Submitted: 20 September, 2024 Accepted: 24 October, 2024 Published: 15 February, 2025 DOI:10.22514/ejgo.2025.019

ORIGINAL RESEARCH

European Journal of Gynaecological Oncology

Succinic acid might be an inducement for the road to *RIPK* for death through necroptosis in endometrium cancer

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Abstract

Background: Succinic acid, a natural agent has gained attention in cancer research. It induces apoptotic cell death in endometrial cancer. Our aim was to understand the specific cellular pathway triggered by succinic acid at the molecular level. **Methods**: The seventeen genes involved in death pathways were examined by real time quantitative reverse transcription polymerase chain reaction (RT-qPCR) after treating endometrial cancer (CRL-2923) and healthy (MRC-5) cells with 5 μM and 10 μM of succinic acid. Apoptotic relationship was analyzed with the cell death detection Enzyme-Linked Immunosorbent Assay (ELISA) kit to understand the effects of succinic acid on survival. **Results**: The gene expression of *tumor necrosis factor receptor superfamily member 10b* (*TNFRSF10B*) and *mitochondrial carrier 1* (*MTCH1*) increased by 3.62 and 3.31-folds, respectively, after adding 5 μM of succinic acid to CRL-2923 cells. A 9.94-fold upregulation was measured by the changes in the *receptor-interacting serine/threonine kinase 1* (*RIPK*) gene ($p = 0.008$). The necrotic members, *poly* (*ADP-ribose*) *polymerase 1* (*PARP1*) and *mixed lineage kinase domain like pseudokinase* (*MLKL*), genes expression decreased after adding 5 and 10 μM of succinic acid, particularly 10 μM succinic acid exhibited a 4.11-fold decrease in the expression of the *PARP1* gene. **Conclusions**: The intersections of the death pathways form a valuable key spot for cancer treatment. After succinic acid treatment, the interaction between the death pathways with *RIPK* gene have shown promising results for RIPK family. Ultimately, elucidating these cellular pathways, whose functionality is important in every physiological or pathological situation, by evaluating them from different perspectives is among the important needs of the current literature. Therefore, the effects of succinic acid via RIPK should be considered for targeting and/or modulating.

Keywords

Succinic acid; Endometrial cancer; Cell death; Necroptosis; *RIPK*

1. Introduction

Endometrium cancer (EC) is the most common cancer among gynecological cancers [1]. According to the American Cancer Society for 2024, 67,880 new cases of endometrial cancer and 13,250 deaths related to this cancer are estimated [2]. According to the data from developed countries, endometrial cancer in the female pelvis is associated with several malignancies, many of which are gynecological tumors (95%) like adenocarcinomas [3, 4]. EC is classified into two groups, Type I and Type II, based on histomorphological features, pathogenesis and prognosis [5, 6]. Estrogenic stimulation is the primary mechanism involved in Type I, and 80%–85% of all EC cases are Type I. Type I EC is often associated with estrogen and endometrial hyperplasia [6, 7]. Type II tumors, on the other hand, occur in the postmenopausal period in advanced age and are generally high-grade, invasive and aggressive cancers [6].

The preferred choice for early-stage and localized EC cases is surgery and/or radiotherapy [8]. Additionally, systemic treatments, such as chemotherapy, endocrine therapy, or immunotherapy, are preferred in advanced and metastatic cases [6]. Therefore, different combinations of these treatment methods could be applied according to the type of the disease, its course, cancer stage, and disease spread [9]. The preferred systemic treatments prevent tumor recurrence and metastasis [6]. Although the prominent treatment options provide efficient results in the first stages, there are many threats in later periods, such as developing resistance or the tumor's aggressive nature. Concordantly, current studies focus on minimizing the side effects of these clinically used therapies and increasing their selective efficacy. Several studies have highlighted that using natural products of plant or animal origin is advantageous in many ways.

Succinic acid, a natural product, is widely used in food, health, surfactant, biodegradable plastics, and many other industrial fields [10, 11]. Moreover, in the last few decades, succinic acid has attracted researchers' attention in terms of its clinical and molecular efficacy. There are studies on using various forms of succinic acid as a therapeutic agent in different types of cancer to inhibit tumor cell proliferation, induce apoptosis, or increase the levels of anticancer drugs, alone or in combination with anticancer drugs clinically [12–18]. In 2018, we reported our study in which we demonstrated the *in vitro* anticancer activity of succinic acid on endometrial cancer cells [16] for the first time, and subsequently investigated the anticancer activities of succinic acid in different types of cancer [17, 18]. The most exciting finding of our succinic acid studies in terms of being an anticancer candidate, we demonstrated that succinic acid triggered apoptotic activity and high antiproliferative activity in endometrial cancer [16], leukemia [17], and renal cancer [18] cells. Moreover, current studies show that succinate form, which is formed as an intermediate metabolite in the tricarboxylic acid cycle, rather than *in vitro* activity of succinic acid, plays a role as a signal molecule in the cellular metabolism, inflammation, immune response, and carcinogenesis [19–21]. Furthermore, the fact that succinate is an essential fighting member in hypoxic conditions showed that it is a part of crucial pathways in human metabolism [19]. After demonstrating the promising capacity of succinic acid on different cell types in previous studies, we focused on the molecular level expression changes of well-known and seventeen essential genes encoding proteins involved in apoptosis, necrosis and autophagy pathways with succinic acid. Thus, in this study, we aimed to find out which genes succinic acid acts on inducing cell death and to investigate its potential for further targeting-based studies.

2. Materials and methods

2.1 Cells and culture conditions

Endometrial cancer cell line (CRL-2923) and MRC-5 healthy cell line (RRID: CVCL_0440) were newly obtained from the American Type Culture Collection (ATCC), and they were mycoplasma-free and authenticated. All cell culture procedures were carried out in accordance with the guidelines within the scope of international good laboratory practices. The culture mediums include Roswell Park Memorial Institute (RPMI) 1640 for CRL-2923 and minimum essential medium eagle (EMEM) for MRC-5 with 10% fetal bovine serum (FBS), 1% antibiotic, and 1% L-glutamine. The cells were grown in a carbon dioxide incubator at 37 ℃ with 5% CO_2. The media were renewed every two days until the cells reached sufficient density and size.

2.2 Creating an experiment group and succinic acid treatment

The cells were collected from culture flasks by trypsinization and seeded into 96 well plates (10,000 cells/well) after they attained confluency above 80%. Then, three research groups included 5 μM succinic acid treatment, 10 μM succinic acid treatment, and untreated control groups. All the groups, except the untreated group, were administered succinic acid at 5 μM and 10 μM doses and 48 hours incubation period, as determined in our previous investigations using the CRL-2923 endometrial cancer cells [16]. The doses and the time were established before because searching the related genes with the doses was required for further analysis.

2.3 Isolation of RNA from succinic acid-treated and non-treated cells

After a 48 hours succinic acid treatment, the cells were prepared for the RNA isolation step. The Jena Bioscience Total RNA Purification Kit (PP-210S, Jena, TH, Germany) was used, and all procedures were performed according to the kit's protocol. Each sample was extracted by trypsinization, and the cell counts were chosen according to the kit procedure. The cells were pelletized by centrifugation at 5000 rpm for 5 minutes and the supernatant was removed from the cells. Cells were lysed with lysis solution containing 2-mercaptoethanol as recommended by the kit. A 0.6 μL isopropanol per volume was added to the cell lysate and homogenized by vortexing. The mixture was placed in spin columns and centrifuged for 30 seconds at 10,000g. The liquid was removed from the spin column's bottom tube, and a two-stage washing process was performed using the washing buffers included in the kit. A 2-minute centrifugation was performed to remove the washing solutions and ethanol. The spin columns were inserted in fresh sterile DNase-RNase-free microcentrifuge tubes. Finally, 50 μL of elution buffer was added to the spin columns and centrifuged, and RNA samples were transferred to microcentrifuge tubes. The NanoDrop instrument was used to determine the concentration and purity of the RNA samples, which were subsequently kept at −80 °C for future research.

2.4 cDNA synthesis and quantitative polymerase chain reaction (qPCR)

The isolated RNAs were diluted to 1 μg/μL and equilibrated before cDNA synthesis. The SOLIScript RT cDNA synthesis kit was used, and the synthesis was performed following the kit protocol. The obtained cDNA samples were transferred to 96 well plates for qPCR. The 5× HOT FIREPol® EvaGreen® qPCR Mix Plus kit (08-24-00001, Tartu, Estonia) from Solis Biodyne was used to amplify an acquired single-chain cDNA. The qPCR analysis was performed by reacting cDNA samples from each group (treated with 5, 10 μM succinic acid, and untreated cells) with the primers of genes associated with apoptosis, autophagy and necrosis pathways listed in Table 1. In addition to these genes, *β-actin* was used as an internal control for relative quantitation. Following this, 0.5 μL forward primer, 0.5 μL reverse primer, 13 μL PCR grade water, 4 μL 5× HOT FIREPol® EvaGreen® qPCR Mix Plus, and 2 μL of cDNA samples were added to each tube to make the reaction mixture. Finally, the standard and sample mixes were mixed in a 96 well plate, and the reaction was carried out using the Biorad CFX Connect device (1855201, Foster, CA, USA) under the parameters listed in Table 2.

TABLE 1. Genes analyzed quantitatively in qPCR reaction and their associated pathways.

Cellular Pathway	Genes
Apoptosis	*FAS*, *HRK*, *TNFRSF10A*, *TNFRSF10B*, *MTCH1*, *AKT1*, *Caspase-3*, *Caspase-9*, *BCL-2*, *MDM-2*, *mTOR*
Autophagy	*ATG-3*, *ATG-5*, *BECN*
Necrosis	*PARP1*, *RIPK**, *MLKL*

**Also associated with necroptosis. FAS: Fas Cell Surface Death Receptor; HRK: Harakiri; TNFRSF10A: Tumour necrosis factor ligand receptor superfamily member 10a; TNFRSF10B: Tumour necrosis factor ligand receptor superfamily member 10b; MTCH1: Mitochondrial carrier 1; AKT1: AKT serine/threonine kinase 1; Caspase-3: Cysteine-dependent; aspartate-specific peptidase-3; Caspase-9: Cysteine-dependent; aspartate-specific peptidase-9; BCL-2: B-cell leukemia/lymphoma 2 protein; MDM-2: E3 ubiquitin-protein ligase; mTOR: Mammalian target of rapamycin; ATG-3: Autophagy related 3; ATG-5: Autophagy related 5; BECN: Beclin; PARP1: Poly (ADP-ribose) polymerase; RIPK: Receptor-interacting serine/threonine kinase 1; MLKL: Mixed lineage kinase domain like pseudokinase.*

TABLE 2. Real time PCR conditions.

Steps	Temperature (ºC)	Time	Cycle
First Activation	95	12 min	1
Denaturation	95	15 s	40
Annealing	(It varies depending on the melting point of the primer)	20 s	40
Elongation	72	20 s	40

2.5 Cell death detection Enzyme-Linked Immunosorbent Assay (ELISA)^{PLUS} kit

A Cell Death Detection ELISA^{PLUS} (Roche, 11774425001D2, Mannheim, BW, Germany) kit for detecting histone-associated DNA fragments outside the cytoplasm after apoptosis stimulation was used to examine whether succinic acid has possible apoptotic effects on the growth of EC cancer cells. Apoptosis produces 180 bp DNA fragments that are divided into multiples. Histone proteins keep DNA in a compact state called nucleosomal DNA (H2A, H2B, H3 and H4). Anti-DNA peroxidase (POD) antibody binds one or two-stranded DNA, whereas histone-targeted antibodies react with H1, H2A, H2B, H3 and H4 histone proteins. After succinic acid treatment, the cell-killing activity was assessed by looking for mono- and oligonucleosomes in cell supernatants of the CRL-2923, and MRC-5. After performing all steps according to the kit protocol, measurements were made between 405 and 490 nm wavelengths using ELISA reader equipment to detect the color change.

2.6 Statistical analysis

Statistical analyses of our findings were performed using the statistical package for the social sciences (SPSS) version 21.0 (IBM, Chicago, IL, USA) package program. All experiments were performed in triplicate. The statistical significance rate was determined as $p < 0.05$. The comparative Ct ($2^{-\Delta\Delta Ct}$) method was used in the expression value analysis of the relevant genes. The relative amounts of mRNA according to β-*actin* used as a housekeeping gene was calculated based on the ΔCt values. The experimental and control groups were compared with the analysis of variance (ANOVA) method.

3. Results

3.1 Succinic acid's road to the death pathway

To understand the cellular death pathways in EC cells affected by succinic acid treatment, we performed gene expression analysis via qPCR on CRL-2923 EC cells and MRC-5 normal lung fibroblasts. All gene expression findings were compared to non-treated cells for dose groups of 5 μM and 10 μM of succinic acid. The expression results obtained in our study are shown in Figs. 1, 2, respectively, for the EC cell line CRL-2923 and the healthy cell line MRC-5.

It was observed that *tumour necrosis factor ligand receptor superfamily member 10b* (*TNFRSF10B*) and *mitochondrial carrier 1* (*MTCH1*) gene expressions increased by 3.62 and 3.31-folds, respectively, after 5 μM succinic acid administration in CRL-2923 cells ($p = 0.0038$, $p = 0.006$ respectively). After 10 μM succinic acid administration, it was determined that the expression changes in the *TNFRSF10B* and *MTCH1* genes increased compared to the non-treated group but increased less than 5 μM of succinic acid treatment.

In addition, when the changes in autophagy markers (*Autophagy related 3* (*ATG-3*), *autophagy related 5* (*ATG-5*) and *beclin* (*BECN*)) were evaluated, it was concluded that succinic acid was not involved in autophagy or participated in the cell death process. However, decreased *poly* (*ADP-ribose*) *polymerase 1* (*PARP1*) and *mixed lineage kinase domain-like* (*MLKL*) gene expressions play a role in the necrotic process and were observed after administering 5 and 10 μM succinic acid ($p < 0.05$). Especially after 48 hours of incubation with 10 μM of succinic acid, the 4.11-fold decrease in *PARP1* gene expression is significant in indicating succinic acid's suppressive effects on the necrotic process ($p = 0.0042$).

On the other hand, the expression changes in the *RIPK* gene

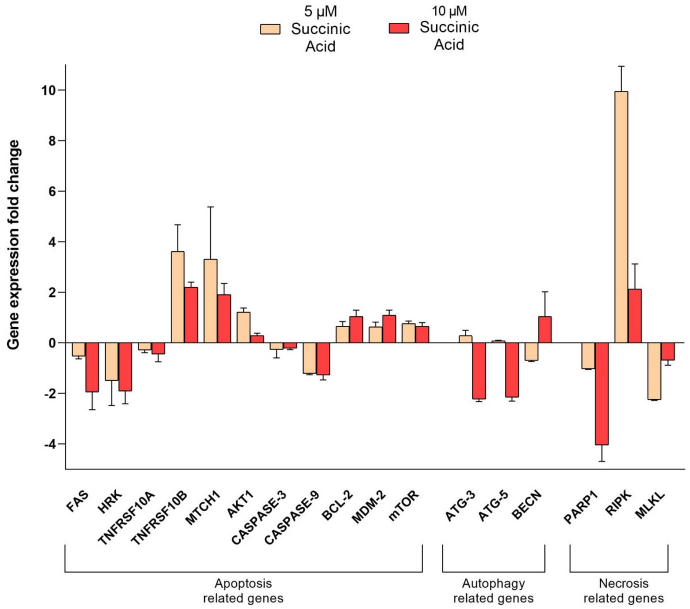

FIGURE 1. Gene expression analysis of CRL-2923 cells. *FAS*: *Fas Cell Surface Death Receptor*; *HRK*: *Harakiri*; *BCL2 Interacting Protein*; *TNFRSF10A*: *Tumour necrosis factor ligand receptor superfamily member 10a*; *TNFRSF10B*: *Tumour necrosis factor ligand receptor superfamily member 10B*; *MTCH1*: *Mitochondrial carrier 1*; *AKT-1*: *AKT serine/threonine kinase 1*; *Caspase-3*: *Cysteine-dependent*; *aspartate-specific peptidase-3*; *Caspase-9*: *Cysteine-dependent*; *aspartate-specific peptidase-9*; *BCL-2*: *B-cell leukemia/lymphoma 2 protein*; *MDM-2*: *E3 ubiquitin-protein ligase*; *mTOR*: *Mammalian target of rapamycin*; *ATG3*: *Autophagy related 3*; *ATG5*: *Autophagy related 5*; *BECN*: *Beclin*; *PARP1*: *Poly (ADP-ribose) polymerase*; *RIPK*: *Receptor-interacting serine/threonine kinase 1*; *MLKL*: *Mixed lineage kinase domain like pseudokinase.*

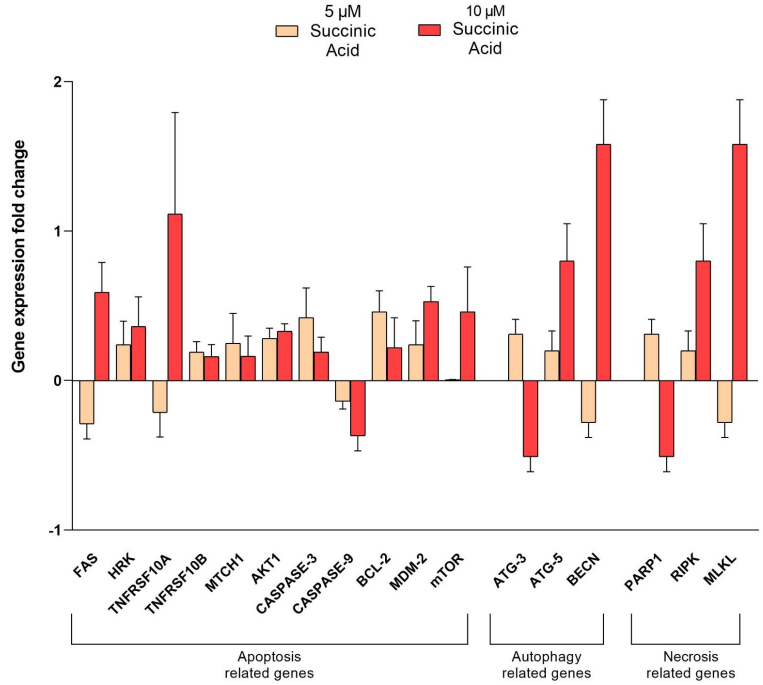

FIGURE 2. Gene expression analysis of MRC-5 cells. *FAS*: *Fas Cell Surface Death Receptor*; *HRK*: *Harakiri*; *BCL2 Interacting Protein*; *TNFRSF10A*: *Tumour necrosis factor ligand receptor superfamily member 10a; TNFRSF10B*: *Tumour necrosis factor ligand receptor superfamily member 10B*; *MTCH1*: *Mitochondrial carrier 1*; *AKT-1*: *AKT serine/threonine kinase 1*; *Caspase-3*: *Cysteine-dependent*; *aspartate-specific peptidase-3*; *Caspase-9*: *Cysteine-dependent*; *aspartate-specific peptidase-9*; *BCL-2*: *B-cell leukemia/lymphoma 2 protein*; *MDM2*: *E3 ubiquitin-protein ligase*; *mTOR*: *Mammalian target of rapamycin*; *ATG3*: *Autophagy related 3*; *ATG5*: *Autophagy related 5*; *BECN*: *Beclin*; *PARP1*: *Poly (ADP-ribose) polymerase*; *RIPK*: *Receptor-interacting serine/threonine kinase 1*; *MLKL*: *Mixed lineage kinase domain like pseudokinase.*

play a role in the necrotic process, showing more exciting results than other necrosis markers. Although the three cell death mechanisms and their associated signaling pathways have been well-studied, cell death in the whole organism proceeds as a complex interaction of these pathways. In our findings shown in Fig. 3, when the fold changes in the *RIPK* gene were evaluated after 5 μM succinic acid treatment, a 0.04-fold downregulation was measured for MRC-5, while a 9.94-fold upregulation was measured for CRL-2923 ($p = 0.008$).

3.2 Succinic acid increases the apoptotic nucleosomal enrichment factor

To measure apoptotic cell death, the CRL-2923 endometrial cancer and MRC-5 healthy cells were incubated with succinic acid for 48 hours, and changes in nucleosomal enrichment factor were analyzed using a Cell Death Detection ELISA^{PLUS} assay. The obtained results are given in Fig. 4 for the CRL-2923. According to the results of 5 μM and 10 μM succinic acid doses treatment on CRL-2923, it was observed that the enrichment factor showed a very slight increase ($p > 0.05$). Even though the increasing level was not observed at the statistical significance limit, the expression results with well-known apoptotic genes showed similarly increased results. For MRC-5 cells, the enrichment factor was slightly decreased after 5 μM and 10 μM of succinic acid treatments compared to the control groups ($p > 0.05$).

4. Discussion

Succinic acid is a promising candidate for cancer research [22, 23]. Several studies have been reported in the literature demonstrating the combination of various forms of succinic acid with chemotherapeutic agents in different cancer types [14, 15, 24, 25]. Among the outstanding studies in the last two decades, it has been emphasized that the ester compound of succinate with alpha-tocopherol has an anticancer effect in various types of cancer [26].

The *in vitro* activity of succinic acid against some cancer cells has been studied in our laboratory since 2013, and

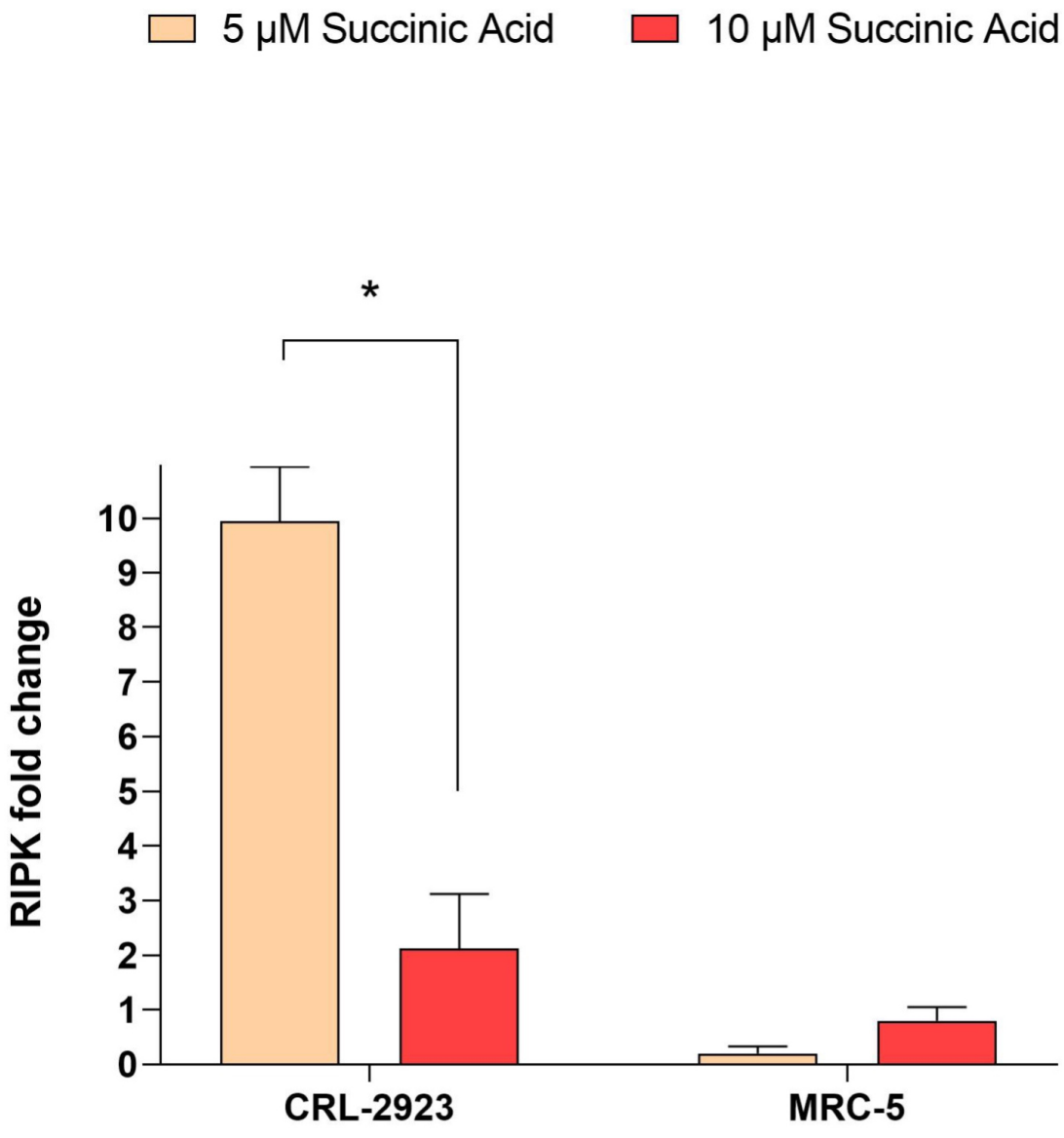

FIGURE 3. Comparison of *RIPK* gene expression in MRC-5 and CRL-2923 cells after succinic acid application. *RIPK: Receptor-interacting serine/threonine kinase 1.* *: signs a significant difference in meaning between the two groups ($p < 0.05$).

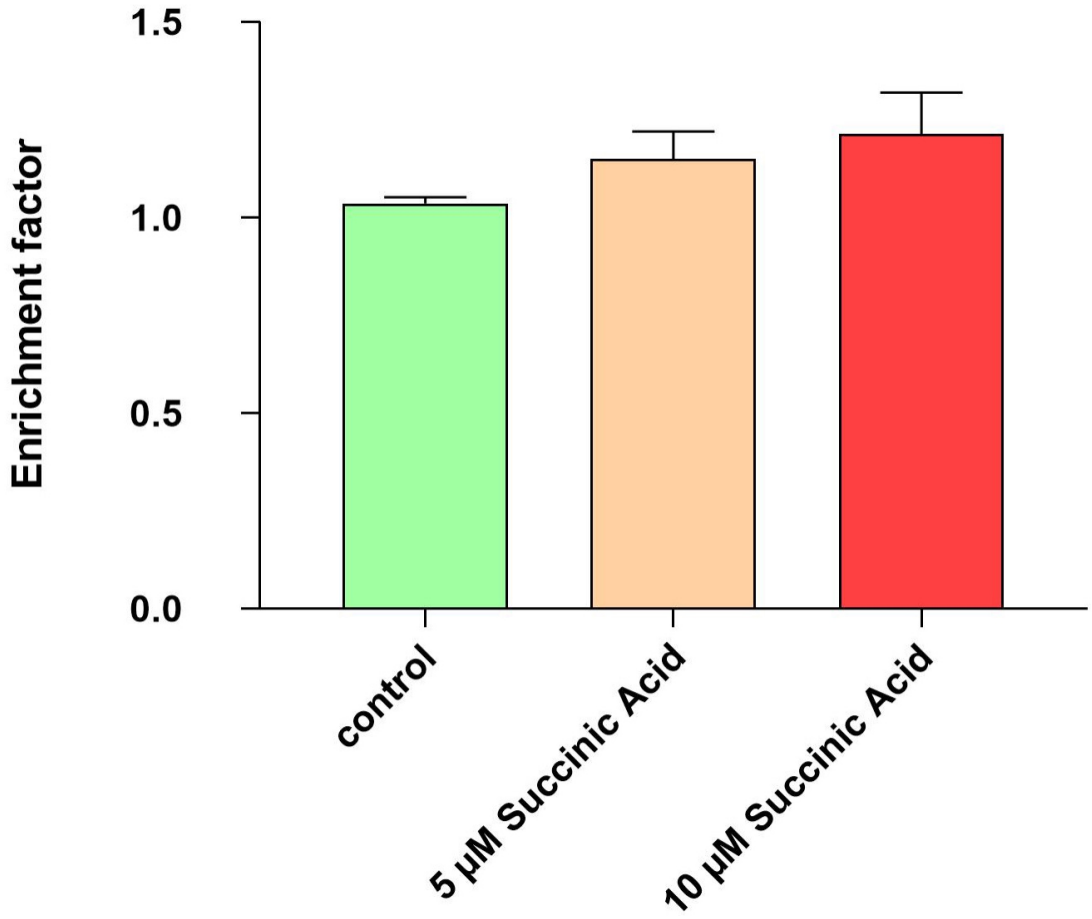

FIGURE 4. Apoptotic activity of succinic acid on CRL-2923.

the findings in endometrial cancer [16], T cell lymphoblastic leukemia [17], and renal cancer [18] cells have been published in recent years. Furthermore, succinic acid's behavior on basic and well-known genes associated with cellular death pathways in EC have been the major focus in this study. However, in this study, it was observed that 5 μM succinic acid produced results that supported our previous study [16] on gene expression. Thus, we took the role of succinic acid on cell death one step further.

The *TNFRSF10B* gene is a member of the tumor necrosis factor (TNF)-receptor superfamily, and its encoded protein structurally contains an intracellular death domain [27]. TNFRSF10B receptor can be activated by tumor necrosis factor-associated apoptosis-inducing ligand tumor necrosis factor superfamily/tumor necrosis factor-related apoptosis-inducing ligand/Apo2 ligand or tumour necrosis factor-related apoptosis-inducing ligand (TNFSF10/TRAIL/APO-2L), which can stimulate apoptosis [28]. It is known that death domains in the structure of this protein are essential for initiating Fas cell surface death receptor (FAS) and TNF-mediated apoptosis in the cells [28]. *MTCH1* gene, another candidate gene in our study, encoded a member of the mitochondrial transporter family. The protein encoded by this gene is localized to the mitochondrial outer membrane and induces apoptosis independently of the proapoptotic proteins, Bax and Bak [29]. According to our findings, the increase in *TNFRSF10B* and *MTCH1* expressions indicated several apoptosis inductions. Therefore, stimulation of succinic acid might not support the functions through the apoptosis pathway and mitochondria-mediated apoptosis. Essentially, *TNFRSF10B* and *MTCH1* findings demonstrate the function of succinic acid through a specific pathway rather than direct apoptosis stimulation, such as necroptosis that works with the complex in which *caspase 8* and *RIPK* are involved and activate the apoptotic pathway.

On the other hand, it was observed that *PARP1* and *MLKL* gene expressions, which are selected as cellular necrosis markers, were decreased after 5 μM and 10 μM succinic acid administrations, indicating that succinic acid might play a suppressive role in the necrotic process. According to our findings, the *PARP1* gene, which encodes the PARP1 protein, plays a role in essential cellular processes, such as DNA repair, maintenance of genome stability, and induction of cell death, which decreased a 4.11-fold after 10 μM succinic acid administration. Ogino *et al.* [30] demonstrated a change in the *PARP1* gene due to some amino acid substitutions in sixteen human germ cell tumors. Accordingly, the response of germ cells to chemotherapy and radiotherapy may be altered [30]. Nonetheless, specific mutations observed in cancer types, such as ovarian cell carcinoma, uterine endometrioid carcinoma, gastric cancer, hepatocellular carcinoma, and breast cancer, impair DNA damage repair mechanisms associated with *PARP* sensitivity [8, 31]. According to cancer studies, it is believed that *PARP* inhibition will be the focus for future directions for these types of cancer [8, 32]. Furthermore, apoptosis resistance, frequently encountered in multifactorial diseases

such as cancer, which causes drug resistance and similar adverse effects in the treatment process, constitutes a significant problem, especially in treatment efficacy and healing periods. Therefore, necroptosis, an alternative mode of programmed cell death, may overcome the resistance to apoptosis and is modifiable. Although the role of necroptosis in cancer treatments is complex, it is effective in triggering and/or strengthening antitumor immunity. This necroptosis efficiency depends on the type of cancer and the presence of critical regulators in the necroptotic pathway. Decreasing levels of *PARP1* and *MLKL* expression is another finding which showed promising results for the necrotic process that somehow cells might undergo programmed death due to the action of succinic acid. MLKL protein, a known and well-studied molecule of the necroptotic process, initiates the necroptotic process by interacting with different upstream stimuli, such as *RIPK*, transcriptional regulation of the human (TRIF) or Z-DNA binding protein 1 (ZBP-1) [33]. During RIPK and MLKL function as necroptosis machinery, caspase 8 blocks this process. In the absence of caspase 8, because of the interaction of RIPK and MLKL and other molecules, necrosome is formed, and necroptosis occurs. On the other hand, with the stimulation of the complex in which caspase 8 is involved, RIPK stimulation is directed, leading to the activation of the apoptotic pathway. From this perspective, the need to examine and elucidate the function of caspase 8 with further studies showing both gene expression and protein levels has come to the fore.

The RIPK family is a seven-member enzyme family; each has a conserved kinase domain with a wide range of activities in cellular signaling pathways, from cell survival to death-deciding mechanisms [34]. In recent years, studies on genetic changes in RIP kinase members and their relationship with carcinogenesis have revealed that RIPK might play an essential role in cancer development and progression [35–37]. The members of the RIPK family bind to different molecules in downstream pathways and determine whether a cell will continue to survive or die by apoptosis or necrosis. In other words, promoting cellular survival could induce necroptosis in different groups. For instance, while ubiquitination of RIPK by a complex consisting of TNF-A, TNFR, tumor necrosis factor receptor type 1-associated death domain protein (TRADD), TNF receptor associated factor 2 (TRAF2) and cellular inhibitor of apoptosis proteins (cIAPs) stimulates survival via the nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, deubiquitinating by another complex containing caspase-8 and Fas-associated protein with death domain (FADD) activates the apoptotic cell death pathway. On the other hand, upon inactivating of the caspase activity, the interaction of two members of the RIPK family causes necroptosis by forming a necrosome complex. All these features indicated that RIPK's could show a dual role in these vital pathways. It revealed that, from a different perspective, the preference for apoptosis, necrosis, and/or necroptosis processes could be modulated via RIPK. An essential finding was that only one RIPK was found, and there was almost no change in healthy cells, while there was a high concentration in the endometrium cells. The other reason we have decided to break down our judgments on *RIPK* after seeing our results of *MLKL* expression level. First, we have thought this situation was necrosis and resulting in a pathological effect on the process. Notwithstanding, when the relationship between MLKL and RIPK in necroptosis was evaluated via the pathway, we overthrew these judgments and turned to necroptosis pathway. RIPK's activity is modified by the presence of other proteins with which it interacts downstream. For instance, it does not work unidirectionally in cellular death processes, suggesting that the activity of succinic acid on RIPK and cellular death processes may lead to different points. In this study, due to budget constraints, further protein analyzes, and functional studies could not be performed, and the effectiveness of succinic acid could only be studied on a wide gene panel, including genes that play a role in cell death pathways. However, it is scheduled to be added in our further studies.

5. Conclusions

We observed that succinic acid has the potential to influence both apoptosis and necroptosis through *RIPK* and other members of this pathway. This situation emphasizes once again that the interaction of succinic acid with different cellular pathways has a potential that should not be overlooked for research of targeting-based anticancer treatment strategies.

AVAILABILITY OF DATA AND MATERIALS

Datasets are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

SB and BC—designed the research study. GKK and BE—performed the research, project development, and all experimental work. RA—provided help and advice on the data analysis. BC—provided resources, analyzed the data and review manuscript. GKK and SB—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Due to the fact that the study has no human or animal material, no ethical approval is required. The cell lines used in the study, Endometrial cancer (CRL-2923) cells and healthy fibroblast cells (MRC-5), were commercially obtained from the ATCC.

ACKNOWLEDGMENT

We would like to thank Acibadem Mehmet Ali Aydinlar University Publication Office for the English spellchecked and editing.

FUNDING

This study was funded by the Scientific Research Projects Coordination Unit of Istanbul University (Grant number: 34139).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Sinem Bireller, Goksu Kasarci Kavsara, Baris Ertugrul, Rukset Attar, Bedia Cakmakoglu. Succinic acid might be an inducement for the road to *RIPK* for death through necroptosis in endometrium cancer. European Journal of Gynaecological Oncology. 2025; 46(2): 30-37. doi: 10.22514/ejgo.2025.019.