system. In peripheral tissues, SIRT1 is involved in metabolic control. PGC-1α acts as a central inducer in oxidative metabolism and mitochondrial biogenesis in cells. As mentioned previously, SIRT1 interacts with PGC-1α and this affects cellular metabolism and mitochondrial transcription. Puigserver et al. showed that SIRT1 bonds with PGC-1α to enhance the production of hepatic gluconeogenesis during fasting. Similarly, the deacetylation of PGC-1α by SIRT1 is essential for the activation of fatty acid oxidation genes in peripheral skeletal muscle mitochondria [16,45]. Wu et al. proved that SIRT1 is involved in β-cell regeneration of patients with diabetes. They found that SIRT1 enhances β-cell regeneration by activating AMPK mediated fatty acid oxidation to activate endocrine progenitor cell differentiation [40]. Plenty of studies provided

dynamical insights as SIRT1 deacetylation of PGC-1α [46-48].

5.5.2 SIRT1 and DNA repair

Evidence proved that SIRT1 serves as a central regulator of DNA repair and DNA damage response (DDR) in the nucleus and that mitochondria restore chromatin to maintain genomic stability. SIRT1 phosphorylation delicately modifies the frequency of replication origins and prevents over-replication [80]. Homo sapiens SIRT1 could modulate DNA binding and stable DNA replication factor, while both have synergistically effect on the DNA replication fork initiation [81]. SIRT1 deacetylates DNA topoisomerase binding protein 1 (TopBP1), resulting in DNA replication inhibition and checkpoint inactivation [82].

SIRT1 recruits plenty of DNA repair proteins to cope with DNA damage response process. For instance, SIRT1 deacetylated Nibrin (a protein associated with double strand DNA break repair), Ku70, and other proteins related to DNA repair. So it acts as a mediator in various aspects of the DNA damage response in a dynamic regulation [83-88]. In addition, Oberdoerffer and his colleagues observed that redistribution of SIRT1 and other intracellular chromatin-modifying proteins in the nucleus under various pressure from intracellular and extracellular ultimately contributes to DNA breaks repair [89]. SIRT1 can manipulate the chromatin state by modulating epigenetic change and gene expressions such as the histone modifications H4K16Ac and H3K4me3 [90]. SIRT1 inhibition induces p53 activation, which increases DNA damage and decreases levels of DNA repair enzymes in human embryonic stem cells. As a consequence, it results in apoptosis of cells [91]. SIRT1 deacetylates mouse double minute 2 homolog (MDM2), inducing MDM2 degradation and thereby prevents p53 degradation as well [92]. In epigenetics, SIRT1 serves as

an essential epigenetic regulator to restore DNA damage [93].

5.5.3 SIRT1 and the modulation of autophagy

Accumulating evidence has proved that SIRT1 regulates autophagy in the cellular process [94-98]. Microtubule-associated protein 1A/1B-light chain 3 (LC3) acts as an initiator of autophagy. Under the insufficient external nutrients environment, SIRT1 deacetylation of LC3 subsequently formed LC3 and diabetes- and obesity-regulated nuclear factor (DOR) complex, leading to LC3-DOR complex shuttle from nuclear to cytoplasm. Furthermore, SIRT1 deacetylates LC3 and other autophagy factors. As a result, autophagosomy is formed [99]. SIRT1 activates AMPK and inhibits inositol-requiring enzyme 1 α (IRE1α). Consequently, it advanced autophagy processes and decreased apoptosis hedge from cell hypoxic stress [100]. SIRT1 is essential for adipose triglyceride lipase (ATGL)-mediated signaling to promotion autophagy. Meanwhile, SIRT1 mediates the effects of ATGL to manipulate hepatic lipid metabolism [101].

5.5.4 SIRT1, cell senescence and aging processes

SIRT1 is involved in increasing life span and delays aging in saccharomyces cerevisiae, drosophila, and in rodents [102-104]. Loss of SIRT1 shortens life span of Saccharomyces cerevisiae [105]. Standard diet with resveratrol in mice did not extend lifespan but delayed age-related deterioration [106,107]. As a key regulator protein, the level of SIRT1 in mRNA and protein is under close supervision. SIRT1 changed with age yet. Not only the level of SIRT1 altered with age in various tissue but also the activity of SIRT1 is decreased with age [26,108,109]. Hyperglycaemia-induced down-regulation of SIRT1 increases oxidative stress in diabetes-induced endothelial senescence [110]. However, the activation of SIRT1 can reverse aging. Gano et al. found out that SRT1720 (an activator of SIRT1) enhanced cyclooxygenase-2 level

and reduced excessive superoxide production as well as inflammation factor, which improves the outcome of vascular endothelial function in aging mice [111].

5.5.5 SIRT1 and the regulation of inflammation and immune

NF-κB remains a central protein of the inflammatory response. SIRT1 deacetylates p65 and modulates the transcription level of the inflammatory factors and cytokine such as interleukines and tumor necrosis factor α (TNF-α) [29,112,113]. There is a closely coordination crosstalk that occurs between NF-κB and SIRT1 in the manipulate of energy metabolism and cancer inflammation. NF-κB signaling stimulates glucose metabolism and increases the energy supply in the process of acute inflammation, while the activation of SIRT1 inhibits NF-κB in the inflammation resolution phases. SIRT1 activates AMPK, PPAR-α, and PGC-1α and as a result, it inhibits NF-κB and thereby suppresses inflammation process. Furthermore, NF-κB lessens SIRT1 activity and promotes inflammatory process by a high level of miR-34a, IFNγ, and ROS [14].

SIRT1 may contribute to the anti-inflammatory factors in neuroinflammation [114]. In pancreatic damage, SIRT1 reduces inflammation and oxidative stress while enhancing pancreatic β-cells' insulin release [115]. In airway inflammation, there seems to be a dual role of SIRT1 as proinflammatory and anti-inflammatory actor. For example, in asthma, proinflammatory actions of SIRT1 increase the level of hypoxia-inducible factor 1 α (HIF-1α) while decreasing proliferator-activated receptor gama (PPARγ) activity. The anti-inflammatory actions of SIRT1 decline the acetylation of GATA binding protein 3(GATA3) and expression of NF-κB [9]. Regarding CD4+ T-Cells, SIRT1 inhibits the promotor of Bcl-2 associated transcription factor 1(Bclaf1) after stimulation of the T-Cell receptor, thus regulating Bclaf1 in T-Cell development and homeostasis [116,117]. Inhibition of SIRT1

promotes IL-9-producing CD4+ T-Cell differentiation [118], while SIRT1 inhibitors enrich the function of Treg cells to support immune tolerance [119]. Transcription factors p65 also bonded with SIRT1 contributes to net immunosuppressive effects in T-Cells [120]. In CD8+ memory T-Cell, SIRT1 is linked to cell metabolic reprogramming [121].

SIRT1 plays an anti-inflammatory role in myeloid cells. As mentioned above, SIRT1 bonds with and deacetylates the NF-κB/p65. Knockout of SIRT1 renders hyperacetylated NF-κB and promotes an inflammatory response in mice [122]. Similar, in inflammatory bowel disease, the deletion of SIRT1 in macrophages prompts transcriptional activation of TNF-α [123]. Additionally, reduced expression of SIRT1 through deacetylating NF-κB may sustain aberrant chromatin structure and functions in chronic inflammation and cancer [124]. SIRT1 restrains the activity of activator protein-1(AP-1) as well as the level of cyclooxygenase-2, and it ameliorates macrophage function [125].

In innate immune response, SIRT1 regulates myeloid-derived suppressor cells (MDSCs) through NF-κB and PGC-1signaling to influence the MDSCs. In adaptive immune cells, SIRT1 mediates the differentiation of T-Cell subsets with other factors. Therefore, we can hold a viewpoint that the SIRT1 deacetylation makes a bridge between the innate immune response and adaptive immune response [126,127].

5.6 Role of SIRT1 in non-cancer diseases

SIRT1 has been considered promising therapeutic targets and received considerable attention in research in recent decades due to inflammation, metabolic disease, and neurodegeneration impact on the whole community with the aging population expand [9,128-130].

In cardiovascular diseases, dietary restriction is a useful intervention. SIRT1 as a

primary cardiovascular protective factor connected with mammalian target of rapamycin (mTOR), AMPK, and endothelial nitric oxide synthase constitutes a cardiovascular protective signal network [131]. Vitamin D (1,25(OH)2-D3) has a protective effect on diabetic cardiomyopathy through SIRT1-mediated signal pathway [132]. SIRT1 has a negative effect on restraining the development of cardiac hypertrophy through acetylation and phosphorylation of protein kinase C, zeta (PKC-ζ) [133]. While a new meta-analysis of available clinical trials has suggested that resveratrol supplementation does not bring any profit to cardiovascular risk factors [134].

In diabetes, SIRT1 serves as maintenance of glucose and lipid metabolism such as gluconeogenesis, insulin secretion, lipid synthesis, and cholesterol transport [135]. Therefore, SIRT1 is a promising pharmacological therapeutic approach to alleviate insulin resistance and treat type 2 diabetes mellitus. SIRT1 suppressing NF-κB signaling protects pancreatic β-cells from oxidative stress and inflammatory cytokines [136]. SIRT1 deacetylates PGC-1α and activates PPARα, promoting fatty acid oxidation as well as mitochondrial biogenesis and inducing adiponectin. As a consequence, it regulates metabolic homeostasis and reduces oxidative stress against insulin resistance, obesity, and diabetes [45,137-140]. Based on the experiment, more drugs and clinical trials can be expected to ameliorate diabetes.

In neurodegeneration disorder, the neuroprotective effect of SIRT1 has been proved in many neurological diseases such as ischemic stroke and age-related diseases. SIRT1 is widely distributed in neuronal and glial cells. SIRT1 modulates the multiple nerve cellular physiological function such as neural progenitor growth, axon elongation, and dendritic branching [141]. Loss of SIRT1 impairs memory function, weakens short-term memory and damages long-term memory [142]. In addition,

SIRT1 works as a critical mediator in synaptic plasticity, cognitive functions [142], blood-brain barrier permeability [143], and memory function [144]. SIRT1 reduces the formation of α-synuclein aggregates in Parkinson's disease patients [145]. Amyloid-beta (Aβ) peptides generation and accumulation in the brain leads to progressive neurodegenerative disorder and eventually impairment cognition. SIRT1 inhibiting NF-κB signaling results in decreasing amyloid-beta toxicity in microglia, which mitigates the progress of Alzheimer's disease [146]. In addition, SIRT1 combined with PGC-1α, p53, and tau, which protects neurons against oxidative stress, reduce mitochondrial dysfunction and prevent neuronal apoptosis [22,147-150].

In ischemic injury, evidence directly showed that the infarct area in SIRT1 knockout mice was increased compared to the control [151]. Lots of studies proved that treatment with the Sirt1-activator resveratrol in vivo and vitro could reduce ischemic infarcts, increase angiogenesis, decrease in oxidative stress, and enhance the level of glucose and adenosine triphosphate. Eventually, SIRT1 improved neurological functions [152-156].

5.7 Role of SIRT1 in cancer

Downregulation of SIRT1 expression in numerous human malignancies has been identified [157]. SIRT1 is closely connected to tumorigenesis and metastasis. The effect of SIRT1 on cancer remains complicated and controversial due to the fact that it serves either as a tumor suppressor or as a stimulator in cancer cells. The effect of SIRT1 on cancer highly depends on upstream or downstream factors and proteins as well as on its spatial distribution and tumor types [158,159]. AMPK and SIRT1 were considered as energy sensor net to regulate cancer cell metabolism [46,160]. As previously described, NF-κB and SIRT1 play a bridging role between innate immunity and energy metabolism. There is antagonistic crosstalk between immunity and

metabolism [14,161]. For cancer immunotherapy, it is possible to find an approach to cure cancer, because SIRT1 is involved in T-Cell differentiation, immune tolerance, and cell reprogramming [118,121,162].

5.7.1 SIRT1 as a tumor promotor

SIRT1 maintains cancer genomic instability and promotes cancer evolution by resisting cell apoptosis, sustaining proliferation signaling, evading growth suppressors, stimulating angiogenesis, promoting invasion, inducing metastasis, downregulating cellular energetics, and altering the cancer microenvironment, etc.

The increased expression of SIRT1 distributes in various cancer types such as breast cancer [163,164], prostate cancer [165,166], ovarian cancer, colon cancer [167], hepatocellular carcinoma (HCC) [168] etc. Furthermore, SIRT1 overexpression is connected with advanced tumors and distant metastases, which often implies poor clinical outcomes and prognosis [169]. It affects various cell processes through regulating numerous genes and proteins like p53, NF-κB, PGC1-α [170].

Breast cancer

SIRT1 is closely related to breast cancer. For example, SIRT1 inhibits p53 and activates DNA polymerase delta 1. As a consequence, it induces proliferation, metastasis, and increases aggressiveness of breast cancer [171].

Deleted in breast cancer 1 (DBC1) and SIRT1 is related to poor outcome for breast cancer [163]. Furthermore, DBC1 specifically inhibits SIRT1 and eventually induces apoptotic cell death [172]. SIRT1 could increase the level of matrix metalloproteinase-2 (MMP-2), and MMP-2 enhances tumor aggressiveness and distant metastasis by degrading the extracellular matrix. It restrains cancer cell apoptosis and enhances the ability to resist oxidative stress [173]. It is reported that HIF-1α is deacetylated and inactivated by SIRT1, and that decreased HIF-1α results

in elevated levels of aromatase in breast tissues in postmenopausal women [174].

Hepatic cancer

Hepatocellular carcinoma (HCC) is the second most lethal tumor globally [175]. A low level of SIRT1 is expressed in healthy people liver but SIRT1 is highly expressed in HCC cell lines and patients [176,177]. Chen and his group noticed that overexpression of SIRT1 elevated tumorigenesis and chemo resistance in HCC [168]. Numerous factors and proteins binding to SIRT1 influence the HCC cell behavior [178]. SIRT1 plays a substantial role not only in tumorigenesis, but also in differentiation, migration, and apoptosis by activating many signaling pathways [179,180].

High expression of SIRT1 in hepatocellular carcinoma stem cells (CSC) contributes to enhancing the self-renewal and tumorigenic potential of CSC [181]. The mechanism of inducing and maintaining self-renewal in hepatic CSC is also connected with SIRT1 regulation of sex determining region Y-box transcription factor 2 (SOX2). Furthermore, overexpression of exogenous SIRT1 restores the self-renewal potential of non-CSC [182]. In addition, the interaction between SIRT1 and the mitochondrial ribosomal protein S5 axis modulates the metabolic ability of hepatic CSC [183].

Lung cancer

Chen and his group member reported that overexpression of SIRT1 works as an activator in lung cancer tumorigenesis, and they demonstrated further that a high level of SIRT1 is connected with aggressiveness and metastasis in lung adenocarcinoma [184]. SIRT1 not only promotes metastasis in non-small cell lung cancer (NSCLC) but is also overexpressed in brain metastatic tissues of NSCLC [185]. The latest researches pointed out that a higher level of SIRT1 and AMPK are associated with the growth and development of NSCLC [186]. SIRT1 positively enhances tumor growth

and metastasis and plays a positive regulatory role.

For the issue of chemoresistance in lung cancer, SIRT1 elevates human lung cancer sensitivity of the anticancer effects to cisplatin [187]. SIRT1 deacetylates and stabilizes X-ray repair cross-complementing protein 1 (XRCC1) to increase the chemoresistance of lung cancer cells [188]. More research about SIRT1 is to be expected for drug resistance studies in tumors.

Colorectal cancer

Recent studies have suggested that SIRT1 is an appropriate marker for predicting poor outcomes in non-colorectal gastrointestinal cancer, but this phenomenon could not be observed in colorectal cancer (CRC) [189]. Several factors and proteins are involved in regulating SIRT1 to ultimately affect colorectal cancer cell behavior. Several factors and proteins are involved in the regulation of SIRT1 influence colorectal cancer cell behavior. Nicotinamide phosphoribosyltransferase (NAMPT), an enzyme converting nicotinamide, regulates colon cancer stem cell properties and resistance to therapy through SIRT1 and poly adenosine diphosphate ribose polymerase (PARP) [190]. SIRT1 is involved in hypoxia promotion colorectal cancer cell migration. Phosphorylated NF-κB expression promotes invasion and progression through SIRT11-inducing angiogenesis [191]. SIRT1 bonded with NF-κB decreases MMP-2 and promotes colorectal cancer cell invasion [192].

Prostate cancer

In prostate cancer, the increased SIRT1 expression induces neuroendocrine differentiation of prostate cancer through activating protein kinase B (Akt). Besides, the interaction between Akt and SIRT1 facilitates neuroendocrine prostate cancer tumorigenesis when basic helix-loop-helix protein 37 (N-Myc) is blocked [193]. Blyes group found that elevating of SIRT1 with decreased expression of E-cadherin in

prostate cancer thereby damages the epithelial morphology concomitant, and plenty of mesenchymal markers also increased, eventually contributing to prostate cancer cell metastasis [194].

5.7.2 SIRT1 as a tumor suppressor

As reviewed above, plenty of strong evidence exists to elucidate SIRT1 as a tumor promoter. However, some papers published that SIRT1 can also function as a tumor suppressor.

Breast cancer

Although numerous papers have demonstrated that there is an increase in SIRT1 in breast cancer development, a high level of SIRT1 has also been reported to inhibit breast cancer cell growth and proliferation. Rifai reported that an inverse relationship between SIRT1 overexpression and breast cancer aggressiveness [195]. In human breast carcinoma, SIRT1 showed a correlation to epigenetic markers such as H3K4ac, H3K9ac, and H4K16ac as a breast cancer-related gene promoters [196]. To find a promising therapeutic avenue to conquer breast cancer and to prolong survival years of patients, numerous compounds and treatment had been proposed. Treatment of doxorubicin combined with resveratrol effectively changes the fate of cancer with inhibiting cell growth, suppressing cell migration, and promoting cell apoptosis [197]. In addition, the same phenomenon was found in the treatment with doxorubicin combined with nicotinamide [198]. Also, Fatehi et al. demonstrated that activation of SIRT1 increased the efficacy and sensitivity of chemo-radio-therapy in triple-negative breast cancer, especially when patients were pretreated by Interleukin-6 [199].

Hepatic cancer

In the normal liver, SIRT1 modulates lipid homeostasis through peroxisome proliferators-activated receptor alpha (PPARα). SIRT1 interacts with PPARα and

activates PPARα coactivator PGC-1α. In knockout SIRT1 mice, various impaired liver functions such as hepatic steatosis and liver inflammation were observed [200]. In malignant HCC, SIRT1 and β-Catenin were co-overexpressed but SIRT1 suppressed Wnt-β-Catenin [201]. SIRT1 impacted on polarization M1-like macrophage through reinforcing infiltration and inhibited HCC metastasis. In addition, SIRT1 reinforced NF-κB stimulation, increasing its downstream phosphorylation of p65, and some kinases [202].

Therapeutic drugs have been suggested to suppress tumor development in the recent years. Resveratrol inhibits HCC cell proliferation and migration. This chemical compound elevated SIRT1 expression and decreased the downstream proteins to regulate post-translational modification [203]. Low dose of metformin prompted hepatoma cell senescence through activation of AMPK and inactivation of SIRT1. Functionally, AMPK negatively regulation of the level of SIRT1 prompted metformin-induced senescence in HCC xenografts [204].

Lung cancer

A recent study showed that SIRT1-positive patients with NSCLC had longer survival time [205]. SIRT1 overexpression postponed the appearance of K-Ras-driven lung adenocarcinomas, decreasing the number and size of carcinoma and extending survival time of mice [206]. SIRT1 inversely regulates the protein expression of the NF-κB signaling pathway, which facilitates apoptotic death of lung cancer cells [207]. In lung cancer, H_2O_2 activation of AMPK and subsequent SIRT1 phosphorylation inhibits the deacetylation activity of p53, which likewise regulates programmed cell death [208]. Resveratrol induced apoptosis and autophagy in lung cancer via inhibiting the mTOR and its downstream proteins [209]. In NSCLC, metformin combined with tenovin-6 inhibited SIRT1 expression induced caspase-3-dependent

apoptosis, increased p53 acetylation, and subsequently enhanced p53 stability, promoting apoptosis [210]. MiR-138 decreased SIRT1 levels to inactivity of AMPK and promoted the mTOR phosphorylation. At last, it lowers the level of SIRT1 and lifts autophagy of NSCLC [211].

Colorectal cancer

SIRT1 inhibits the proliferation and tumor formation of colorectal cancer (CRC) [212]. In the colorectal cancer, cells which expresses wild-type p53, SIRT1 inhibitors decreased the anti-tumor effects of multiple chemotherapeutic drugs [213]. Leptin induces SIRT1 expression via stimulating nuclear factor erythroid 2-related factor 2 (Nrf2) which induces obesity-associated colon carcinogenesis [214]. Butyrate suppresses the growth of cells and induces the apoptosis of CRC by inactivation of mTOR signaling. This process is mediated by SIRT1 downregulation [215].

Prostate cancer

Overexpressed SIRT1 reverses epithelial–mesenchymal transition and prevents prostate cancer progression. miR-204 targets SIRT1 and thus decreases deacetylation of p53 in prostate cancer cells. As a consequence, acetylated p53 upregulates the expression of apoptotic proteins followed by induction of mitochondrial apoptosis [216].

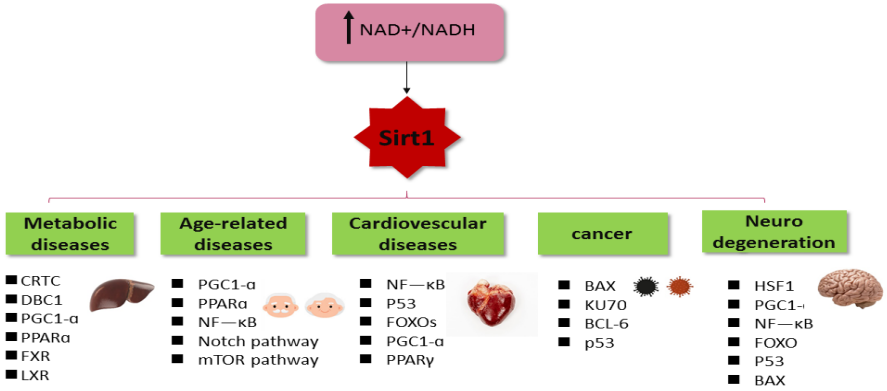


Figure 4. The major studied cytokines, proteins, and signaling pathway of SIRT1 in the metabolic diseases, age-related diseases, cardiovascular diseases, cancer, and neurodegeneration. Adapted from [22,28]

5.8 SIRT1 and RXR

Retinoid X receptor (RXR), nuclear hormone receptor, serves as a famous transcription factor. It promotes transcription of the downstream target gene. Three isoforms of RXR are represented in humans: RXRα, RXRβ, and RXRγ. Among these subtypes, RXRα is the first RXR subtype, whose structure was identified [217]. Despite the distributions of RXR subtypes are different, their functions are the same and overlap [218].

The RXR heterodimers can be divided into two main groups: permissive heterodimers (for example, RXR/PPAR) and non-permissive heterodimers (for instance, RXR/ Vitamin D receptor) [218]. The discrepancy between permissive and non-permissive heterodimers is whether these ligands interact strongly constitutively with RXR [219]. RXR and its numerous ligands hold a central position in genomic and non-genomic functions in the cell. RXR works as an important mediator in the development of certain cancers. It is overexpressed during cancer progression [220,221]. For example, RXR overexpression is found in 70% of ductal breast cancer [218]. A high level of RXR also is found in endometrial cancer [222].

Besides, there are many co-regulators between SIRT1 and RXR in cancer. The vitamin D receptor (VDR) is overexpressed in all gynecologic cancers and vitamin D-related signaling pathways predict the risk and clinical outcome of gynecologic cancers as well [223]. An interaction between RXR and VDR has been demonstrated, suggesting that they exert an effects on ovarian cancer [224]. SIRT1 enzymatically promotes 1,25-dihydroxyvitamin D 3 signaling through deacetylation of the vitamin D receptor [225]. Another example is PPARγ: RXR binds to PPARγ and they affect cancer biology and lipid metabolism [226,227]. PPARγ/SIRT1 participates in the progression of cancer cell behavior through activating mitochondrial dysfunction as

well [228]. However, the exact interaction between SIRT1 and RXR is not clear until now.

5.9 Aims of this study

SIRT1 is known for its participation in the modulation of many cellular physiological processes, including growth, migration, differentiation, and apoptosis. It plays an evolving role in certain cancers. However, the effect of SIRT1 on the survival and proliferation of ovarian cancer cells remains controversial issues, and its role in ovarian cancer is unclear. Besides, the treatment of ovarian cancer is still an unsolved problem, especially for platinum resistant ovarian cancer.

Therefore, it is necessary to study the function and effect of SIRT1 in ovarian cancer. Resveratrol, a well-known SIRT 1 activator can help us to understand the underlying mechanisms of SIRT1 activation in ovarian cancer. It also can serve as a target in cancer therapy.

Purpose: Role of SIRT1 and its overexpression in ovarian cancer.

Aims:

1. Examining the expression of SIRT1 in ovarian cancer and correlation with histopathological data including survival data
2. Treating ovarian cancer in vitro by resveratrol and collecting laboratory data to study the role of SIRT1 and its interaction with resveratrol.

6. Materials and methods

6.1 Clinical samples

After institutional ethics committee approval, we included 123 female patients ranged in age from 20 to 88 (median age was 59 years) who received a diagnosis of ovarian cancer, and who received surgery because of ovarian cancer. All the ovarian cancer specimens were collected for histopathological diagnostics during surgery. Because other ovarian histological subtypes samples were quite rare only serous and mucinous samples were included. As positive controls for immunohistochemically staining, palatine tonsil for SIRT1 and first-trimester placenta for retinoid X receptor (RXR) staining were used. The overall median survival year of the patients was 2.67 years. All the clinical samples were collected from 1990 to 2002 at the Department of Gynecology and Obstetrics, Ludwig-Maximilians-University of Munich, Germany. Histopathological data were cited by the original record from 1990-2002, resulting in a grading G1 to G3 for serous carcinomas (Today serous ovarian cancer is distinguished in high grade and low grade). The follow-up data for statistical analyses were provided by the Munich cancer registry and retrieved from medical records.

6.2 Ethics Approval

Ethical approval for this study was obtained from the local ethics committee of the Ludwig-Maximilians University of Munich (APPROVAL NUMBER 227-09 and 18-392). The study was performed and conducted conforming to the Declaration of Helsinki.

6.3 Chemicals and antibodies

A2780	ATCC, USA
ABC detection kit	Vectastain, USA
anti-SIRT1 rabbit IgG	Atlas Antibody, Sweden
anti-RXR rabbit IgG	PPMX, Japan
Bradford reagent	Bio-Rad, USA
BrdU (Bromodeoxyuridine) kit	Roche, Switzerland
BCIP/NBT -chromogen substrate solution	Vector, Germany
CASEIN	Vector, Germany
A2780cis	ATCC, USA
DMSO	SERVA, Germany
Elx800 universal Microplate Reader	BioTek,USA
Dulbecco's Phosphate Buffered Saline	Gibco, USA
Fetal Bovine Serum	Biochrom, Germany
FragEL™ DNA Fragmentation Detection Kit	MERCK, USA
GAPDH	GeneTex, USA
GelScan V6.0 1D Analysis Software	SERVA, Germany
HRP-Polymer-Kit (mouse/rabbit)	Zytomed Systems, Germany
Mini Protean 3 System	Bio-Rad, USA
Mini Trans-Blot Filtterpaper	Bio-Rad, USA
MTT	Sigma, USA

M30 CytoDeath	Roche, Switzerland
Methanol	J.T.Baker,Germany
Phosphate-buffered saline (PBS)	Sigma, USA
PVDF membrane	Roche, Switzerland
Precast Gel Mini-Protein TGX	Bio-Rad,USA
Protein Standard ladder	Fermentas,Germany
Resveratrol	Sigma, USA
RIPA buffer	Sigma-Aldrich, St. Luis, USA
Roti-Load1 4x-concentrated proben buffer	Roche, Switzerland
RPMI1640	Gibco, USA
Tris/Glycine/SDS 10 x buffer	Bio-Rad, USA
Tris/Glycine10 x buffer	Bio-Rad, USA
UWB1.289	ATCC, USA
Vectastain ABC-AmP Reagent	Bio-Rad, USA

6.4 Immunohistochemistry

Paraffin-embedded slides of 3μm were dewaxed in xylol. The slides were washed in 100% ethanol for 5 seconds. All the sample slides were immersed in 3% methanol/ hydrogen peroxide for 20 minutes and rehydrated in a descending row of alcohols. For heat-induced antigen retrieval, sodium citrate buffer (distilled water with 0.1 M citric acid + 0.1 M sodium citrate; pH=6.0) was utilized in a pressure cooker. After washing the slides with phosphate-buffered saline (PBS), blocked them for 30 minutes with a blocking solution. These slides were incubated with primary polyclonal anti-SIRT1 (rabbit,1:300 dilution) and polyclonal anti-RXR (rabbit,1:300 dilution) as

primary antibodies for 16 hours at 4°C overnight. After washing 3 times, the secondary antibodies of HRP-polymer were utilized to detect reactivity. HRP-conjugated secondary antibodies were used for 30 minutes at room temperature, followed by another washing step. Subsequently, the bound antibody complexes were detected by the chromogen-3,3'-diaminobenzidin (DAB) substrate, whereas Mayer's hemalum (2min) served as a counterstain. After washing with water, all the slides were dehydrated in an ascending row of alcohols and soaked with paraffin oil ultimately. Immunostaining was detected with the substrate and the chromogen-3, 3'-diaminobenzidine after 3 minutes.

Tonsil follicles were utilized as a positive control for Sirt1. The First-trimester placenta was utilized as a positive control for the staining of RXR. Positive cells presented a brownish color, while the negative control and unstained cells showed blue. Negative controls were conducted with the same control tissues.

Under light microscopy, a semi-quantitative immune-reactivity-score (IRS score) was utilized as analysis: intensity of the staining was multiplied with the percentage range of positive stained cells.

Immune-reactivity-score

Score	Grades	Percentage range of positive stained cells
0	No staining	No staining
1	Weak staining	1-10%
2	Moderate staining	11–50%
3	Strong staining	51–80%
4	Null	81-100%

6.5 Cell culture

Human ovarian cancer cell lines A2780, UWB1.289 and A2780cis were chosen for our study as model cells. A2780 (serous) and UWB1.289 (mucinous) were cultured with RPMI 1640+10% fetal bovine serum, while A2780cis were cultivated from with RPMI 1640 + 10% FBS + 1 μM Carboplatin for the anti-chemical cell. All cell lines were sowed into 96-well plates for MTT and BrdU as well as 6-well plates for Western blot. After 20 hours incubated at 37 °C in 5% CO_2, the cell culture medium was changed with fresh RPMI 1640 with resveratrol for the remaining 24 hours. The dimethyl sulfoxide (DMSO, 0.5%) always worked as our vehicle control.

6.6 ELISAs

6.6.1 Cell viability assay

The three ovarian cancer cell lines were sowed at the density of 1.5×10^4 cells per well in 96-well plates with medium (RPMI1640 + 10% FBS). Then these plates were put into an incubator at 37 °C in 5% CO_2. After 20 hours later, 50μM and 100μM of resveratrol were treated. All the cells were incubated at 37°C in 5% CO_2 for 24 hours. Untreated cells were plated in RPMI1640. The dimethyl sulfoxide (DMSO, 0.5%) worked as vehicle control. To observe the viability of cells, 20μg MTT was treated to the plates for 1.5 hours at 37 °C incubator. Subsequently, removing MTT from the plates thoroughly, 200μL DMSO was added to each well. By using Elx800 universal

Microplate Reader, the optical density was checked at 595 nm. Each experiment was carried out in triplicate.

6.6.2 Marker of proliferation: BrdU

A2780, UWB1.289 and A2780cis ovarian cancer cells were cultured on 96-well plates with 50 µM and 100 µM dose of resveratrol. Cell density is 1.0×10^4 cells/well. To mark the DNA replication of these cells, we added BrdU in a medium (RPMI1640 without FBS + Resveratrol) at 37 °C incubator for 2 hours. The final dose of BrdU was 10 µM. After removing BrdU and adding, 200µl per well FixDenat incubation of cells took place for 30 minutes at room temperature. Subsequently, the FixDenat solution was eliminated and 100µl per well anti-BrdU-POD working solution was performed. After incubation for 1.5 hours at room temperature, wells were washed 3 times completely with phosphate-buffered saline (PBS). 100µ/well substrate solution BrdU was implemented and 37°C CO_2 incubation for 20 minutes was performed. 25µl of 1M H_2SO_4 was added to each well. Finally, the absorbance of the samples was estimated at 450nm by an ELISA reader.

6.7 Apoptosis assay

6.7.1 M30 staining

Caspase-cleaved cytokeratin 18 was utilized to detect apoptosis. All the ovarian cancer cells (A2780, UWB1.289 and A2780cis) were sowed on 96-well plates at a

density of 1.0×10^4 cells/well. After cultivated for 20 hours, M30 CytoDeath (1:1000 dilution) was utilized to detect the apoptotic death cells. Stimulated cells with resveratrol (50μM and 100μM) were put in the incubator at 37°C in 5% CO_2 for 24 hours. The dimethyl sulfoxide (DMSO, 0.5%) worked as our vehicle control. Added 20μl of MTT into each well. Putting the plates on the shaker for 5 minutes to intensive mixing. Afterwards, the plates with MTT were in an incubator at 37°C in 5% CO_2 for 1.5 hours. After removing the supernatant from plates, added DMSO 200μl/well in the plates and intensive mixing it for 5 minutes. By using Elx800 universal Microplate Reader, the optical density measured the absorbance of cells at 595nm. Each experiment was carried out 5 times.

6.7.2 TUNEL

Terminal deoxynucleotidyl transferase (TdT) deoxyuridine triphosphate (dUTP) Nick-End Labeling (TUNEL) assay was used to measure and estimate apoptotic death cells which are undergoing extensive DNA degradation during apoptosis. TUNEL assay is an acceptable and reliable assay for establishing apoptosis in situ. The main procedure included permeabilizing, binding of labeled dUTPs onto the fragmented DNA using TdT, and detecting the labeled dUTPs. TUNEL staining was performed to assess in situ DNA fragmentation using FragEL™ DNA Fragmentation Detection Kit.

The procedure as follows:

1. Fixation

The specimen was fixed with methanol, then washed with TBS once only.

2.Permeabilization

2 mg/ml Proteinase K (1:100) were diluted in 10 mM Tris pH 8 (mixed 1 µl of 2 mg/ml Proteinase K + 99 µl 10 mM Tris per specimen). The specimen was immersed with 100 µl of 20 µg/ml proteinase K. Subsequently, these specimens were incubated at room temperature for 5 minutes. The slide was washed three times with TBS. Next, the specimen was immersed with 3 % hydrogen peroxide in methanol for 5 minutes for inactivation of endogenous peroxidase. The slide was washed three times with TBS.

3.Equilibration + labeling reaction

The entire specimen was immersed with 100 µl of 1X TdT Equilibration Buffer (20 µl 5X Buffer + 80 µl dH2O per specimen). Then the specimen was incubated at room temperature for 20 minutes. After removing the 1X TdT Equilibration Buffer, 60 µl of TdT Labeling Reaction Mixture (57 µl TdT Labeling Reaction Mix + 3 µl TdT Enzyme) was immediately applied onto the specimen and incubated for 1.5 hours at 37 °C. The specimen was washed three times with TBS.

4.Termination of the labeling reaction

The Stop Buffer should be prewarmed at 37°C for 5 minutes if there were precipitates presented. The entire specimen was soaked with 100 µl stop solution for 5 minutes at room temperature. Then washed the slides only once with TBS. Notice: the buffer was carefully removed and the glass slide around the specimen was dried.

5. Detection

The entire specimen was blocked with 100 µl of Blocking Buffer and was incubated at room temperature for 10 minutes. After removing the Blocking Buffer, 100 µl of the 1X conjugate (2 µl 50X Conjugate + 98 µl Blocking Buffer) was applied to each specimen. All the slides were put into a humidified chamber and were incubated at room temperature for 30 minutes. Before finishing incubation, DAB solution was prepared (dissolved one tablet of DAB + one tablet of H_2O_2/Urea in 1 ml of TAP/FAUCET H_2O). Then the slide was washed one time with TBS and gently removed superfluous liquid around the specimen. The entire specimen was soaked with 100 µl of DAB solution and was incubated at room temperature for 10-15 minutes. The specimen was washed once with dH_2O.

The entire specimen was immediately soaked with 100 µl of methyl green counterstain solution and was incubated at room temperature for only 3 minutes. After removing the unnecessary counterstain, the specimen was placed in a Coplin jar slide holder. Subsequently, the specimen was dipped into 100% ethanol 2-4 times. The

specimen was blotted briefly on an absorbent paper. Then the previous step was repeated, and the specimen was dipped into 100% fresh ethanol 2-4 times. The specimen was blotted briefly on an absorbent paper. For the next step, the specimen was dipped 2-4 times into xylene. Removed redundant xylene carefully from the specimen. Finally, a glass coverslip was covered on the specimen with mounting media. Each experiment was carried out 5 times.

Under light microscopy, apoptosis cells showed a brownish color, while the normal cells and unstained cells presented blue.

6.8 Western blotting

Cultured cells (A2780, A2780cis, and UWB1.289 cells) were lysed with radio-immuno-precipitation assay buffer and protease inhibitors on ice. The lysate was centrifuged at 13 000 × g for 15 minutes in 4°C to collect the soluble fraction and supernatant. 20 μg of cell lysates per well were prepared. The lysates were separated in 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Subsequently, they were transferred to a polyvinylidene fluoride membrane. This membrane was blocked in 10% casein. Finally, incubation with the primary antibodies anti-SIRT1 (rabbit,1:1000) and anti-RXR (rabbit,1:1000) took place for 16 h at 4°C overnight.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an enzyme of 37kDa, was utilized as a housekeeping gene. Mouse monoclonal anti-GAPDH antibody was

diluted (1:1000) in 10% Casein for 16 hours in 4°C overnight. Afterwards, the membrane was thoroughly washed three times by casein. Next, incubation of the membrane with the goat-anti-rabbit secondary antibody (1:1000) was performed for 45 minutes at room temperature. Subsequently, removed the rest secondary antibody from the membrane, reaction with Vectastain ABC-AmP Reagent for 20 minutes was the next step. The membrane was conjugated with alkaline phosphatase and checked with 5-bromo-4-chloro-3′-indolylphosphate/nitro-blue tetrazolium (BCIP/NBT)-chromogen substrate solution. By using the GelScan V6.0 1D Analysis Software, the western blots were scanned and measured. The band intensities of SIRT1 and RXR were normalized with band intensities of GAPDH. The western blots experiments were repeated three times.

6.9 Statistics

Statistical analyses were performed using SPSS 25.0 software. For the clinical data and pathological data were presented as the median ± SD, independent T test was used to clinical and pathological data between related groups. Survival rates were determined using the Kaplan-Meier curves. Spearman's test was utilized to compare the IRS scores of SIRT1 and RXR staining in ovarian cancer patients. Data were collected as the means ± SEM from at least 3 independent experiments. Wilcoxon test was utilized for the evaluation of SIRT1 and RXR expression between related groups. *P-value <0.05* was considered to be statistically significant.

7. Results

7.1 Correlation of RXR and SIRT1 expression with clinical and pathological data

We obtained 123 cases to analyze SIRT1 and RXR expression in ovarian cancer (110 serous and 13 mucinous) (**table 1**) aged between 20 and 88 years. SIRT1 staining is detected in the cytoplasm and nucleus (**table 2**). 115 (93.5%) cases showed co-staining of SIRT1 in the cytoplasm and nucleus, while in 8 cases (6.5%) SIRT1 expression was detected neither in cytoplasm nor in nucleus. In among cases of the examined subcategories (serous, high grade, low grade, and different FIGO stages; **table 2**; **figure 5 A-D**) the median IRS of SIRT1 expression was 4 in the nucleus and cytoplasm, respectively. No significant differences were found about histological subtype (p=0.915), FIGO stage (p=0.568) or grading (p=0.147 and 0.585) in cytoplasmic as well as in nuclear expression (histology: p=0.639; FIGO: p=0.408; grading: p=0.475 and 0.699) (**table 2**). In cytoplasm, high level of SIRT1 (IRS≥4) were observed in 61.0% (75 cases) and low level of SIRT1 (IRS<4) were detected in 39.0% (48 cases). In contrast, in the nucleus, 66.7% of the samples showed a high level of SIRT1 (IRS≥4; 82 cases compared to 33.3 % with a low level (IRS<4); 41 cases).

Regarding RXR staining, 114 cases of ovarian cancer showed RXR expression (see **table 2**) while 4 cases did not have any RXR staining, and 5 cases could not be evaluated. Among the 114 cases, the median IRS was two. 44 cases (35.7%) were identified with a high level of RXR (IRS ≥3) while 74 cases were identified with a low RXR level (IRS<3). The median IRS in serous specimens was 2 (p=0.816) while in mucinous carcinomas it was 4 and 3.5 (p=0.690; **table 2**; **figure 5 E-G**). No significant differences were found regarding FIGO stage (p=0.405) or grading (p=0.816 and 0.690). A significant positive correlation was identified (p=0.006; **table 3**) between co-expression nuclear SIRT1 and RXR in IRS staining.

Table 1. Patients' characteristics.

	N	%
Subtype		
Serous	110	89.4
---Low-grade	24	19.5
---High-grade	80	65.0
---NA's	6	4.9
Mucinous	13	10.6
---Grade G1	6	4.9
---Grade G2	6	4.9
---Grade G3	0	0
---NA's	1	0.8
Age		
≥60	61	49.6
<60	62	50.4
FIGO		
I/II	29	23.6
III/IV	92	74.8
NA's	2	1.6
Progression (18 years)		
No progression	101	82.1
Progression	21	17.1
NA's	1	0.1
Survival (18 years)		
Right censured	38	30.1
Died	84	68.3
NA's	1	0.1

Table 2. Expression profile of RXR and SIRT1 staining regarding clinical and pathological characteristics

	SIRT1 cytoplasm		**SIRT1 nucleus**		**RXR Nucleus**	
	Median (+/- SD)	**p**	**Median (+/- SD)**	**p**	**Median (+/- SD)**	**p**
Histology		0.915		0.639		0.424
serous	4 (+/- 1.94)		4 (+/- 1.81)		2 (+/- 0.15)	
mucinous	4 (+/- 3.23)		4 (+/- 2.39)		3,5 (+/- 0.50)	
FIGO		0.568		0.408		0.405
I/II	4 (+/- 2.43)		4 (+/- 1.78)		2 (+/- 1.48)	
III/IV	4 (+/- 1.98)		4 (+/- 1.91)		2 (+/- 1.55)	
Grading						
---serous		0.147		0.475		0.816
Low grade	4(+/-0.34)		4(+/-0.38)		2(+/- 0.35)	
High grade	4(+/-0.24)		4(+/-0.22)		2(+/-0.17)	
---mucinous		0.585		0.699		0.690
G1	6(+/-2.04)		4(+/-1.36)		4(+/-0.87)	
G2	3.5(+/-0.96)		4(+/-0.91)		3.5(+/-0.52)	
G3	0		0		0	

Table 3. Spearmann's Correlation analysis between SIRT 1 and RXR.

	SIRT1nucleus	**SIRT1cytoplasm**
RXR-α		
Correlation coefficient	-0.259	-0.163
p	**0.006**	0.085

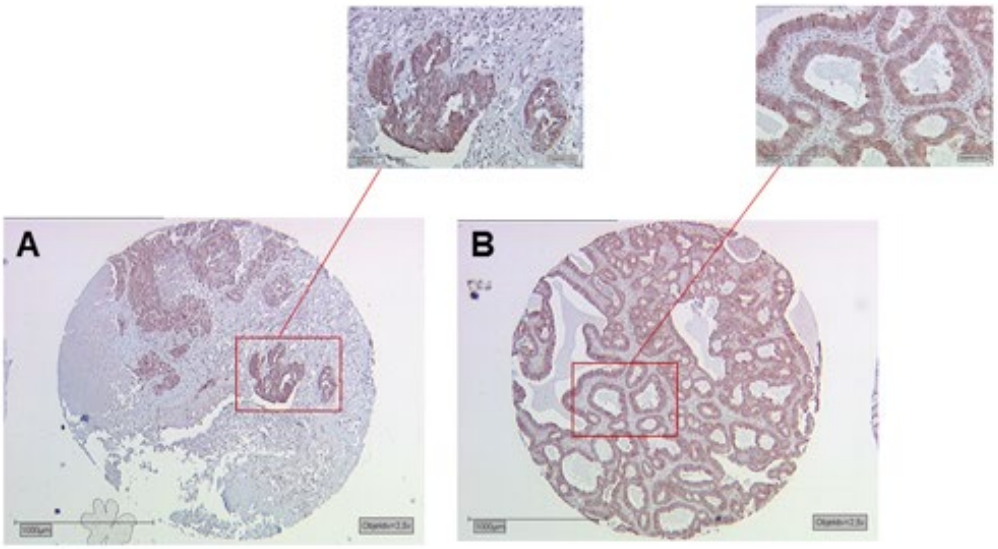
Table 4. Multivariate analysis

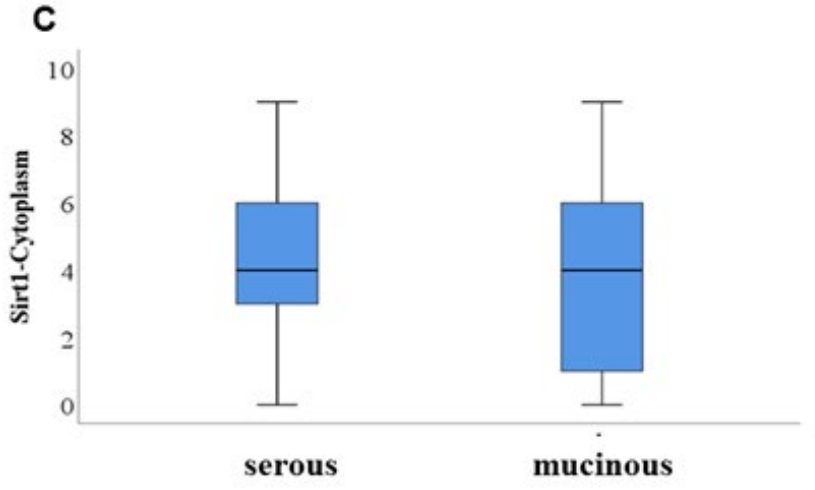
Covariate	Coefficient (Bi)	Exp(B)	95%CI Lower	Upper	p-value
Subtype	0.109	1.115	0.642	1.937	0.699
FIGO(I/II vs. III/IV)	1.327	3.771	1.956	7.271	**0.000**
Grade	-0.604	0.547	0.355	0.843	**0.006**
Age (<60 vs. ≥60 years)	0.359	1.432	0.944	2.170	0.091
SIRT1cytoplasm	0.004	1.004	0.868	1.161	0.959
SIRT1nucleus	0.035	0.965	0.821	1.135	0.670
RXR nucleus	-0.096	0.908	0.908	1.057	0.213

* *FIGO (Federation International of Gynecology and Obstetrics)*
Grade (Low grade vs. High grade)

No significance has been found regarding the prognostic outcome of SIRT1-cytoplasm (p=0.959), SIRT1-nucleus (p=0.670), and RXR-nucleus (p=0.213) alone (**table 4**).

As shown in the Kaplan-Meier curve (**figure 6**), co-expression of SIRT1and RXR-nucleus were related with better outcome in overall survival rates and longer survival time in the late stage of ovarian cancer patients (FIGO III/IV). This was detected for both, cytoplasmic SIRT1 expression (p= 0.026; **figure 6A**) and nuclear SIRT1 expression (p=0.041; **figure 6B**).


A
B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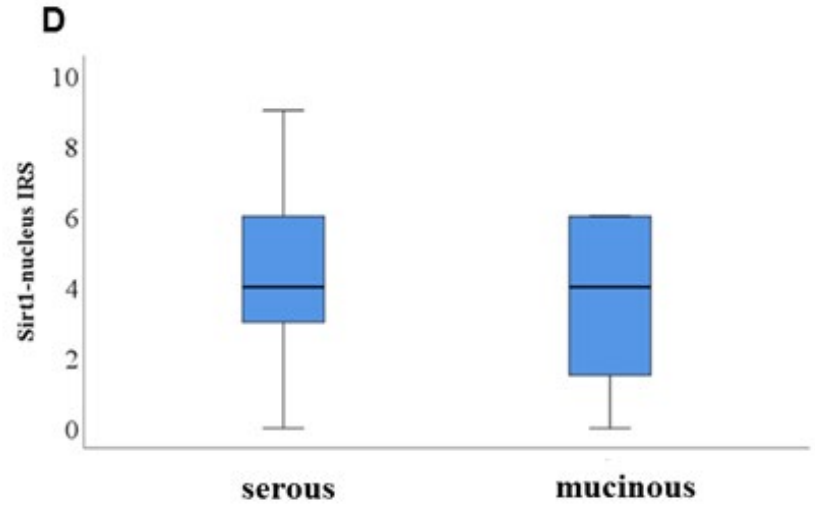
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mucinous

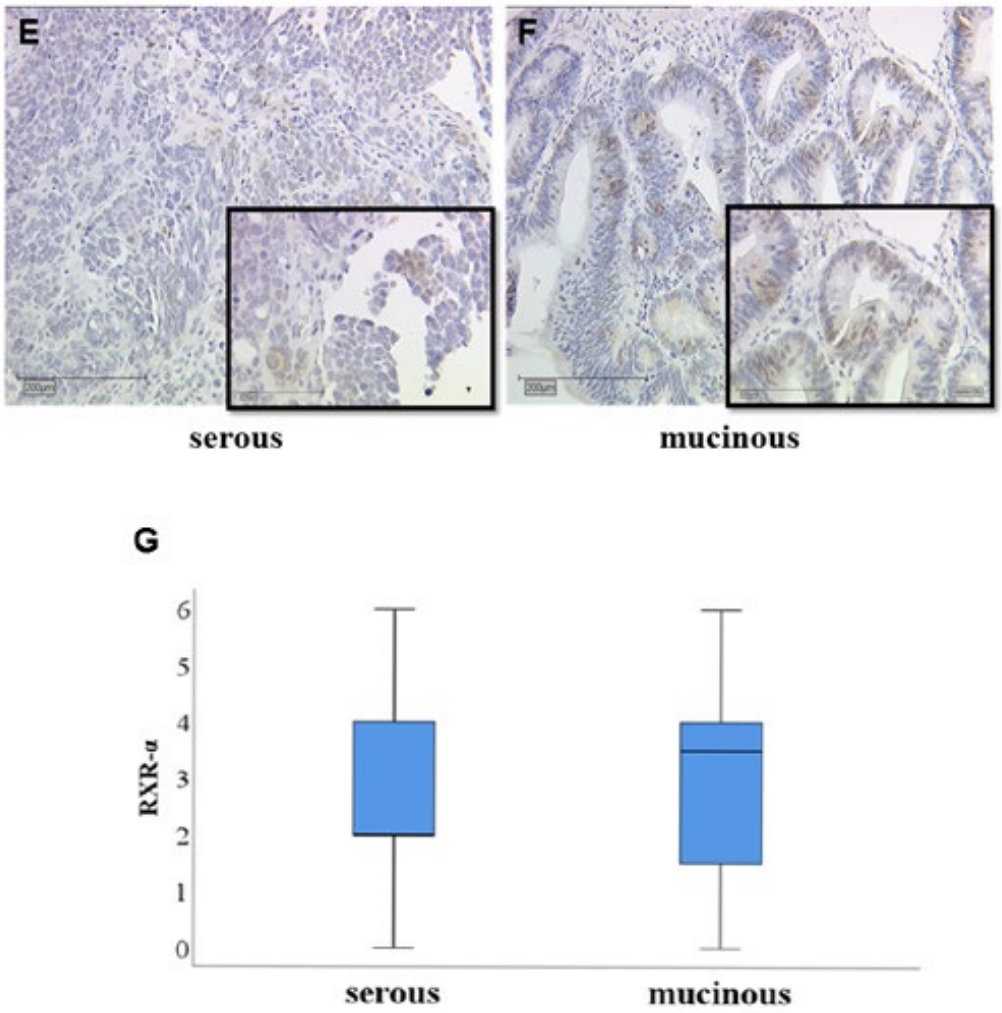

Figure 5. Representative images of SIRT1 and RXR (immunohistochemistry) of ovarian cancer samples. **A:** SIRT1 expression in serous ovarian cancer on a TMA with a 2.5 and 10x magnification. **B:** SIRT1 expression in mucinous ovarian cancer on a TMA with a 2.5 and 10x magnification. **C and D:** boxplot; SIRT1 expression with a median IRS of 4 in mucinous and serous ovarian carcinoma on TMA. **E:** RXR expression in serous ovarian cancer on slide with a 10 and 25x magnification. **F:** RXR expression in mucinous ovarian cancer on slide with a 10 and 25x magnification. **G:** boxplot; RXR expression with a median IRS of 2 and 3 in mucinous and serous ovarian carcinoma on slides.

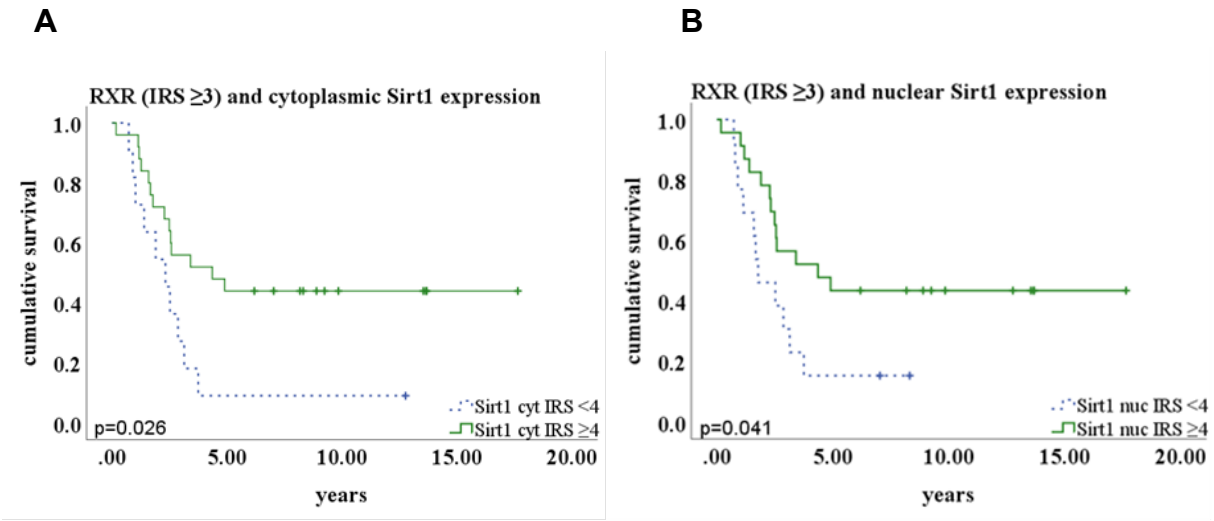

Figure 6. Cytoplasmic SIRT1 expression and overall survival (A; p=0.026) versus overall survival in patients with nuclear RXR-expression (B; p=0.041).

7. 2 Correlation of RXR and SIRT1 expression with laboratory data

All cell-lines (A2780, A2780cis, and UWB1.289) were treated with resveratrol for 24 hours. The MTT assay results revealed that the viability of all cell-lines declined dose-dependent (**figure 7**). Cell apoptosis was measured by BrdU, M30, and TUNEL. The cell apoptosis detected by BrdU ELISA showed that in cells treated with 100μM resveratrol, the apoptotic characteristics were significantly improved (**figure 8**, $p<0.003$). Besides, cell morphology observations showed that in cells treated with 100 μM resveratrol, apoptosis markers (brown cytoplasm, marked by M30) were significantly higher (**figure 9**; $p<0.05$). After resveratrol treatment, the percentage of TUNEL stained cells decreased, which means that the rate of apoptosis increased (**figure 10**; p=0.043).

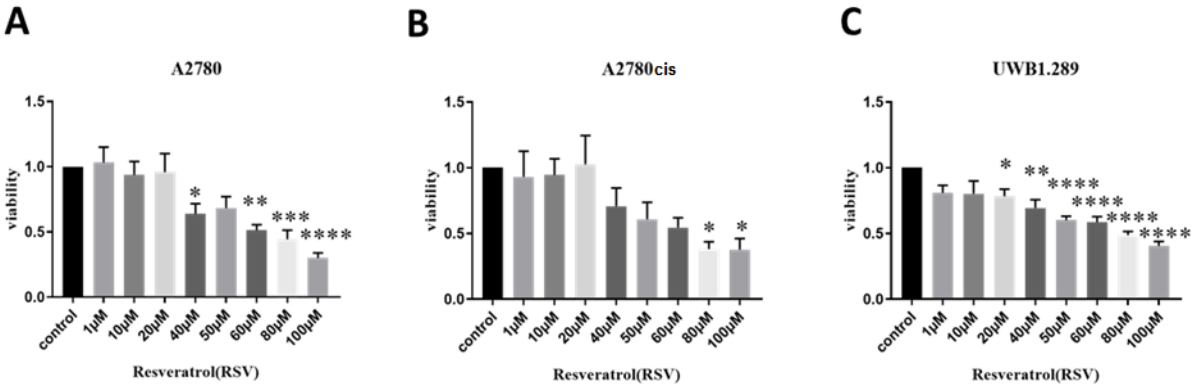

Figure 7. Cytotoxicity of resveratrol in ovarian cancer cells**.** Cell-lines were treated with resveratrol in different concentrations (0 to 100μM) for 24 hours. MTT assay for cell viability. **A**: A2780 (*A2780 control vs. RSV 40μM p=0.0032; **A2780 control vs RSV 60μM p=0.002; *** A2780 control vs. RSV 80μM p=0.0004; ****A2780 control vs. RSV 100μM p<0.0001), **B**: A2780cis (*A2780cis control vs RSV 80μM/RSV 100μM p<0.0001) and **C**: UWB1.289 (*UWB1.289 control vs. RSV 20μM p=0.0326; **UWB1.289 control vs. RSV 40μM p=0.0013; ****UWB1.289 control vs. RSV 50/60/80/100μM p<0.0001). The data are graphed as the means ± SEM. N=3. *p < 0.05.

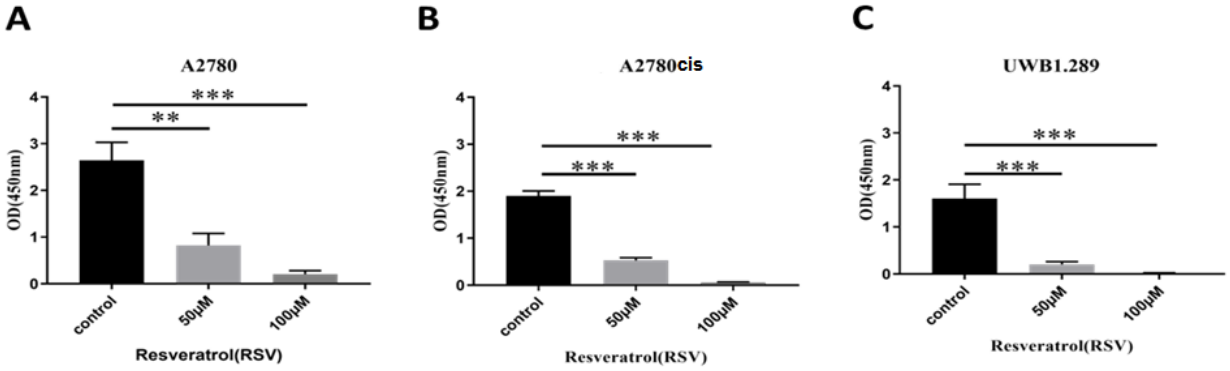

Figure 8. Cell apoptosis measured by BrdU**.** Cell-lines were seeded onto 96-well plates at a density of 1.0×10^4 cells per well. The cells were treated with resveratrol (50μM and 100 μM) for 24 hours and BrdU (final concentration is 10μM) was added of the treatment. BrdU in corporation was determined by measuring at the absorbance at 450nm. **A**: A2780 (**A2780 control vs. RSV 50μM p=0.0019; ***A2780 control vs. RSV 100μM p=0.003); **B**: A2780cis (**A2780cis control vs. RSV 50/100μM p<0.0001); **C**: UWB1.289 (***UWB1.289 control vs. RSV 50μM p=0.0007; ***UWB1.289 control vs. RSV 100μM p=0.0003). Representative results are graphed as the means ± SEM. (N=3) *p < 0.05.

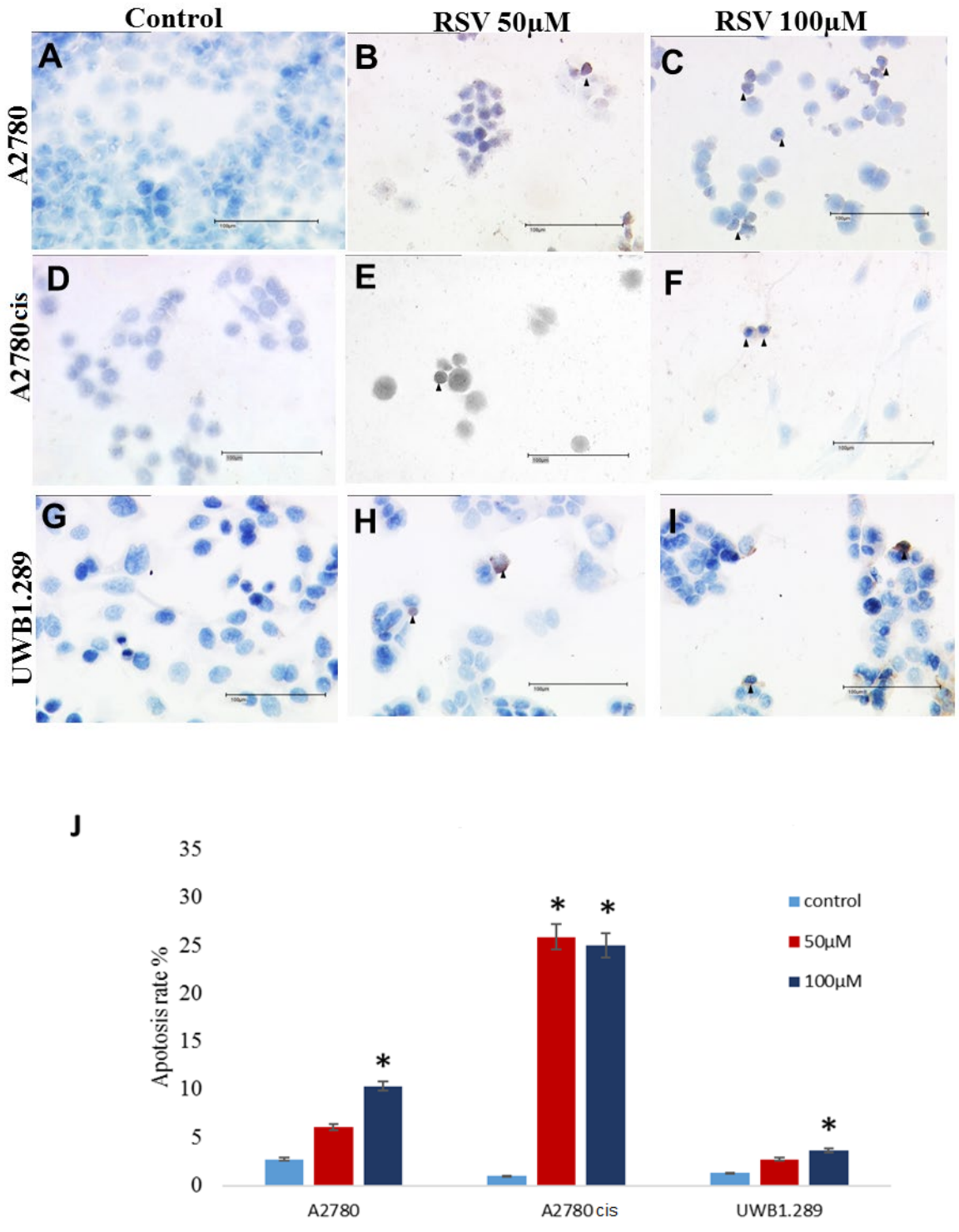

Figure 9. Effect of RSV-treatment and M30 identification on A2780 (**A-C**), A2780cis (**D-F**) and UWB1.289 (**G-I**) cells with 50 µM and 100 µM resveratrol for 24 hours. (N=5). Apoptosis rates in dependent of RSV concentration are shown in a boxplot **(J)**. The data are presented as means ± SEM. *p < 0.05.

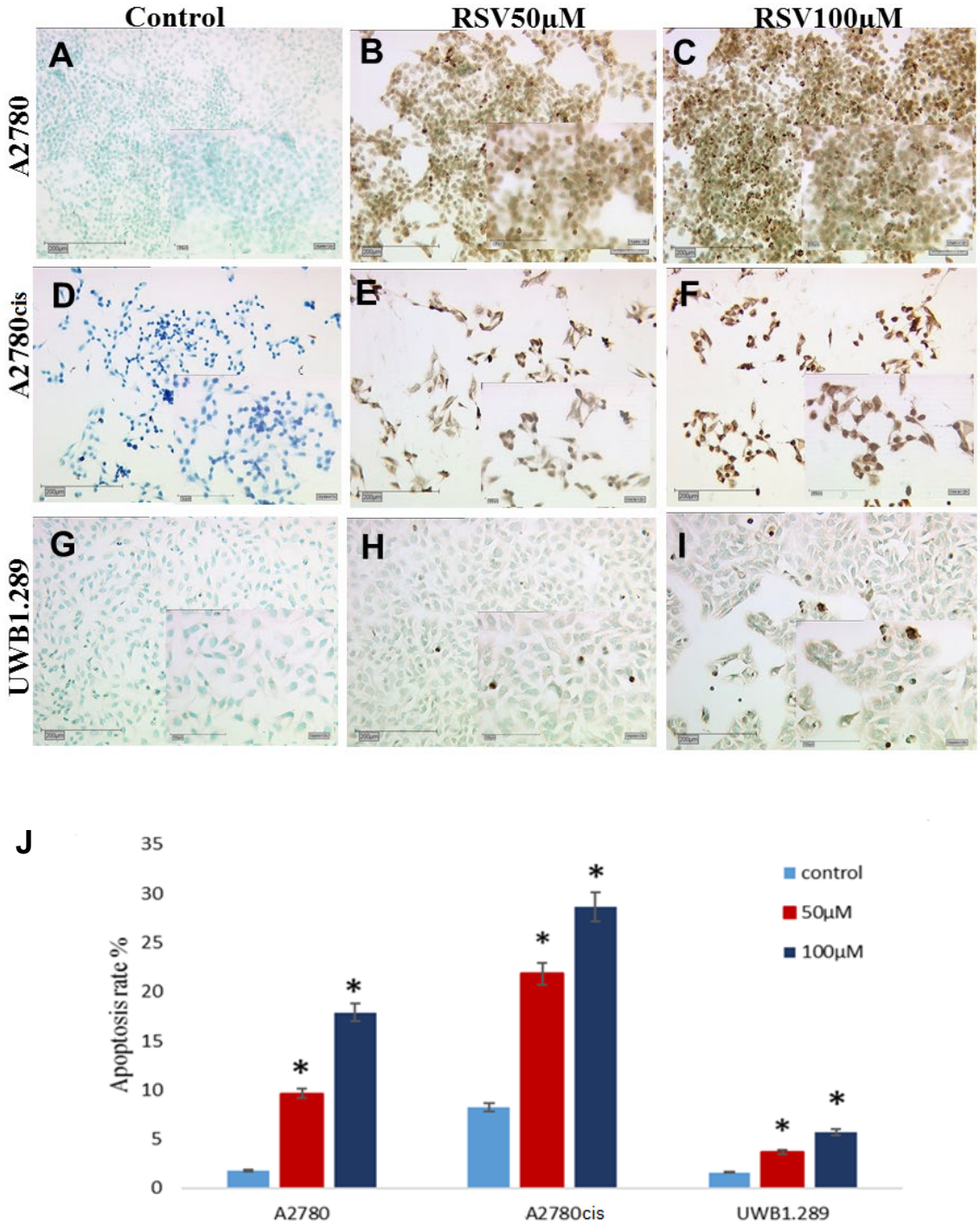

Figure 10. Apoptosis of A2780 (**A-C**), A2780cis (**D-F**) and UWB1.289 (**G-I**) by TUNEL assay. All images are at 2.5× magnification with an insert at 10× magnification. Apoptosis rates in dependence of RSV concentration are shown in a boxplot (**J**). The experiments results are presented as means ± SEM. (N=5) *p= 0.043.

7.3 The interaction between SIRT1 and RXR

All the cell lysates were collected and analyzed by Western blotting with primary antibodies against SIRT1 and RXR. As shown in the western blot results (**figure 11G**), resveratrol (100μM) treatment in A2780 cells contribute to a significant decrease of the expression of SIRT1 (p=0.208 and 0.025 **figure 11A**). No significant change in SIRT1 expression was observed when A2780cis (p=0.327 and 0.069 **figure 11B**) and UWB1.289 (p=0.401 and 0.575 **figure 11C**) were treated with resveratrol. Compared to the control, RXR protein levels increased dramatically after A2780cis cells were treated for 24 hours with resveratrol 50μM and 100μM (p =0.012 and 0.017 **figure 11E**). After treatment with resveratrol 50μM or 100μM for 24 hours, the expression of RXR in A2780 (p=0.208 and 0.069) and UWB1.289 (p=0.093 and 0.069) fails to reach conventional levels of statistical significance (**figure 11D and 11F**).

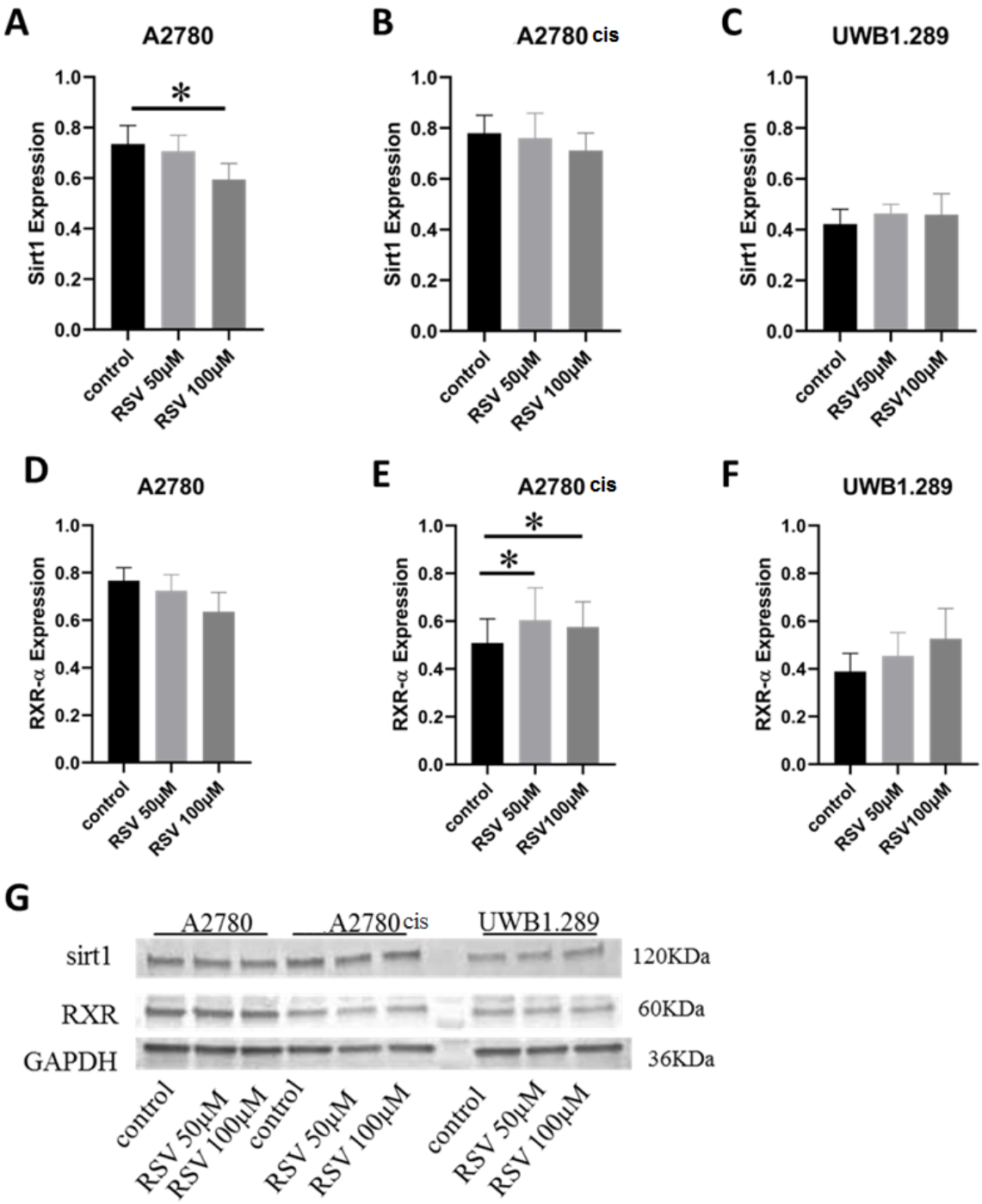

Figure 11. Ovarian cancer cells were treated with RSV 50μM and RSV 100μM for 24 hours. Expression of SIRT1 in A2780 cells (**A**), A2780cis (**B**) and UWB1.289 (**C**) cell-lines. RXR-expression in A2780 (**D**), A2780cis (**E**) and UWB1.289 (**F**) cell-lines after RSV treatment. Finally, expressions were analyzed by western blotting (**G**). Representative results are expressed as the means ± SEM. *p< 0.05.

8. Discussion

8.1 Comprehensive summary of the results

Ovarian cancer is the third common gynecologic cancer after cervical and uterine cancer in women globally. Until now, the incidence rates and mortality rates of ovarian cancer remain high [229]. So far, the function of SIRT1 in ovarian cancer remains controversial. Both the treatment of ovarian cancer and the prognosis of platinum-resistant patients need to be improved and enhanced.

In the current study, we reported that the protein level of nuclear RXR and SIRT1 in advanced ovarian cancer was associated with significantly longer overall survival after diagnosis. In this study, resveratrol, a polyphenol, could reduce the growth and increase the apoptosis of ovarian cancer cell lines according to the results of BrdU, M30, and TUNEL. Resveratrol (100μM, 24h) increased the level of RXR in anti-chemo cell A2780cis while decreased the expression of SIRT1 in A2780.

Most scientists believe that SIRT1 shuttles between the nucleus and cytoplasm; however, live-cell imaging shows that SIRT1 is localized predominantly in cellular nucleus. Furthermore, Sun and his colleagues demonstrated that the absence of cytoplasmic macromolecular crowding effect and hypotonic dwelling make SIRT1 in the nucleus leak into the cytoplasm [7]. This suggests that SIRT1 in the cytoplasm can be released from the nucleus. Our immunohistological results show that SIRT1 is localized both in the nucleus and cytoplasm. We support Sun's viewpoint because the experimental step of immunohistochemistry may allow SIRT1 to leak from the nucleus into the cytoplasm. However, we cannot assert that SIRT1 is present only in the nucleus and we cannot exclude the possibility of some uncovered new principles regarding the subcellular localization of SIRT1.

8.2 Resveratrol partially inhibited proliferation and induced apoptosis in ovarian cancer cell

Resveratrol is a compound that directly activates the antioxidant properties of SIRT1, and the antitumor effect of this compound restrains cell proliferation and induces apoptotic death in cancer cells. Numerous studies have demonstrated that resveratrol has the effect of inhibiting cell growth and proliferation, and even increasing apoptosis in various types of cancer cells. For example, resveratrol enhanced the efficacy of chemosensitivity and prevented cancer aggressiveness [230]. Alternatively, resveratrol induced apoptotic death in ovarian cancer cells [231,232]. Similarly, the current experimental results support that resveratrol prevents cell proliferation and causes ovarian cancer cell apoptotic death, suggesting an antitumor effect of resveratrol during the progression of ovarian cancer. However, a recent study has shown that resveratrol can effectively inhibit ovarian cancer cells in vitro, while such inhibition could not be observed for ovarian cancer cells in vivo [233]. Considering the different experimental settings in vivo and in vitro, numerous experiments and researches should be implemented to clarify the effect of resveratrol on ovarian cancer.

Resveratrol effectively affects multiple signaling pathways, thereby inhibiting cancer cell proliferation, migration, angiogenesis, and even inducing apoptosis. These biological properties of cells can be achieved by regulating various signaling pathways and proteins associated with SIRT1. Evidence suggests that resveratrol inhibits ovarian cancer and various proteins in a SIRT1-dependent manner. Resveratrol enhances antitumor drug sensitivity by preventing epithelial-mesenchymal transition and regulating the SIRT1-β-catenin axis and its downstream factors in cancer [197]. Resveratrol regulates cancer cell growth, proliferation, and protein translation by

acting SIRT1-mediated AMPK signaling [234]. RXR binds to PGC-1α and SIRT1, activates oxidative metabolic genes, and ultimately determines mitochondria-triggered cell death [235]. However, in the current experiments, we did not find a clear link between RXR and SIRT1, and further experiments are needed to confirm the relationship between them.

Ohshiro et al. reported that in lung cancer cells treated with 100μM resveratrol 24 h inhibited cell growth and caused apoptosis [236]. In the current study, we utilized the same dose (100μM) and duration (24 h) to treat ovarian cancer cells. Our results were consistent with that of Ohshiro's work. We confirm that resveratrol prevented ovarian cancer cell growth and induced apoptosis. It maybe refers to the increasing of reactive oxygen species (ROS) by resveratrol. Additionally, one study has also shown that resveratrol can promote ROS over-production (2-3 times), which can cause cell death [237]. Compared with our study (50-100μM, 24 hours), cancer cells exposed to a higher dose (200μM-500μM) and a longer duration (48 hours) maybe have more stress on cell behavior.

Ovarian cancer was classified into four subtypes by histopathological examination, including serous, clear cell, endometrioid, and mucinous. We choose mucinous and serous cell lines in our experiment. In the study, we observed that the ovarian cancer cell lines either A2780 or UWB1.289 have different anti-drug resistance. A2780 cell line was more resistant to platin compared with UWB1.289 cells, and this result was consistent with previous study. Based on the data of the apoptosis assay, we concluded that the mucinous cell line (A2780) appeared to be more sensitive when treated with resveratrol compared to the serous cell line (UWB1.289). This may be since the fact that mucinous cells grow faster than serous cells. In addition, the

apoptosis rate was much higher for A2780cis than for A2780. For resveratrol, the effect of apoptosis seems to be synergistic to cisplatin [238].

8.3 Resveratrol did not decrease SIRT1 expression in carboplatin-resistant cell lines.

SIRT1 facilitates ovarian cancer cells' invasiveness and chemoresistance. This protein represents a poor outcome of advanced ovarian carcinoma as well [239-241]. Besides, SIRT1 is an important subcellular target of resveratrol. So, we chose SIRT1 to study the influence of resveratrol in the ovarian cancer cell. Stimulation with resveratrol is associated with a lower SIRT1 level in the mucinous ovarian cancer cell line (A2780), while no significant difference was found in anti-drug mucinous ovarian cancer cell lines (A2780cis). Pizarro confirmed that the reduction of SIRT1 stimulated by resveratrol was not related to apoptosis [242]. In addition, Bjorklund's work proved that the treatment of 40μM resveratrol induced potentiation of platinum for ovarian cancer not connected to the SIRT1 expression [243]. Our results were inconsistent with these findings. In this study, we stimulated ovarian cancer cells with a higher resveratrol dose (100μM). However, these findings cannot explain why resveratrol does not lower SIRT1 expression in chemical-resistant cell lines (A2780cis). Considering the poor survival rates of patients, further study should be expected.

Li and his group found out that breast cancer type 1 (BRCA1) inactivation decreased SIRT1 levels in ovarian cancer [244]. Recent studies further demonstrated that BRCA1 mutant mice tumor has decreased level of SIRT1 [245]. Our results were inconsistent with these findings. In our study, the expression of SIRT1 did not significantly change when UWB1.289 was exposed to resveratrol. It may be one reason that BRCA1-null is in these serous ovarian cancer lines and that it is not sensitive to resveratrol. The other reason is that UWB1.289 cells are less proliferation

compared with A2780 cells. The doubling time of UWB1.289 is 36 hours compare to that of A2780 18 hours.

As an essential factor located in the nucleus, SIRT1 affects many nuclear factors and proteins. For example, SIRT1 deacetylates vitamin D receptor (VDR) [225] and bonds PPARα [246,247]. Meanwhile, RXR dimerized VDR [248,249]. RXR acts as a critical mediator in ovarian cancer growth suppression [250]. RXR expression declines during the progression of epithelial ovarian cancer [251]. Recent studies have proved that resveratrol could either bind to RXR or can modulate RXR dimerization [252]. Retinoid X Receptor (RXR)-α-dependent mechanisms could restrain proliferation and induce apoptosis [253]. The "rexinoid apoptosis" participates in the activation of both inducible nitric oxide synthase (iNOS) and eNOS by RXR-PPARγ. Consequently, plenty of apoptotic NO contributes to cell apoptosis [254]. In addition, the activation of PPARγ and the formation of heterodimers with retinoid X receptors (RXRs) generate the anti-tumor effect of cancer [255]. In the current study, we evaluated resveratrol-stimulated repression of apoptosis of the ovarian cancer cells. Our results suggest that RXRα plays an indispensable role in the regulation of apoptotic cell death in human ovarian cancer.

Oka and his group found that SIRT1 competes with RXRα to dimerize with PPARα [246] and other proteins, and then influences cellular metabolism. Our results are consistent with this study. In the present study, co-expression of both RXR and SIRT1 is closely related to better a clinical outcome and survival rate in advanced stages of ovarian cancer. Either SIRT1 or RXR is related to poor outcome and bad prognosis in ovarian cancer, while the interaction of these two proteins can ameliorate the poor outcome.

Together with the results of our studies on ovarian cancer cells, previous reports suggest that resveratrol can be a promising chemical even clinically utilized in the treatment of anti-drug ovarian cancer. However, these findings should be confirmed in plenty of specimens and experiments. Altogether, further studies are warranted to understand the mechanism behind resveratrol and its role in the anti-cancer effect in combination with platinum.

8.4 The mechanisms inducing apoptosis of mucinous and serous

Several lines of evidence suggest that resveratrol has a suppressive effect on ovarian cancer cells and that various signaling pathways are involved in this inhibitory effect. Recently more studies started to investigate the anti-tumor effect of resveratrol. Recently, Kueck's group proved that resveratrol makes an anti-tumor effect through inhibiting glucose metabolism in human ovarian cancer cells [256]. Resveratrol inhibits cellular mitochondrial respiration, even in cancer cells. The cytotoxic effects of resveratrol on cancer cells are regulated by SIRT1 and also inhibited mitochondrial complex I inhibition [257]. In vitro, SIRT1 deacetylated kruppel-like factor 4 (KLF4) to activate Claudin-5. By this, it suppressed metastasis and invasion of ovarian cancer cells [260]. A high level of SIRT1 effectively inhibited high mobility group box 1 protein (HMGB1) acetylation, therefore inhibiting ovarian cancer migration, invasion, and angiogenesis [261]. Resveratrol inhibited IL-6-induced metastasis of ovarian cancer cells [258]. The apoptotic effect of resveratrol on ovarian cancer cells may be related to the suppression of galectin-3 and stimulation of microRNA transcription [259].

The results show that serous cancer cells treated with resveratrol does not affect the expression of SIRT1 and RXR at the protein expression level, we can see apoptosis from the results of our experiments, though. SIRT1 could not responsible for resveratrol-induced cancer cell death. Therefore, we suppose that resveratrol induces

the apoptosis of serous cancer cells maybe not be explained by SIRT1 and RXR. These data show that the mechanisms inducing apoptosis of mucinous are different from those causing apoptosis of serous. Our findings are consistent with other studies showing the different effects of resveratrol on induction of apoptotic death relying on the kinds and species of human cancer cells [260]. The pharmacologic and pharmacokinetic characteristic of resveratrol has been studied in detail in numerous aspect [230,231,256,258], however, we cannot exclude the possibility that some unrevealed inhibition effects of resveratrol may increase apoptosis. In general, further studies aiming at understanding the mechanism of the anti-cancer effect of resveratrol are required.

One shortcoming of our study is, that the samples and its data are based on classifications between 1990 and 2002. Additionally, the majority of all included patients suffered from ovarian cancer in an advanced stage. More studies about ovarian cancer should be performed for the next step.

Ovarian cancer is a kind of malignant tumor in gynecology with a high mortality rate. 80% of patients are diagnosed as advanced (i e: FIGO stage III~IV); the chance of relapse is 70% within 2 years after the initial treatment, 70% of patients will die within 5 years [261]. This results from the asymptomatic and delayed onset of symptoms as well as from a lack of proper screening. Age, family history, high level of hormones, inflammation in the ovary, unhealthy lifestyle such as alcohol, caffeine, and cigarette intake lead to the incidence of ovarian cancer [262]. The 5-year survival rate of ovarian cancer has not been significantly improved globally in recent years, still hovering between 30% and 40%. Although widely used of oral contraceptives and the decline in menopausal hormone supplementation [263,264]. Cytoreductive surgery and systemic chemotherapy are the mainstream clinical treatment and therapy for this

malignant tumor. And chemotherapy is still the essential treatment of ovarian cancer in clinical management. One important future direction of ovarian cancer is a combination therapy. This therapeutics consists of traditional chemotherapy drugs with natural compounds. Furthermore, it has been reported that combination therapies are beneficial in the treatment of cancer. On the one hand, these new therapeutics help to aim at more signaling pathways participated in tumor development and treatment, and on the other hand, they can limit and reduce the toxic effects of chemotherapeutic drugs on the patient [265]. In addition, molecular targeted therapy is currently mainly used for the treatment of relapsed patients, and immunotherapy in cancer still needs more research. In addition, plenty of clinical trials that immunotherapy, bevacizumab, and inhibitor combination therapies are under the supervision of several organizations. From bench to bedside is still a long journey for the treatment of ovarian cancer. In a word, numerous therapies emerging predict that the treatment of ovarian cancer has been entering a new era.

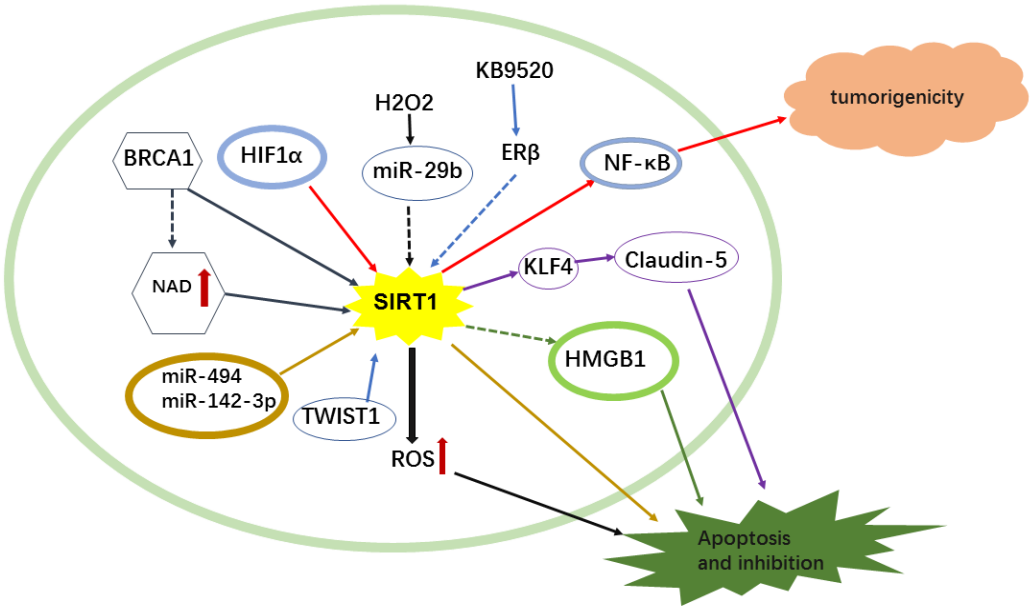

Figure 12. SIRT1 in ovarian cancer. ⟶ , positive regulation; ⟶ , negative regulation. BRCA1, Breast cancer type 1 susceptibility protein; ERβ, Estrogen receptor beta; HMGB1, High mobility group box 1 protein; HIF1α, Hypoxia-inducible factor 1-alph; H2O2, Hydrogen peroxide; KLF4, Kruppel-like factor 4; NAD, Nicotinamide adenine dinucleotide; Nrf2, nuclear factor erythroid 2-related factor 2; SIRT1, sirtuin1; TWIST1, Twist-related protein 1.

8.5 Conclusion

In summary, this study provides evidence that the combination of nuclear RXRα and SIRT1 expression is connected to better overall survival rates for advanced ovarian cancer. Resveratrol induces apoptosis and reduces the proliferation of human ovarian cancer cell lines, which is closely connected to the decreased expression of SIRT1 in mucinous ovarian cancer and the increased expression of RXR in mucinous and carboplatin-resistant ovarian cancer cells. New strategies should be developed to improve the understanding of resistance mechanisms and to improve drug treatment. Undoubtedly, new research for the effective treatment of ovarian cancer is needed.

9. Summary

SIRT1 has been the popular subject of the scientific research field since its detection in 1990. The function of this protein varies greatly depending on the tissue. Recent studies focused on SIRT1, especially in tumors. SIRT1 has been revealed to modulate cancer cell growth, metastasis, and invasion in numerous tumors. However, SIRT1 has been barely studied in gynecological tumors.

The purpose of our study was to evaluate the effect of SIRT1 on ovarian cancer. First, we analyzed the relationship between the intensity of SIRT1 and RXR-alpha staining and clinical prognosis in ovarian cancer by immunohistochemistry. To further study the therapeutic effects of SIRT1 activator Resveratrol, we used MTT, M30, BrdU, and other methods to test the function of resveratrol on ovarian cancer cell lines A2780, UWB1, 289, and A2780cis, including proliferation and apoptosis. Finally, the relationship between RXR-alpha and SIRT1 proteins in ovarian cancer treated with Resveratrol was studied by western blot.

Studies have shown that nuclear RXR-alpha and SIRT1-expression are significantly connected with better outcome and overall survival rates in advanced ovarian cancer. Resveratrol had a direct effect on ovarian cancer: it suppressed the growth and proliferation of ovarian cancer cells and even increased their apoptosis. At the protein level, resveratrol (100 μM, 24h) up-regulated the expression of RXR-alpha in the anti-carboplatin cell line A2780cis and down-regulated the expression of SIRT1 in A2780. In conclusion, SIRT1 may have a suppressive role in ovarian cancer, especially in advanced ovarian cancer. In the recent years, the scientific interest of SIRT1 research has been increasingly applied to therapeutic applications, and there is a vision to use SIRT1 activators in the future: in the case of tumor diseases, for

tumor treatment. However, the influence and function of these proteins must be better understood. Thus, further studies are warranted.

10. Zusammenfassung

SIRT1 ist seit seiner Entdeckung im Jahr 1990 Gegenstand wissenschaftlicher Forschung. Die Funktion dieses Proteins variiert je nach Gewebe stark. Neuere Studien konzentrierten sich auf die Rolle von SIRT1 bei Tumoren. Es wurde gezeigt, dass SIRT1 die Proliferation, Migration und Invasion von Tumorzellen bei zahlreichen Krebsarten reguliert. In gynäkologischen Tumoren wurde SIRT1 bisher jedoch kaum untersucht.

Das Ziel unserer dieser Studie war es, die Wirkung von SIRT1 auf den Eierstockkrebs zu untersuchen. Zunächst analysierten wir retrospektiv die Beziehung zwischen der Intensität der SIRT1- und RXR-alpha-Färbung und der klinischen Prognose bei Eierstockkrebs durch immunhistochemische Methoden. Um die therapeutischen Wirkungen des SIRT1-Aktivators Resveratrol weiter zu untersuchen, verwendeten wir MTT, M30, Brdu und andere Methoden, um die Wirkungen von Resveratrol auf die Eierstockkrebs-Zelllinien A2780, UWB1, 289 und cis-A2780, einschließlich Proliferation und Apoptose, zu testen. Schließlich wurde die Beziehung zwischen RXR-alpha und SIRT1-Proteinen bei mit Resveratrol behandeltem Eierstockkrebs durch Western Blot analysiert.

Studien haben gezeigt, dass die nukleäre RXR-alpha und SIRT1-Expression signifikant mit besseren Gesamtüberlebensraten bei fortgeschrittenem Eierstockkrebs assoziiert waren. Resveratrol reduzierte die Proliferation von Eierstockkrebszellen und erhöhte deren Apoptose. Auf Proteinebene regulierte Resveratrol (100 μM, 24 h) die Expression von RXR-alpha in der Anti-Carboplatin-Zelllinie A2780cis hoch und regulierte die Expression von SIRT1 in A2780 herunter. Zusammenfassend kann SIRT1 eine supprimierende Rolle bei Eierstockkrebs spielen, insbesondere in fortgeschrittenen Stadien.

In den letzten Jahren wurde das wissenschaftliche Interesse der SIRT1-Forschung zunehmend auf therapeutische Anwendungen übertragen und es besteht die Vision, SIRT1-Aktivatoren in Zukunft zur Tumorbehandlung einzusetzen. Der Einfluss und die Funktion dieser Proteine müssen jedoch besser verstanden werden, daher sind weitere Studien erforderlich.

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