T.C.
DOKUZ EYLÜL UNIVERSITY
IZMIR INTERNATIONAL
BIOMEDICINE AND GENOME
INSTITUTE

THE EFFECTS OF RUTHENIUM COMPLEXES IN OVARIAN CANCER

GÜLNUR ÇIRAK

MOLECULAR BIOLOGY AND GENETICS MASTER'S PROGRAM

MASTER OF SCIENCE THESIS

İZMİR-2019

2017850043

T.C.
DOKUZ EYLÜL UNIVERSITY
IZMIR INTERNATIONAL
BIOMEDICINE AND GENOME
INSTITUTE

THE EFFECTS OF RUTHENIUM COMPLEXES IN OVARIAN CANCER

MOLECULAR BIOLOGY AND GENETICS
MASTER'S PROGRAM

MASTER OF SCIENCE THESIS

GÜLNUR ÇIRAK

Prof. Hülya AYAR KAYALI

2017850043

T.C.
DOKUZ EYLÜL ÜNİVERSİTESİ

İZMİR ULUSLARARASI BİYOTIP VE GENOM ENSTİTÜSÜ

YÜKSEK LİSANS TEZ SAVUNMA SINAVI TUTANAĞI

Dokuz Eylül Üniversitesi İzmir Uluslararası Biyotıp ve Genom Enstitüsü Genom Bilimleri ve Moleküler Biyoteknoloji Anabilim Dalı, Moleküler Biyoloji ve Genetik Yüksek Lisans Programı öğrencisi 2017850043 numaralı Gülnur ÇİRAK "**THE EFFECTS OF RUTHENIUM COMPLEXES IN OVARIAN CANCER**" konulu Yüksek Lisans tezini 25.10.2019 tarihinde yapılan savunma sınavı sonucunda başarılı olmuştur.

BAŞKAN
Prof. Dr. Hülya AYAR KAYALI
Dokuz Eylül Üniversitesi İzmir Uluslararası Biyotıp ve Genom Enstitüsü

ÜYE
Prof. Dr. Elif ŞUBAŞI
Dokuz Eylül Üniversitesi
Anorganik Kimya Anabilim Dalı

ÜYE
Prof. Dr. Sinan AKGÖL
Ege Üniversitesi
Biyokimya Ana Bilim Dalı

YEDEK ÜYE
Prof. Dr. Nur Arslan
Dokuz Eylül Üniversitesi
Moleküler Tıp Anabilim Dalı

YEDEK ÜYE
Prof. Dr. Şenay ŞANLIER
Ege Üniversitesi
Biyokimya Ana Bilim Dalı

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

ATP……..……Adenosine 5'-triphosphate
CAR……...…….….Carboplatin
D-2HG……..…D-2-hydroxyglutarate
DMSO……..……Dimethyl sulfoxide
DNPH……2, 4-dinitrophenylhydrazine
ETC…..……Electron transport chain
FAD……Flavine Adenine Dinucleotide
FH……Fumarate hydratase
HGSC……..High-grade serous carcinoma
HIF-1a……...Hypoxia-inducible factor 1-alpha
HPLC……High-Performance Liquid Chromatography
IDH…….……Isocitrate dehydrogenase
mtDNA……..Mitochondrial DNA
MTT…….....……3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NADPH……Nicotinamide Adenosine Dinucleotide Phosphate
NAMI-A……..……New Anti-tumor Metastasis Inhibitor A
NEAA……Non-essential amino acid
OAA……..……..…….Oxaloacetate
OSE…….Ovarian surface epithelium
OXPHOS…….……..……Oxidative phosphorylation
PAX……Paclitaxel
PPP…….……..……Pentose phosphate pathway
SDH……Succinate dehydrogenase
TCA…….…..……Tricarboxylic acid
αKG…….……alpha-Ketoglutarate
OC……..Ovarian Cancer

ACKNOWLEDGEMENTS

First of all, I would like thank my advisor Prof. Dr. Hülya AYAR KAYALI, for giving me an opportunity to be part of this experience. I am grateful for her patience, advice, motivation, and encouragement.

I would like to thank my colleagues and everyone in IBG for their help and support throughout my research. I also want to thank our postdoctoral researcher Dr. Zehra TAVŞAN, for her guidance and generous support.

I want to express my special thanks to Ivan de ABREU LIMA Jr. for his love, unreplaceable support, friendship, and his encouragement to follow my dreams. He was always standing by me in my hard times.

Last but not least, I would like to express my sincere gratitude to my family for their unconditional support and love in every moment of my life.

Gülnur ÇIRAK

THE EFFECTS OF RUTHENIUM COMPLEXES IN OVARIAN CANCER

Gülnur Çırak, Dokuz Eylül University İzmir International Biomedicine and Genome Institute, Balçova, 35340, İzmir/TURKEY

ABSTRACT

Ovarian cancer has a high death incidence among females worldwide. Although first line chemotherapy with platinum/taxane combination has been used successfully in ovarian cancer, the majority of the patients relapsed and gained chemotherapy resistance. Lately, ruthenium complexes have emerged as encouraging chemotherapeutic agents to replace the platinum-based chemotherapy regarding their selective mechanisms of action along with lower toxicity, and the potential to overcome chemotherapy resistance.

It is accepted that cancer is a heterogeneous disease, and it also exhibits characteristic variations in metabolic phenotype from one cell to another. Because cancer cells repeatedly reprogram to adapt to challenging environmental changes.

In this thesis, we aimed to examine the effects of chemotherapeutic agents on ovarian cell lines. Therefore, we evaluated the alterations in the levels of glycolysis metabolites such as glucose, pyruvate; critical intermediates of TCA cycle such as OAA, αKG, succinate, fumarate, malate, and the metabolite of anaerobic respiration, lactate, with respect to the Ru, PAX, CAR concentrations and incubation time. The results proved that each ovarian cell line displayed distinct metabolic phenotypes of energy, and thus, each ovarian cell line responded in a unique way to chemotherapeutic agents. Additionally, ruthenium complexes showed better anti-tumor activity than carboplatin and lower toxicity against healthy cells than paclitaxel. Among ruthenium agents, complex 1a demonstrated the most promising results against cisplatin-resistant ovarian cancer cells.

Key Words: Ruthenium, Ovarian cancer, TCA cycle

RUTENYUM KOMPLEKSLERİNİN OVARYUM KANSERİ ÜZERİNDEKİ ETKİSİ

Gülnur Çırak, Dokuz Eylül Üniversitesi İzmir Uluslararası Biyotıp ve Genom Enstitüsü, Balçova, 35340, İzmir/TÜRKİYE

ÖZET

Ovaryum kanseri, Dünya çapındaki en ölümcül kanserlerden biridir. Ovaryum kanseri tedavisinde platin/taksol kombinasyonu her ne kadar ilk aşamada başarılı olsada, hastaların büyük bir kısmı kemoterapiye direnç kazanarak, kanseri nüks etmiştir. Son yıllarda rutenyum kompleksleri gösterdiği seçici anti-kanser aktivitesi, daha az toksik olması ve dirençli hücreleri öldürebilme potensiyeli ile platin-bazlı bileşenlerin yerini alabilecek umut vadeden kemoterapi ajanları olarak ortaya çıkmıştır.

Kanserin heterojen yapıda bir hastalık olduğu kabul edilmiş bir tanımdır. Her kanser hücresi, kendisine özgü bir metabolik fenotip gösterir. Çünkü kanser hücreleri, sürekli olarak metabolizmalarını çevresel etkenlere göre programlayarak adaptasyon sağlarlar.

Bu çalışmada amacımız, kemoterapik ajanların ovaryum hücreleri üzerindeki etkilerini gözlemlemektir. Bu nedenle belli konsantrasyonlarda ve zaman aralıklarında rutenyum, paklitaksel ve karboplatin uygulanmış ovaryum hücrelerindeki glikoliz metabolitlerinden glukoz ve piruvat; Krebs döngüsünün metabolitlerinden oksaloasetat, fumarat, malat, süksinat ve alfa-ketoglutarat; oksijensiz solunum metaboliti olan laktat seviyeleri ölçüldü. Elde ettiğimiz sonuçlar, her ovaryum hücre hattının benzersiz bir enerji metabolizmasının olduğunu ve bu yüzden de her hücre hattının kemoterapiye özgün yanıtlar verdiğini gösterdi. Ek olarak, rutenyum komplekslerinin karboplatinden daha iyi bir anti-kanser ilacı olduğu ve paklitaksele kıyasla sağlıklı hücrelere daha az toksik olduğunu gösterdi. Uygulanmış rutenyumlar arasında sisplatin dirençli ovaryum hücrelerine karşı en umut vadeden sonucları veren kompleks 1a oldu.

Anahtar Sözcükler: Ovaryum kanseri, Rutenyum, Krebs döngüsü

1. INTRODUCTION AND AIM

Cancer caused an estimated 9.6 million deaths in 2018 and still is the second major reason for death in the world (World Health Organization, 2018). Ovarian cancer (OC) is the fifth major cause of death associated with cancer among females in the world. Moreover, nearly 1.3% of females will be diagnosed with OC someday in their life, according to 2014-2016 data (WHO, 2018). Its 5-year relative survival rate is 47% due to late stage (such as stage III or IV) diagnose (American Cancer Society, 2019).

There is consequential proof showing that metal-based chemotherapeutics are encouraging opponents for cancer treatment. Platinum-derived complexes, such as cisplatin, and carboplatin, have been used on numerous kinds of cancers such as ovary, colon, and stomach (Jung & Lippard 2007; Todd & Lippard, 2009). Even though OC patients were responded mostly fine to the first line chemotherapy with platinum/taxane (around 80-90%), the most significant part of patients relapsed and developed chemotherapy resistance (Herzog, 2004; Banno et al., 2014; Stronach et al., 2015). For that reason, designing chemotherapeutic compounds with better efficiency to conquer the drug resistance in OC is a critical and urgent need.

Ruthenium (Ru) complexes are recognized as an alternative to platinum-based compounds, due to their attributes: performing various mechanisms of action, and the potential to conquer the drug resistance, along with lower toxicity. Studies showed that Ruthenium compounds are inhibitors for cell growth in numerous cancer types, such as ovarian, breast, and melanoma (Fernandez et al., 2004; Ang & Dyson, 2006; Bugarcic et al., 2009; Betanzos- Lara et al., 2012).

It is clear now that tumor cells can regulate cellular metabolism under certain circumstances. There is a lack of fundamental researches on the metabolic response of cancer cells to chemotherapeutic agents. Studying the energy metabolism of cancer cells may lead to essential evaluations that would benefit for further treatment applications. In order to evaluate the metabolic response of ovarian cells to ruthenium complexes, the study was examined the alterations of levels of glycolysis metabolites such as glucose, pyruvate; intermediates of tricarboxylic acid (TCA) cycle such as oxaloacetate (OAA), α-ketoglutarate (αKG), malate, fumarate, succinate, and the

metabolite of anaerobic respiration, lactate, with respect to ruthenium (Ru), paclitaxel (PAX), carboplatin (CAR) concentrations and incubation time.

The thesis aims to evaluate the cytotoxicity of certain chemotherapeutic agents and elucidate the effects of these agents on the energy metabolism of ovarian cancer.

2. GENERAL INFORMATION

2.1. Ovarian Cancer

As can be seen in Figure 1, the ovaries are a pair of oval-shaped glands that are located on either side of the uterus. They generate eggs that pass through the fallopian tubes into the uterus. They are also responsible for the production of hormones, such as progesterone and estrogen, which perform an essential part in the menstrual period and pregnancy (National Cancer Institute, 2019).

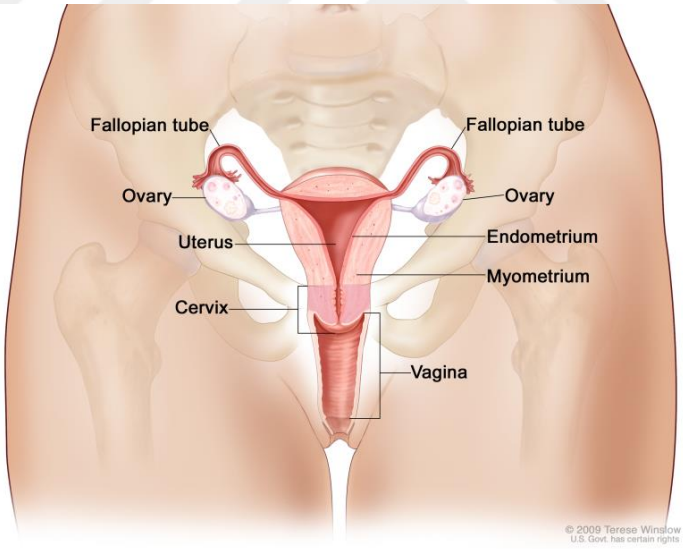

Figure 1 Anatomy of the reproductive system of women (Adapted from National Cancer Institute, 2019).

Ovarian tumors are categorized into three primary division by the anatomic structures from which they originate (Chen et al., 2003):

- The cells of the external surface of the ovary create epithelial-stromal tumors,
- The cells that generate the eggs create germ cell tumors, and
- Connective tissue cells create sex cord-stromal tumors.

Epithelial ovarian cancer is the most common type (60%) among gynecological malignancies (Siegel, Miller, & Jemal, 2018). They are classified as type I and II, based on their unique clinicopathologic and genetic aspects (Kurman & Shih, 2010). Type I exhibit somatic mutations of BRAF, KRAS, or ERBB2 genes, lack of TP53 mutations. They are comprising of low-grade endometrioid, low-grade serous, mucinous carcinomas, and clear cell. They have a good prognosis, although only less than 30% are in this group. On the other hand, tumors in type II are more aggressive and common such as high-grade serous carcinoma (HGSC) (70%). These tumors exhibit repeated mutations in oncogenes, including TP53. Non-epithelial ovarian malignancies are rarer such as sex cord-stromal malignancy. Transitional cell carcinoma was first proposed as a subtype of Brenner tumor, lacked benign factors of Brenner tumors but contained urothelial factors. These tumors are now recognized as a morphologic version of HGSC, and Brenner tumors are recognized as low-grade carcinomas (Brammer et al., 1990; Chen et al., 2003; Prat, 2012; Mutch & Prat, 2014).

2.2. Etiology of Ovarian Cancer

Ovarian cancer has numerous causes, but the most critical risk factor is having a family history of breast or ovarian cancer with BRCA1 or BRCA2 mutations, which accounts for 10% to 15% of all (Hennessy, Coleman, & Markman, 2009). The risk factors for OC summarized in Figure 2. The risk of acquiring OC increases significantly between the ages of 50 and 80 years (Glance, 2009). The studies have shown that the women who had experienced a full-term pregnancy were showed a decreased risk for developing OC compared to women who have not and the risk was minimized with each additional pregnancy (Whittemore, Harris, & Itnyre, 1992; Kvåle, Heuch, Nilssen, & Beral, 1988). While infertility looks as if enhancing the risk of OC in various studies (McGowan et al., 1988; Hartge et al., 1989; Risch, Marrett, & Howe, 1994; Mosgaard et al., 1997), however, there are some others to disagree with it (Kvåle, Heuch, Nilssen, & Beral, 1988; Franceschi et al., 1994). Moreover, some studies were reported a moderate decrease in OC risk among women who breastfed, and the risk decreases more if it is for a more extended period (Wentzensen et al., 2016; Gaitskell et al., 2017). Another study reported that oral contraceptives display a protective effect against OC with the mutations of BRCA1 and BRCA2. A protective effect was also observed for breastfeeding in patients with the mutations of BRCA1 (McLaughlin

et al., 2007). Low parity, late menopause, early menarche, and hormone replacement therapy (HRT) (especially for long-period use) increases OC risk (Lacey et al., 2002; Riman et al., 2002; Glance, 2009). Another study discovered that if all other risk elements had stayed constant, an increase in height and weight would be linked to a three percent acceleration in ovarian cancer frequency for every decade (Beral et al., 2012). In the general population, OC incidence was higher in the whites compared to other ethnic groups (Morris, Sands, & Smith, 2010).

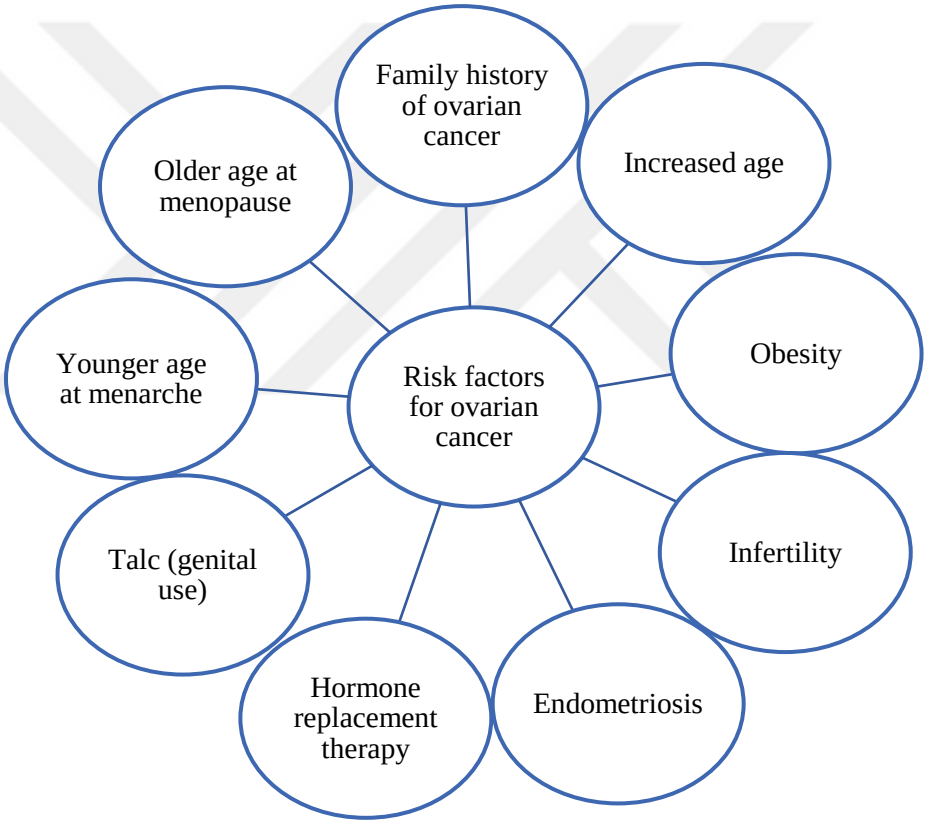

Figure 2 Risk factors associated with Ovarian Cancer

2.3. Epidemiology

Ovarian cancer incidence manifests large geographic variations (Figure 3) (WHO, 2018). The developed countries in North and Central America, and Eastern Europe demonstrated the highest (age-standardized) incidence rates, which rates are usually surpassing 8 per 100,000. Rates are generally moderate in Australia (> 6.7 per 100,000) and South America (~5.5 per 100,000) and

lowest in the Middle East with South Asia and Africa (≤ 3.8 per 100,000). OC rates were also increased in the less developed countries that undergo economic growth and lifestyle alterations. Immigration from less-developed to high-developed countries can also cause a shift in the rates of OC incidence and mortality, which indicates the influence of non-genetic risk factors (Herrinton, Stanford, Schwartz, & Weiss, 1994; Kliewer & Smith, 1995).

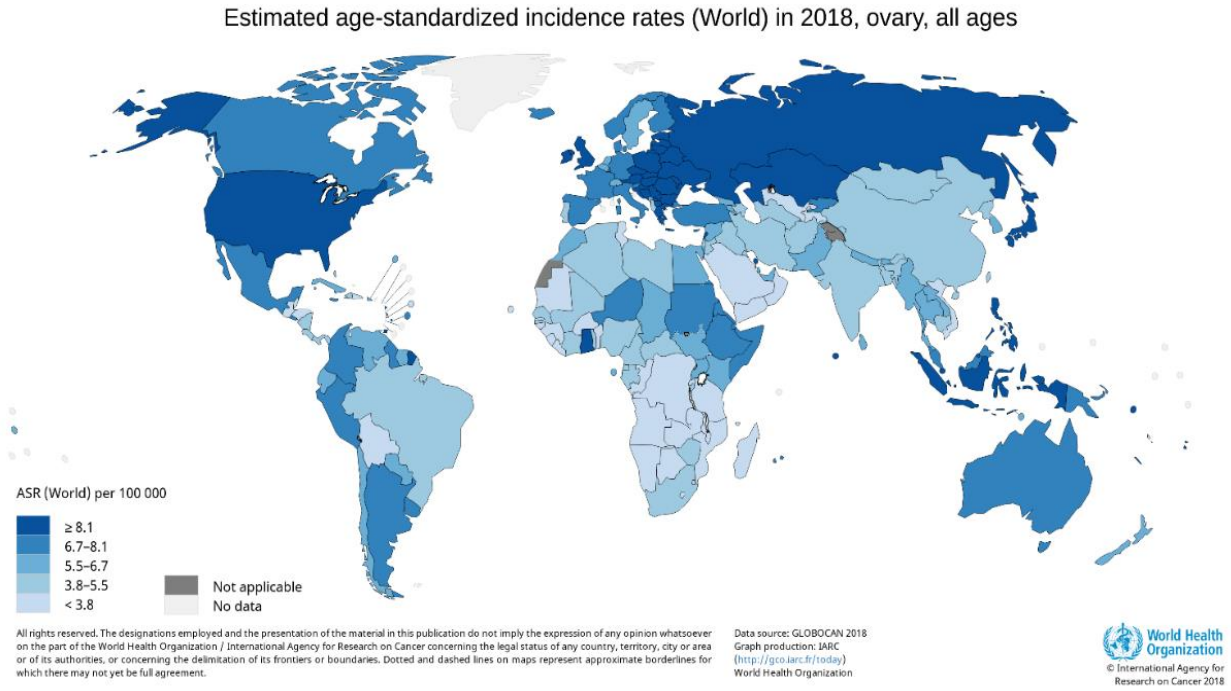

Figure 3 Global Incidence of Ovarian Cancer in 2018 (for all ages). ASR displays an age-standardized rate (WHO, 2018).

2.4. The Staging of Ovarian Cancers

The International Federation of Gynecology and Obstetrics (FIGO) ovarian cancer staging system was announced in 1973 for the first time and then adjusted in the years of 1988 and 2014 (Mutch & Prat, 2014). As it was showed in Table 1 and illustrated briefly in Figure 4, there are four stages to describe ovarian cancer based on the new FIGO classification system (Prat, 2015).

Table 1 FIGO Staging System for Ovarian Cancer (Adapted from Prat, 2015)

Stage IB	"Tumor on the surface of the either or both ovary or fallopian tube, capsule rupture, positive peritoneal ascites."
Stage IIB	"Invasion to pelvic intraperitoneal tissues."
Stage IIIA	"Metastasis to the lymph nodes or microscopic, peritoneal involvement with/out the involvement of lymph nodes."
Stage IIIB	"Macroscopic, peritoneal metastasis equal or bigger than the size of 2 cm with/out the involvement of lymph nodes."
Stage IIIC	"Macroscopic, peritoneal metastasis bigger than the size of 2 cm with/out the involvement of lymph nodes."
Stage IV	"Metastasis to liver, spleen, or extra-abdominal organs."

Stage I is relatively rare because patients are mostly diagnosed at stage III or IV, but it is associated with the most excellent survival rates (Richardson, Scully, Nikrui, & Nelson, 1985). Stage II represents the spread of the tumor into the different areas of the pelvis. It implies that it is not only in the ovaries, but it is also spreading into distinct tissues as well. This stage makes up less than 10 percent of tumors and is deemed curable. Unfortunately, owing to the lack of symptoms, the more significant part of women are generally diagnosed with OC at stage III or IV. Past studies reported that patients who showed non-specific symptoms such as abdominal pain, gastrointestinal symptoms, and bowel habits alterations might lead to a misdiagnose as if suffering from irritable bowel syndrome (Goff, Mandel, Munt, & Melancron, 2000; Olson et al., 2001). At late stages, the tumor has spread outside of the ovaries and pelvis. Stage IV is defined as a distant metastatic form of cancer, which accounts for twelve to twenty-one percent of ovarian cancer patients (Heintz et al., 2006).

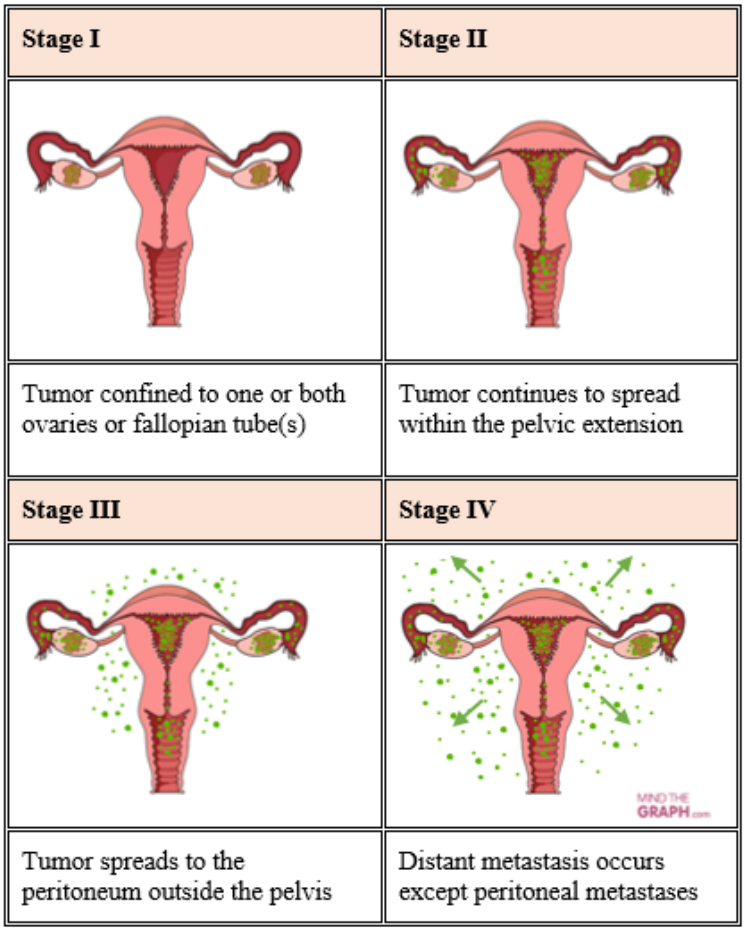

Figure 4 Stages of Ovarian Cancer (Figure created in the Mind the Graph platform, www.mindthegraph.com).

2.5. Cancer Treatment and Chemotherapy

There are three main types of cancer treatment: radiotherapy, surgery, and chemotherapy. Chemotherapy aims to use anti-tumor agents to cure cancer. The purpose is killing the cancer cells precisely without harming the healthy tissue.

The first recorded application of chemotherapy against cancer was to cure lymphoma by the nitrogen mustard in 1942 (Goodman et al., 1984). The significant landmarks of chemotherapy were the introduction of platinum in 1976 (Wiltshaw & Kroner, 1976), cisplatin-based therapy between 1984 and 1986 (Neijt et al., 1984; Williams et al., 1985; Omura et al., 1986), and paclitaxel in 1993 (Einzig et al., 1992; McGuire et al., 1996). These landmarks remarkably improved the survival in

females with advanced stages, and over the previous 20 to 30 years, their combined use has been refined through clinical trial involvement.

2.5.1. Platinum-based Chemotherapeutics

The achievement of platinum as an anti-tumor agent (Galanski, Jakupec, & Keppler, 2005) has inspired research for alternative metallic cytotoxic agents with advanced anti-tumor activity and less toxicity. Some of the antineoplastic agents based on platinum are currently in clinical use, such as cisplatin, carboplatin, and oxaliplatin (Figure 5) (Galanski, 2006). Cisplatin and carboplatin have been used to cure numerous cancers, such as testicular, ovarian, and neck (Muggia, 2009). Generally, these drugs bind at the N7 position of purines (Reedijk, 1987), which leads to unwinding and bending of the DNA (Johnstone, Suntharalingam, & Lippard, 2016). This action inhibits cellular division by preventing transcription and replication of DNA, which probably initiates apoptosis as a result of this action (Alderden, 2006; Amable, 2016).

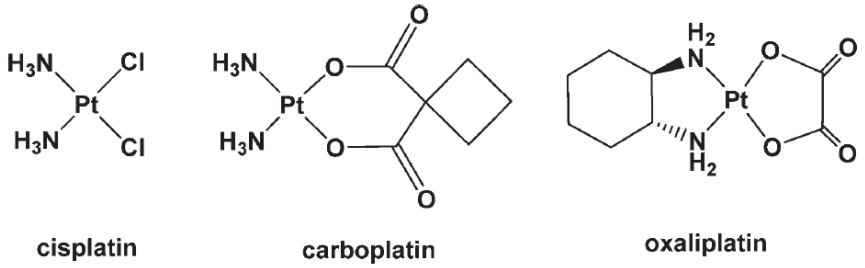

Figure 5 The structures of three platinum drugs that are currently in clinical use (Adapted from Wheate, Walker, Craig, & Oun, 2010).

Even though these heavy metal agents are effective against various cancer types (Muggia, 2009), they display serious side effects such as gastrointestinal symptoms, neuromuscular complications, and nephrotoxicity (Griffin et al., 1996). Furthermore, platinum use is limited by primary and chemoresistance in many tumor kinds (Brabec & Kasparkova, 2005; Chen, Milacic, Frezza, & Dou, 2009). These findings led to a continuous search for non-platinum metals that can expand the range of drug activity (Ott & Gust, 2007).

2.5.1.1. Carboplatin

Carboplatin exhibits a mechanism resembling cisplatin due to forming adducts with the DNA. Carboplatin also favors the N7 position of purines to bind. Nevertheless, carboplatin seems to be less reactive than cisplatin and exhibits less severe side effects. As cisplatin, it contains platinum enclosed by groups of two ammonia and two additional ligands in the cis position. The other two ligands in carboplatin have existed in a ring complex instead of two chlorides in cisplatin. This alteration makes carboplatin less reactive, stable, and lower side effects (nephrotoxicity, neurotoxicity, and ototoxicity) than cisplatin (Go & Adjei, 1999; McEvoy, 2000). A recent study proved that carboplatin favor to form monoadducts opposite to cisplatin, because of this, carboplatin requires a long time to form diadducts with the double-helix (Oliveira, Caquito, & Rocha, 2018).

Carboplatin has been used for many cancer types as ovary (Swenerton et al., 1992; Alberts et al., 1992), lung (Langer et al., 1995), and head tumors (Al-Sarraf et al., 1987), however, it has less efficiency compared to cisplatin in certain circumstances (Bajorin et al., 1993; Bokemeyer et al., 1996; Lokich & Anderson, 1998; Stewart, 2007).

2.5.2. Non-Platinum-based Chemotherapeutics

2.5.2.1. Paclitaxel

Paclitaxel is a chemotherapeutic emerges from the taxane class, which was extracted from the bark of the Pacific yew tree in the 1960s (Cragg, 1998; Suffness, 1994). As a microtubule agent, paclitaxel eliminates the microtubule spindle dynamics in the cell (Manfredi & Horwitz, 1984). The outcome of this is the inhibition of cell division and apoptosis induction. It is effective, particularly against breast, endometrial, bladder cancers, and cervical carcinoma (Hajek, Vorlicek, & Slavik, 1996). Paclitaxel treatment has historically given the first agent with constant activity in platinum-resistant patients (McGuire et al., 1989; Einzig et al., 1992; Trimble et al., 1993; Thigpen et al., 1994). However, the treatment with cisplatin and paclitaxel agents against advanced (stage IV) epithelial ovarian cancer was unsuccessful, and the combination of these drugs was not better than treatments of cisplatin or 24 h paclitaxel individually (David et al., 2007).

2.5.2.2. Ruthenium

Ruthenium complexes are another class of metal compounds. Since they also favor the same position of DNA (N7 of guanine) to bind, their mechanism of action might be similar to cisplatin (Clarke, 1989). Although the similarities between ruthenium and platinum, it is well known that ruthenium has many differences (Heffeter et al., 2008). First, ruthenium seems to favor neoplastic tissues to aggregate rather than healthy tissues, probably through transferrin usage to access to cancer cells (Sava et al., 1984). The study reported that Ru-transferrin complexes carried into the tumor masses. When the receptor (transferrin) attached, the complex detaches Ru, which is later taken into the cancer cell (Sava & Bergamo, 2000). Second, ruthenium maintains its comparatively passive Ru(III) oxidation condition until it arrives at cancer tissue, which has a lower pH and less amount of oxygen than healthy tissues. When it arrives at cancer tissue, reduction to a more active state Ru(II) takes place (Schluga et al., 2006). This reaction, named "activation by reduction", provides a selective tumor targeting, which can be beneficial for hypoxic tumors that are possibly chemotherapy-resistant (Rockwell et al., 2009). Lastly, some of the Ru agents show better efficiency against cancer metastases (Gagliardi et al., 1994). Given these features, ruthenium is anticipated to demonstrate anti-cancer activity and cytotoxicity patterns that are different from those of platinum. These remarkable features may help to overcome platinum resistance together with an extended activity range.

Numerous structure-activity studies showed that three elements are critical to the cytotoxicity of Ruthenium compounds; chloride, N-N donor ligand, and arene ligand (Habtemariam et al., 2006; Ang & Dyson, 2006; Betanzos- Lara et al., 2012; Barry & Sadler, 2013). The Ru complexes that we used in this study containing these elements (Figure 10).

There are only two ruthenium agents, NAMI-A (New Anti-tumor Metastasis Inhibitor A) and KP1019, which introduced to the human clinical trials (Sava, Alessio, Bergamo, & Mestroni, 1999; Pieper, Borsky, & Keppler, 1999). NAMI-A is the first Ru agent that was used in human clinical trials. This Ru (III) complex is carrying an imidazole and DMSO (Dimethyl sulfoxide) coordinated to the ruthenium (Mestroni, Alessio, & Sava, 1998). Another Ru (III) complex, KP1019, contains ruthenium that is coordinated by two indazole heterocycles with nitrogen atoms (Figure 6).

Regardless of their similarities in the structural and chemical base, each agent demonstrates a different anti-tumor phenotype. In preclinical trials, NAMI-A has shown anti-metastatic activities in numerous animal models, but it seems it has no direct cytotoxic effects (Sava, Pacor, Mestroni, & Alessio, 1992; Sava et al., 1995). On the other hand, KP1019 has shown direct cytotoxic effects to an extensive range of primary tumors by promoting apoptosis (Galanski, Arion, Jakupec, & Keppler, 2003; Jakupec et al., 2005; Hartinger et al., 2008).

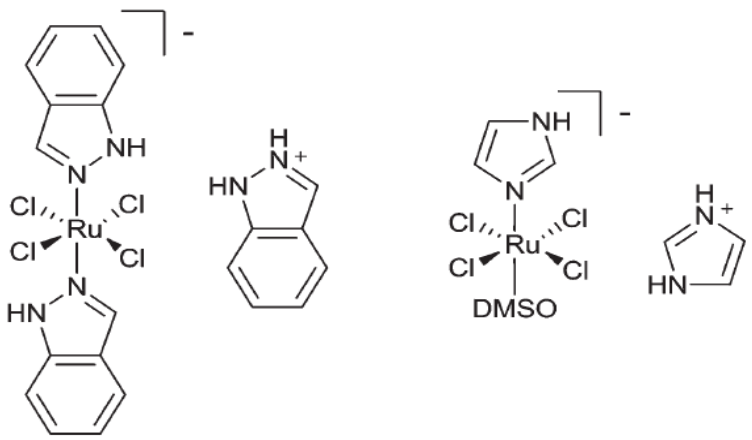

Figure 6 Structures of KP1019 and NAMI-A, respectively (Adapted from Alessio, Mestroni, Bergamo, & Sava, 2004).

A phase I study was started to use NAMI-A in 1999, and it was announced in 2004 (Rademaker-Lakhai et al., 2004). In the study, 24 patients bearing various metastatic tumors (such as colorectal, melanoma, and ovarian) were treated via dose increase protocol. 20 of 24 patients (83%) were successfully treated in this study. However, at high doses, they developed uncomfortable blisters on some body parts that persisted for weeks. Moreover, NAMI-A had other side effects such as anemia, lymphopenia, anorexia, stomatitis, and nausea. Interestingly KP1019 did not show any dose-associated side effects; however, its trial covered only eight patients, and the compound had complications with the solubility.

To confirm if the Ru agents were carried into the cells or not, the cellular uptake of complex 2, 1b, and 1a in OVCAR-3 cell line was examined in a study (Tavsan, Yaman, Subasi, & Ayar Kayalı, 2018). As can be seen in Figure 7, the overlay image demonstrated that the complex 1b efficiently absorbed by OVCAR-3 cells and able to enter into the cytoplasm. The aggregation was detected in cell nuclei. Complex 1a and 2 also showed the same manner (data not shown) as complex 1b. After 8 h, the cells were started to throw out the Ru compounds.

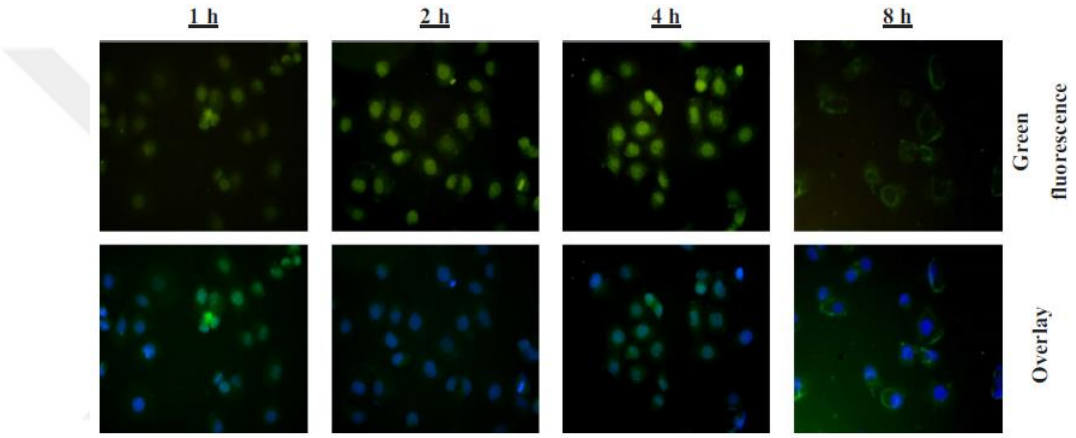

Figure 7 Images of 1b treated OVCAR-3 cells for variable incubation time. DAPI (blue) stain represents nuclei, the green fluorescence stain (green) represents complex 1b, and the overlay demonstrates the cellular relation of these compounds with DNA. (Adapted from Tavsan, Yaman, Subasi, & Ayar Kayalı, 2018).

In conclusion, Ru complexes are potential chemotherapeutics due to their extraordinary qualities allowing a selective entry into the cancer cells along with lower toxicity to healthy tissue. However, many features of these ruthenium agents still waiting to be elucidated for further use.

2.6. Metabolism in Cancer

Reprogrammed metabolism is recognized as a hallmark of cancer due to changed metabolic characteristics that have been seen in numerous cancers (Hanahan & Weinberg, 2011; Pavlova & Thompson, 2016). During the 1920s, Otto Warburg and his colleagues provided evidence for such adaptations when they determined increased glucose uptake by cancer cells (Warburg, Wind, & Negelein, 1927). Warburg et al. (1924) then reported that regardless of the presence of molecular oxygen, glycolysis to produce lactic acid was preferred by cancer cells as if they were simulating the conditions of anaerobic. (Warburg, Posener, & Negelein, 1924). It was called "aerobic

glycolysis" to precisely express this pathway in cancer cells (Warburg, 1956a; Warburg, 1956b). This event is also called “Warburg Effect”. Even though oxidative phosphorylation (OXPHOS) provides a high amount of adenosine 5’-triphosphate (ATP), glycolysis supplies the essential biomolecule precursors that are needed by cancer cells to preserve their high proliferative state (Menendez & Lupu, 2007; Christofk et al., 2008).

Aerobic glycolysis does not cause the suppression of OXPHOS in cancer cells completely. Also, reducing glycolytic ATP production via inhibiting the pyruvate kinase’s activity unsuccessful in stopping tumorigenesis, recommending that the primary function of glycolysis is not the ATP production (Israelsen et al., 2013).

The metabolic shift to aerobic glycolysis is an essential mechanism for cancer cells to synthesize proteins, nucleotides, and lipids. Glucose transporters (such as GLUT1) are overexpressed to compensate, and eventually, uptake of glucose is increased in tumor cells. Increased glucose uptake causes hyperglycemia, which leads to a more acidic and hypoxic environment. The relation between a low pH environment and gain of chemoresistance was examined in many tumor types (Kellenberger et al., 2010). Besides the regulation of glucose uptake and lactic acid production (Vander Heiden, Cantley, & Thompson, 2009; Wu & Zhao, 2013), there are other pathways in cellular metabolism that are reprogrammed in cancer cells such as increased glutamine metabolism and synthesis of fatty acid (Zhao, Butler, & Tan, 2013).

2.6.1. Glucose metabolism

The cells take glucose molecules inside through responsible transporters and then modify and phosphorylate it through a series of biochemical reactions (Berg, Combs, & Scherer, 2002). Glucose can proceed to lipid synthesis by transforming to glycerol or sequentially converted to pyruvate, which is called glycolysis. Glycolysis is a metabolic pathway that does not require molecular oxygen (O_2) and happens in the cytosol of both prokaryotic and eukaryotic organisms. By glycolysis, one glucose molecule breaks down into two pyruvate molecules with $NADH^+$ each, which yields two molecules of ATP. Another way to use glucose is nucleotide synthesis by “pentose phosphate pathway” or nicotinamide adenine dinucleotide phosphate (NADPH)

generation for reductive biosynthesis (Figure 8). Glucose is also necessary for glycosylation via "hexosamine biosynthetic pathway" (Wellen et al., 2010; Dang, 2012).

Oncogenic mutations result in the over-expression of GLUT (Macheda, Rogers, & Best, 2005; Pelicano, Martin, Xu, & Huang, 2006) hence assists the glucose consumption in tumor cells. Even though glycolysis generates a low amount of ATP than mitochondrial OXPHOS, there are certain advantages for tumor cells to prefer aerobic glycolysis (de Souza, Justo, de Araujo, & Cavagis, 2011). First, increased glycolysis rate and lactic acid production would lead to faster tumor growth. Pfeiffer et al. (2001) were proposed that fast but less ATP generation pathway offers particular benefits for circumstances of nutrient deprivation, adding an evolutionary magnitude to glycolysis (Pfeiffer, Schuster, & Bonhoeffer, 2001; Zhou et al., 2012). Second, cancer cells also require metabolic precursors that are necessary for the biosynthesis of molecules to support tumor growth (Deberardinis, Sayed, Ditsworth, & Thompson, 2008).

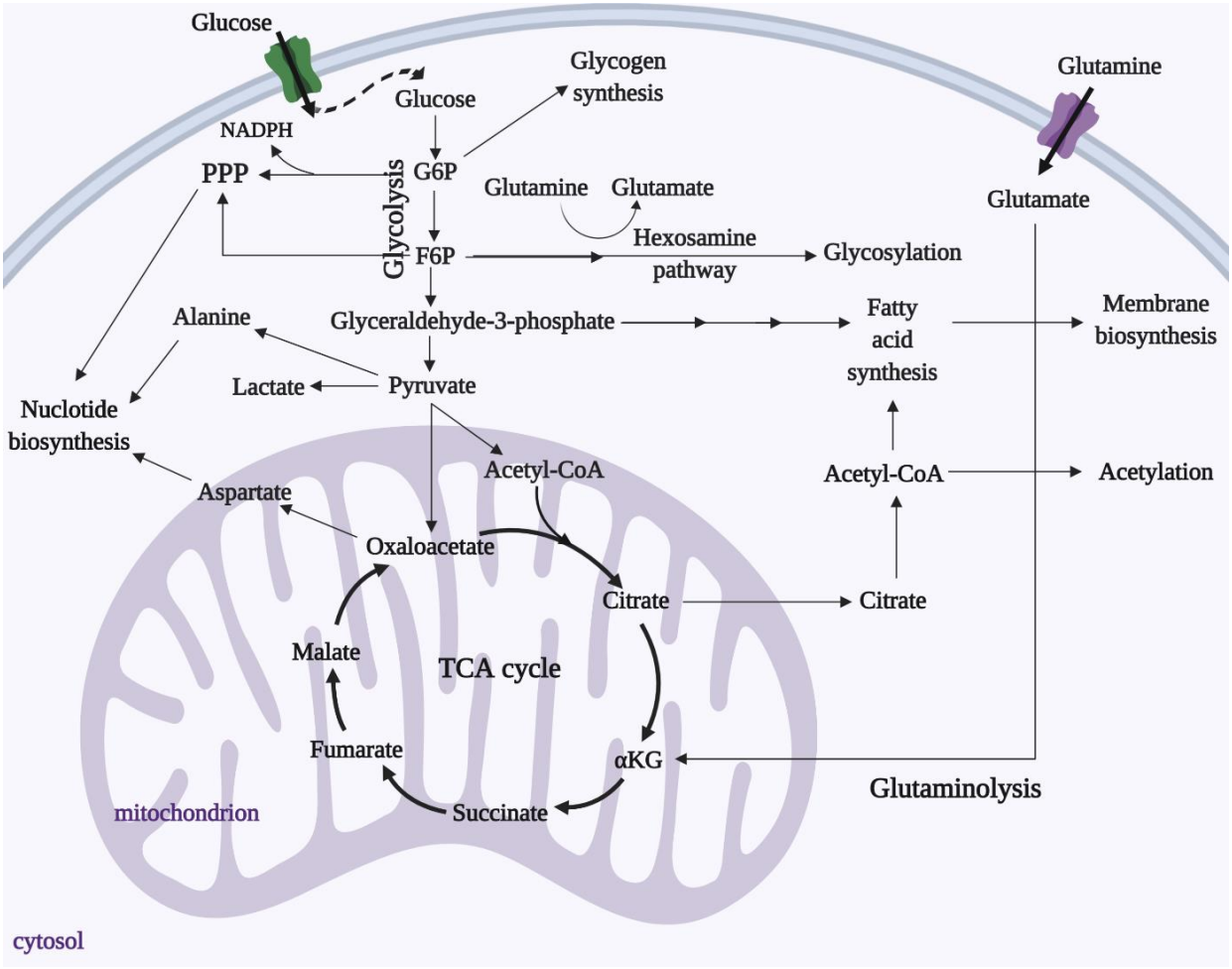

Figure 8 Energy metabolism in the cell (ATP and other cofactors have been removed for simplicity. G6P: glucose-6-phosphate, PPP: pentose phosphate pathway, F6P: fructose-6-phosphate, αKG: alpha-Ketoglutarate, TCA: tricarboxylic acid, NADPH: nicotinamide adenine dinucleotide phosphate. Figure created via biorender, www.biorender.com, and adapted from DeBerardinis & Chandel, 2016).

2.6.2. Tricarboxylic Acid Cycle

The TCA cycle is acknowledged as well as the Krebs or the citric acid cycle. Hans Krebs is the name behind the TCA cycle for his studies and also evident for the importance of glutamine metabolism in animals in 1935. The TCA cycle is an essential pathway in aerobic organisms, which is placed in the mitochondria. After glycolysis, the pyruvate molecule is transformed into acetyl-CoA. With the condensation of acetyl-CoA and OAA, the TCA cycle starts. Intermediates of the TCA cycle mainly consist of OAA, citrate, α-KG, succinyl-CoA, succinate, malate, and fumarate. These metabolites are used in numerous pathways, such as fatty acid synthesis, protein, and

nucleotide biosynthesis (Ahn & Metallo, 2015). TCA cycle produces electrons, carbon skeletons, and CO_2 that can be used to replenish the cycle, which is called "anaplerosis". Citrate can be transformed into acetyl-CoA in the cytosol for the membrane biosynthesis. Oxaloacetate can be transformed to aspartate to use for nucleotide synthesis (Son et al., 2013). Moreover, αKG can be transformed into citrate by reversing the TCA cycle by "reductive carboxylation", that later use in the creation of acetyl-CoA and then fatty acid synthesis to support membrane biosynthesis (Ward et al., 2010). These pathways mainly illustrated in Figure 8.

Mitochondrial dysfunction is commonly observed in cancer cells and crucial for tumor progression (Hu et al., 2012; Lu et al., 2012). Gene mutations in mitochondrial DNA (mtDNA) alter the bioenergetics of cancer cells and help to adapt to environmental changes (Brandon, Baldi, & Wallace, 2006). Succinate dehydrogenase (SDH) and fumarate hydratase (FH) mutations are frequently seen in various types of cancers (Bayley & Devilee, 2010). Therefore it is clear that mitochondrial dysfunction may cause a shift in the cellular metabolism of cancer cells.

2.6.3. Electron Transport Chain

The electron transport chain (ETC) consists of four respiratory complexes that are responsible for the transportation of electrons by redox reactions and also pumps the protons (H^+ ions) into the inner mitochondrial membrane. Molecular oxygen (O_2) has a vital role as the last acceptor of electrons in aerobic respiration. Electron flow creates an electrochemical proton gradient that utilizes a driving force for ATP synthase to phosphorylate ADP (Mitchell, 1961; Nicholls & Budd, 2000; Wallace, 2013). Several metabolic pathways (such as glycolysis and TCA cycle) supply electron donors to ETC.

ETC is identified as an essential pathway that controls signaling, biosynthetic pathways, and bioenergetics during proliferation and metastasis of cancer cells. A study suggests that the crucial role of the ETC in cell proliferation is to support the conversion of aspartate (Birsoy et al., 2015). Aspartate can be used for nucleotide synthesis to support cell proliferation (Son et al., 2013).

Even though the severe inhibition of ETC showed anti-growth properties, the temperate inhibition of ETC that contains heteroplasmic mitochondrial DNA mutations can benefit to

tumorigenesis, possibly by raising the reactive oxygen species levels (Kulmacz, 1989; Petros et al., 2005; Ishikawa et al., 2008).

2.6.4. Anaerobic Respiration

Anaerobic respiration uses electron acceptors that are different from O_2. Even though oxygen has not been using, the reaction process in a respiratory electron transport chain (Slonczewski & Foster, 2011). Other electron acceptors in anaerobes possess lower reduction potentials, thus result in less energy release for each oxidized molecule.

Although highly proliferative cancer cells display high glucose uptake, most of the glucose is transformed into lactate rather than consumed in OXPHOS (Mayers & Vander Heiden, 2015). Raised lactate production leads to less pyruvate availability for the TCA cycle. However, with this conversion, the cells can reproduce NAD, which is needed in the glycolysis pathway.

2.6.5. Amino Acid Metabolism

The non-essential amino acid glutamine as an important anaplerotic substrate can be used in the conversion of glutamate to αKG. Thus it can replenish the TCA cycle via glutaminolysis (Hensley, Wasti, & DeBerardinis, 2013). The cancer cells that are supplied with αKG, oxaloacetate, or pyruvate is sufficient for them to survive in glutamine deprivation conditions, confirming that glutamine assists tumor growth by replenishing TCA cycle intermediates via anaplerosis (Yuneva et al., 2007; Weinberg et al., 2010; Altman, Stine, & Dang., 2016). As reverse reaction can happen, the transformation of alanine to pyruvate and aspartate to oxaloacetate can happen to support the TCA cycle or glycolysis when it is necessary.

Glutamine and glutamate have a fundamental part in non-essential amino acid (NEAA) metabolism, and each can synthesize the other NEAAs (Choi & Coloff, 2019). Many cancer cell types showed a glutamine dependent behavior to sustain their energy needs (Wise & Thompson, 2010). The conversion of glutamine to glutamate happens via the glutaminase enzyme. Glutamate supplies the TCA cycle and assists in the biosynthesis of fatty acids (Wise et al., 2008; Mullen et al., 2012). The studies showed that the tumor-initiating cells were able to endure the glucose deficiency on glutamine-supplemented media due to glutamine usage in the TCA cycle via

glutaminolysis (Roberts et al., 2005; Anderson et al., 2014). It has been proved that hypoxia does not weaken the glutamine metabolism in proliferating cells. Glutamine can supply to fatty acid biosynthesis by reversing the TCA cycle or reductive carboxylation of α-ketoglutarate to citrate via isocitrate dehydrogenase (IDH) (Wise et al., 2011; Metallo et al., 2012; Mullen et al., 2012).

Hudson et al. (2016) observed that the overexpression of glutaminase and more sensitivity to glutamine deprivation in platinum-resistant ovarian cancer cells compared to platinum-sensitive ovarian cancer cells. It was suggested that increased glutamine consumption is an important step to acquire platinum resistance (Hudson et al., 2016).

2.6.6. Metabolism in Ovarian Cancer

The studies proved that the Warburg effect is highly essential for the development of ovarian cancer cells (Deberardinis, Sayed, Ditsworth, & Thompson, 2008; Caneba et al., 2014). Another study proposed that the primary purpose of the Warburg effect is to keep glycolytic intermediates at a high amount to supply for the biosynthesis of macromolecules, which is preferred by fast-cycling cancer cells (Vander Heiden, Cantley, & Thompson, 2009). On the contrary, slow-cycling cells mostly favor OXPHOS as a preferred energy source (Roesch et al., 2013).

In ovarian cancer cells, glucose is broadly used to produce ATP and sustain energy and redox balance (Vander Heiden et al., 2010; Ahn & Metallo, 2015). The study of Ippolito et al. (2016) has found that chemoresistant cells are excellent miners of glucose (Ippolito et al., 2016). Additionally, another study proved that cisplatin-resistant ovarian cancer cells showed higher glucose demand, and they were more delicate to glucose deficiency (Catanzaro et al., 2015). However, Xu et al. (2018) proved that cisplatin-resistant ovarian cancer cells had a greater demand for glucose and demonstrated higher glucose uptake and consumption with decreased lactate production (Xu et al., 2018). Moreover, they were less delicate to glucose deficiency due to their greater stocks of glycogen. These cells displayed exceptional reduces in extracellular lactate and ECAR (extracellular acidification rate - an indicative of glycolysis). Dar et al. (2017) also found that chemoresistant ovarian cancer cells could endure the conditions of limiting glucose and they demonstrated higher OXPHOS activity (Dar et al., 2017). These findings are recommending that the energy metabolism of ovarian cancer cells may differ from one cell line to another.

Accumulation of cellular glycogen was recognized as an aspect of clear cell ovarian carcinoma, which usually generates chemoresistance (Iida et al., 2012). Additionally, glycogen accumulation was increased under hypoxic circumstances (Uekuri et al., 2013). These findings indicate that particular metabolic phenotypes advance the chemoresistance in ovarian cancer.

Fatty acid metabolism was also changed in tumors. In ovarian cancer, unsaturated lipids were elevated (Zhang, Zhang, & Cheng, 2015), which stimulates stemness, while lipid desaturation impairs tumor initiation and cancer progression (Li *et al*., 2017).

3. MATERIALS AND METHODS

3.1. Type of Research

The type of research performed in this study is experimental.

3.2. Time and Place of Research

The experiments performed at Ayar Kayalı Lab at Izmir Biomedicine and Genome Institute between October 2018 and May 2019.

3.3. Study Plan and Calendar

The experimental outline of this study summarized in Figure 9.

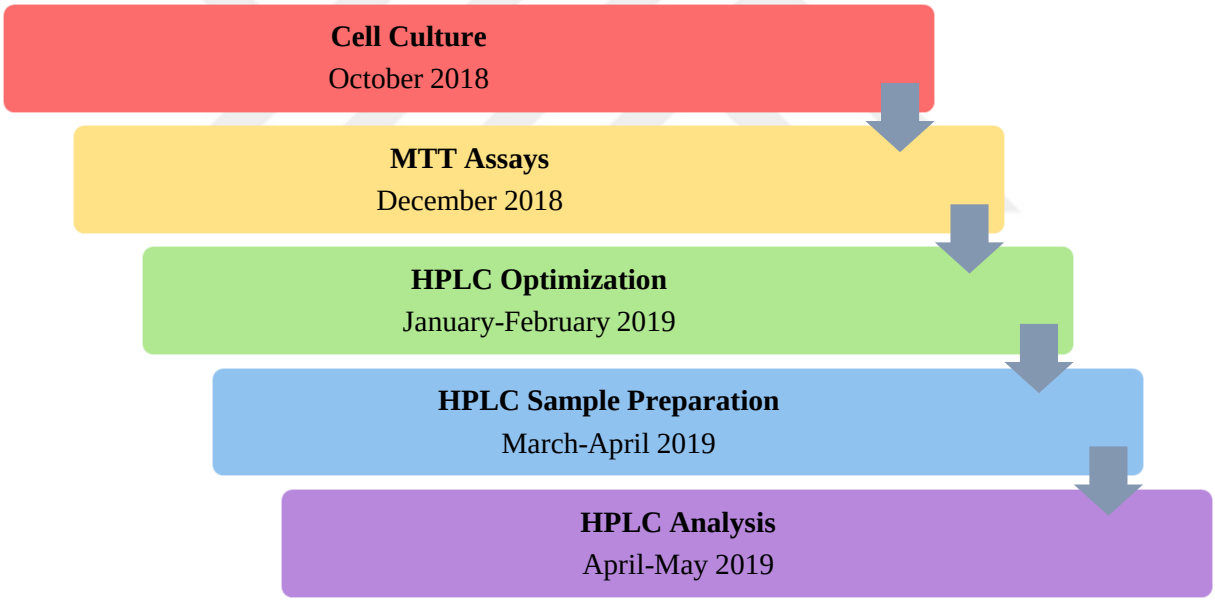

Figure 9 Outline of the study.

3.4. Materials

DNPH was obtained from Sigma-Aldrich (Sternheim, Germany). All standards were High-Performance Liquid Chromatography (HPLC) grade. The synthesis of TSC^1, TSC^2, and ruthenium complexes, $[(\eta^6\text{-}p\text{-cymene})Ru(\eta^1\text{-}S\text{-TSC})Cl_2]$ (1b), $[(\eta^6\text{-}p\text{-cym})Ru(\eta^1\text{-}S\text{-}TSC^2)Cl_2]$ (2) (Yaman, Şen, Karagöz, & Subaşı, 2017), and trans- $[RuCl_2(PPh_3)_2(\eta^2\text{-}\ N,S\text{-}\ TSC)]$ (1a), were described before (Tavsan, Yaman, Subasi, & Ayar Kayalı, 2018). All ruthenium agents (Figure 10) were

kindly supplied by E. Subasi, Department of Chemistry, Faculty of Science, Dokuz Eylül University. Carboplatin (Carbodex®, 150 mg/15 ml) and paclitaxel (Ataxil®, 100 mg/16.7 ml) (Kocak Farma, Istanbul, Turkey) were obtained from a local pharmacy. The chemicals used in this study, as in the following Table 2.

Table 2 The chemicals used in this study.

Chemicals	**Vendors**
DNPH	Sigma-Aldrich
Oxaloacetic acid	Sigma-Aldrich
Lactic acid	Sigma-Aldrich
Succinic acid	Sigma-Aldrich
α-Ketoglutaric acid	Sigma-Aldrich
Fumaric acid	Sigma-Aldrich
Pyruvic acid	Sigma-Aldrich
Sodium Hydroxide (NaOH)	Sigma-Aldrich
Hydrochloric acid (HCl, 37%)	Sigma-Aldrich
H_2SO_4	Sigma-Aldrich
Isopropanol (IPA)	Sigma-Aldrich
Malic acid	Merck
D-(+)-Glucose monohydrate	Merck
Ethanol	Isolab
Carbodex® and Ataxil®	Koçak Farma

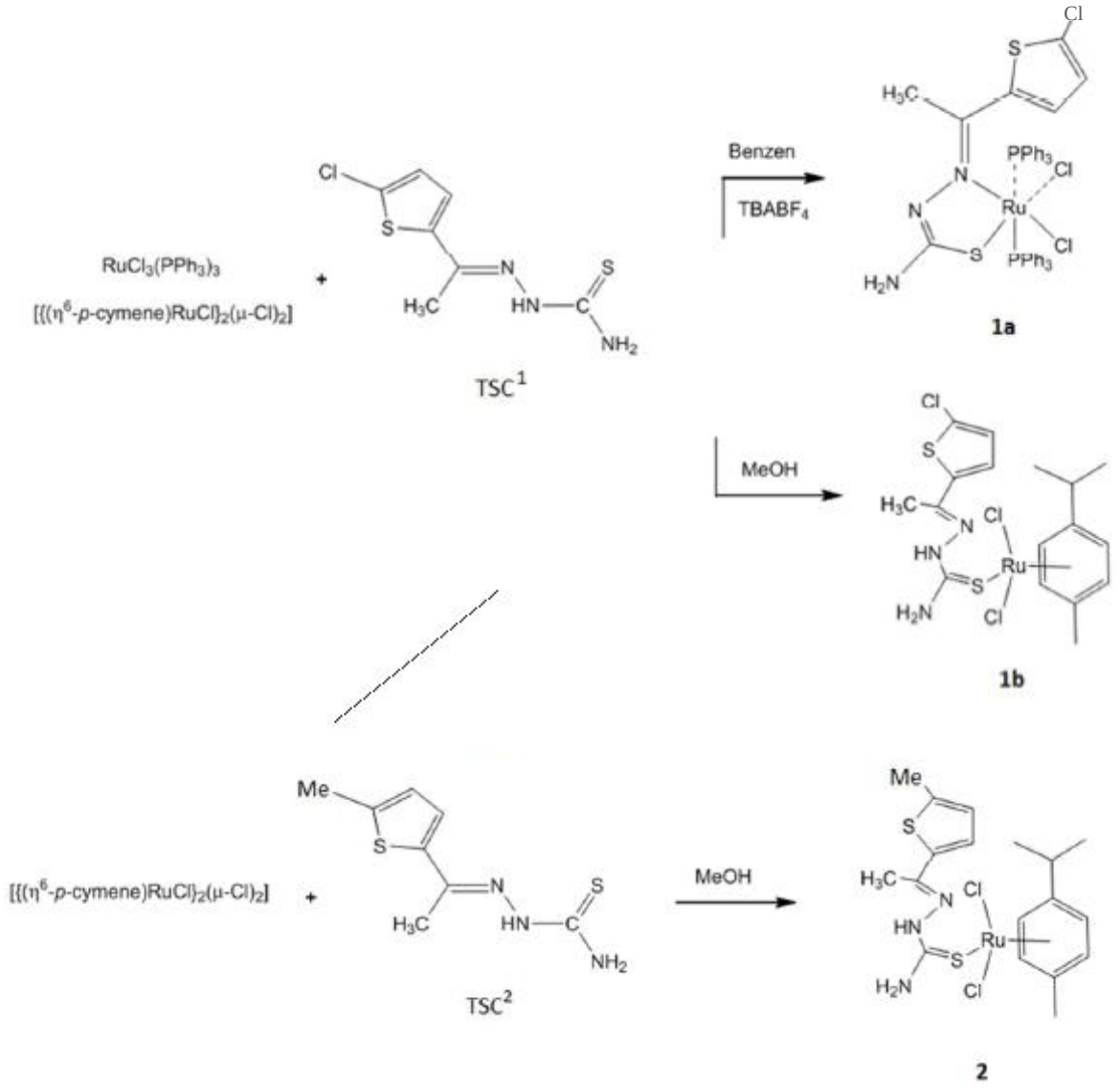

Figure 10 Ru (III) complexes. Complex 1a, 1b, and their ligand are TSC^1, Complex 2, and its ligand is TSC^2 (Adapted from Tavsan, Yaman, Subasi, & Ayar Kayalı, 2018).

3.4.1. Machines

The machines used in this study as in the following Table 3.

Table 3 The machines used in this study.

Machines	**Vendors**
Centrifuge 5810R	Eppendorf

Multiskan™ GO Microplate Spectrophotometer	Thermo Scientific
Centrifuge MicroCL 17R	Thermo Scientific
Balance - basic	Sartorius
HPLC System	Shimadzu
StabiliTherm™ Ovens and Incubators	Thermo Scientific
Axio Vert.A1 Inverted Microscope	ZEISS
Vortex Mixer	Thermo Scientific
Water Bath and Lid	Nüve
Dry Bath Incubator	Allsheng
pH Meter	Hanna

3.4.2. Cell Culture Materials

The cell lines were OSE (human normal ovarian surface epithelium), OVCAR-3, A2780, and A2780cis. Immortalized OSE cell line by SV-40 transfections was purchased from Abm-Good. A2780, A2780cis, and OVCAR-3 cell lines were purchased from the ECACC. Cell culture plates, flasks, cryovials, centrifuge tubes, filter tips, and serological pipettes were purchased from Isolab (Germany). Used materials in cell culture, as in the following Table 4.

Table 4 Used Materials in Cell Culture.

Materials	**Vendors**
DMEM	Sigma-Aldrich
DMSO	Sigma-Aldrich
PBS	Gibco
Trypsin-EDTA	Gibco
FBS	Gibco
Penicillin (100 units/ml)-Streptomycin (100 mg/ml)	Gibco

RPMI-1640	Sigma-Aldrich
MTT	Sigma-Aldrich

3.5. Methods

3.5.1. Cell Culture Conditions

A2780 (ovarian cancer cell line sensitive to cisplatin), A2780cis (ovarian cancer cell line resistant to cisplatin), and OVCAR-3 (ovarian cancer cell line resistant to cisplatin) were grown in RPMI-1640 medium. OSE-SV40 cell line was grown in DMEM growth medium. All growth medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cells were kept in the incubator provides an atmosphere of 5% CO_2 at 37 °C.

3.5.2. Cell Viability Assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983) was used to assess the mitochondrial dehydrogenase activity in the cells. The cells (100 µl/well) were seeded at a density of 7500 cells per well into 96-well plates and allowed them to grow for 24 h. In all assays, the concentration range of complex 1a, 1b, 2, and carboplatin were between 0-100 µM, and the concentration range of paclitaxel was between 0-100 nM. All Ru agents were dissolved in DMSO then diluted in PBS solutions serially. Later, they were diluted in the culture medium. All cells were treated for 24 h and 48 h. During the inclusion of the ruthenium complexes onto the cells, compounds were protected from light. Finally, 100 µL of MTT (5 mg/mL) was added on the cells and then incubated for 3 to 4 h in the incubator. The formazan crystals were dissolved via DMSO, and the absorbance was measured at 540 nm in Multiskan™ GO Microplate Spectrophotometer. The IC_{50} values (concentration of agent causing 50% inhibition of growth) were calculated by curve fitting. IC_{50} values of compounds were used later to treat the cells (for 24 h and 48 h) before collecting samples for HPLC analysis.

3.5.3. Preparation of Samples for HPLC Analysis

A modified protocol (Ganzera et al., 2006) was used for sample preparation. Each cell line was grown to 70% confluency before they were collected. The metabolites were extracted by adding boiling ultra-pure water on frozen cell pellets. The mixture was put inside in a boiling water

bath for 20 min and shook every 5 min. After cooling the sample, it was centrifuged for 20 min at 13,000 rpm. The supernatant was centrifuged once more before HPLC analysis. Extracellular metabolites (such as extracellular glucose) were extracted by collecting the medium of the cells after treatment of 24 h and 48 h. Then, the medium was centrifuged the same as the previous method for intracellular metabolites.

3.5.4. HPLC Conditions for Metabolites

The analysis of organic acids was examined by Shimadzu prominence HPLC system (Kyoto, Japan) equipped with an Alltech IOA-1000 column and a UV detector and a refractive index detector (RID). The conditions were the following: mobile phase 9.0 mM H_2SO_4 solution; injection volume, 20 µL; flow rate, 0.4 mL/min; detection wavelength, 210 nm, and column temperature, 42 °C. Standards were prepared for OAA, α-KG, succinate, fumarate for UV detector, and for glucose, lactate, malate for RID, and the calibration curves were generated (Ayar Kayalı, 2005).

3.5.5. Colorimetric Determination of Pyruvic Acid

The concentration of pyruvate was assessed via 2, 4-dinitrophenylhydrazine (Aras & Ersen, 1975). The protocol was modified and recalculated to apply for smaller, 100 µL, amount (96 well plate). 50 mg 2,4-dinitrophenylhydrazine (DNPH) was dissolved in 50 mL 2N Hydrochloric Acid (HCl, 37%). 30 µL sample added into wells, and then 10 µL DNPH was added to each solution and gently shook for 5 min at room temperature. 60 µL 2N Sodium Hydroxide (NaOH) was added on the mixture and again shook for 10 min at room temperature. Absorbance was measured at 520 nm. Blank and standard solutions of pyruvic acid were prepared in the same way. For the calibration curve, 1.5 mg pyruvic acid was dissolved in 3 mL distilled water (0.5 mg/mL=$50x10^{-2}$ mg/mL). Then it was diluted to concentrations of $0.25x10^{-2}$ mg/mL, $0.5x10^{-2}$ mg/mL, $0.10x10^{-2}$ mg/mL, $2.5x10^{-2}$ mg/mL, $5x10^{-2}$ mg/mL, $10x10^{-2}$ mg/mL solutions. The calibration curve was created for pyruvate by plotting the concentration against the corresponding absorbance, which was used for the detection of the pyruvate amount in samples.

3.5.6. Statistical analysis

Each experiment was conducted at least three times with close outcomes and performed separately in triplicates. The results are average ± S.D of at least three independent experiments.

4. RESULTS

4.1. Cytotoxic Activities of Chemotherapeutic Agents on Ovarian Cell Lines

The aim of the MTT assay was to assess the cytotoxic activity of each agent against human ovarian cell lines (OSE, A2780cis, A2780, and OVCAR-3). Cytotoxic activities of ligands, carboplatin, and paclitaxel were also tested under the same conditions to serve as a comparison. MTT assay was carried out by testing the agents in the concentration range of 0-100 μM, but the concentration range of paclitaxel was 0-100 nM due to its high cytotoxicity. All MTT assay studies conducted for 24 h and 48 h. The inhibitory potency (IC_{50}) (the concentration of agent reduced the cell viability by half) used to express the cytotoxicity.

Figure 11 displays the results of complex 1a treatment in ovarian cell lines for 24 h and 48 h. Inhibition in OSE cells reached the maximum value (90%) at 25 μM at 48 h, and 24 h line reached 88% inhibition at 25 μM (Figure 11A). A2780 cells showed a more delayed response to complex 1a compared to OSE and 89% inhibition observed at 20 μM at 48 h even though 24 h values failed to show the same; it was 70% at 20 μM (Figure 11B). A2780cis cells demonstrated more resistance than A2780 cells, but it was successfully reached to inhibition of 79% at 10 μM at 48 h, and for 24 h line, it was 55% inhibition (Figure 11C). OVCAR-3 cells showed a more resistant profile to treatment compare to other cell lines and reached 68% inhibition at 50 μM at 24 h. It showed 80% inhibition at 48 h at the same dose (Figure 11D). In general, results of 24 h applications were overrun by 48 h in all cell lines (OSE, A2780, A2780cis, OVCAR-3) because of increased cell death at 48 h and high toxicity of complex 1a (Figure 11).

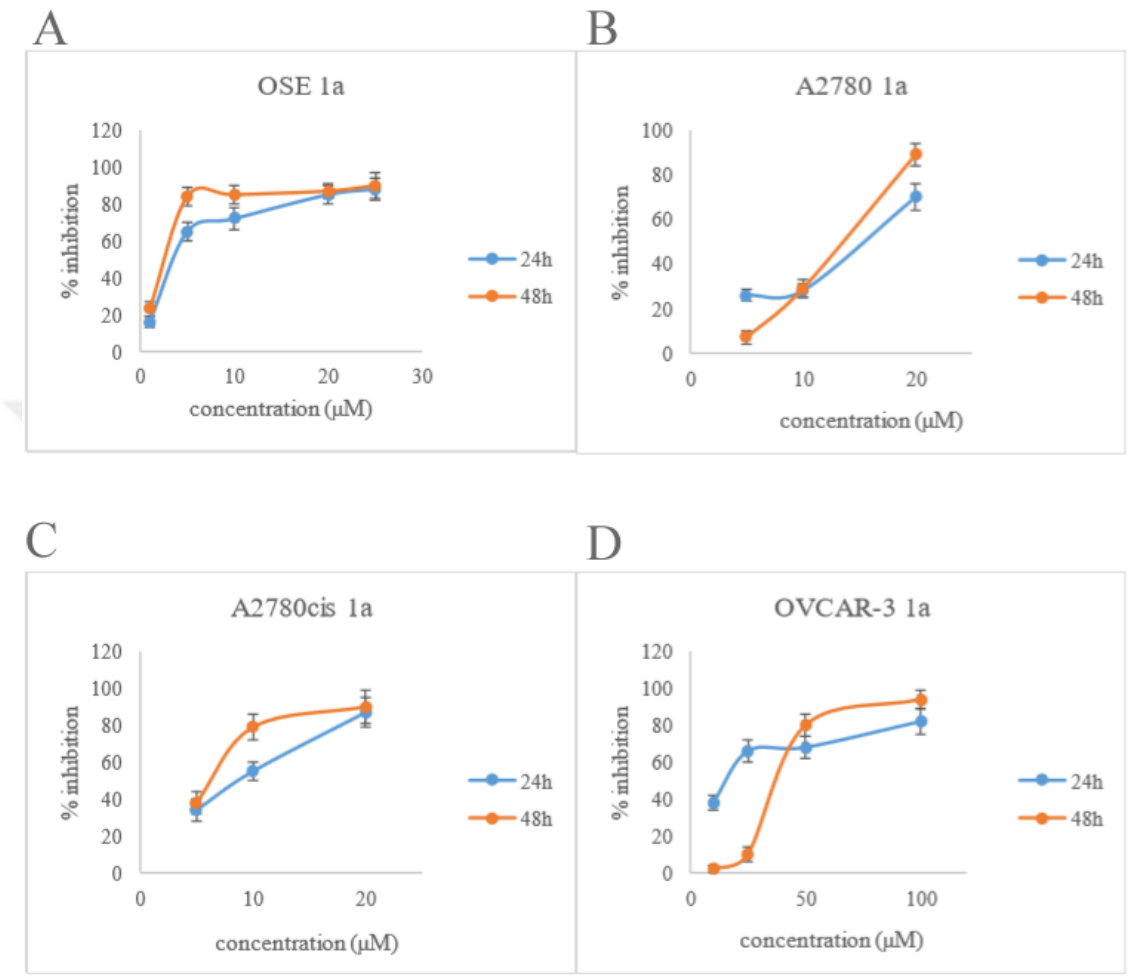

Figure 11 MTT results of complex 1a treatment in ovarian cell lines (A: OSE cell line with treatment of complex 1a for 24 h and 48 h; B: A2780 cell line with treatment of complex 1a for 24 h and 48 h; C: A2780cis cell line with treatment of complex 1a for 24 h and 48 h; D: OVCAR-3 cell line with treatment of complex 1a for 24 h and 48 h).

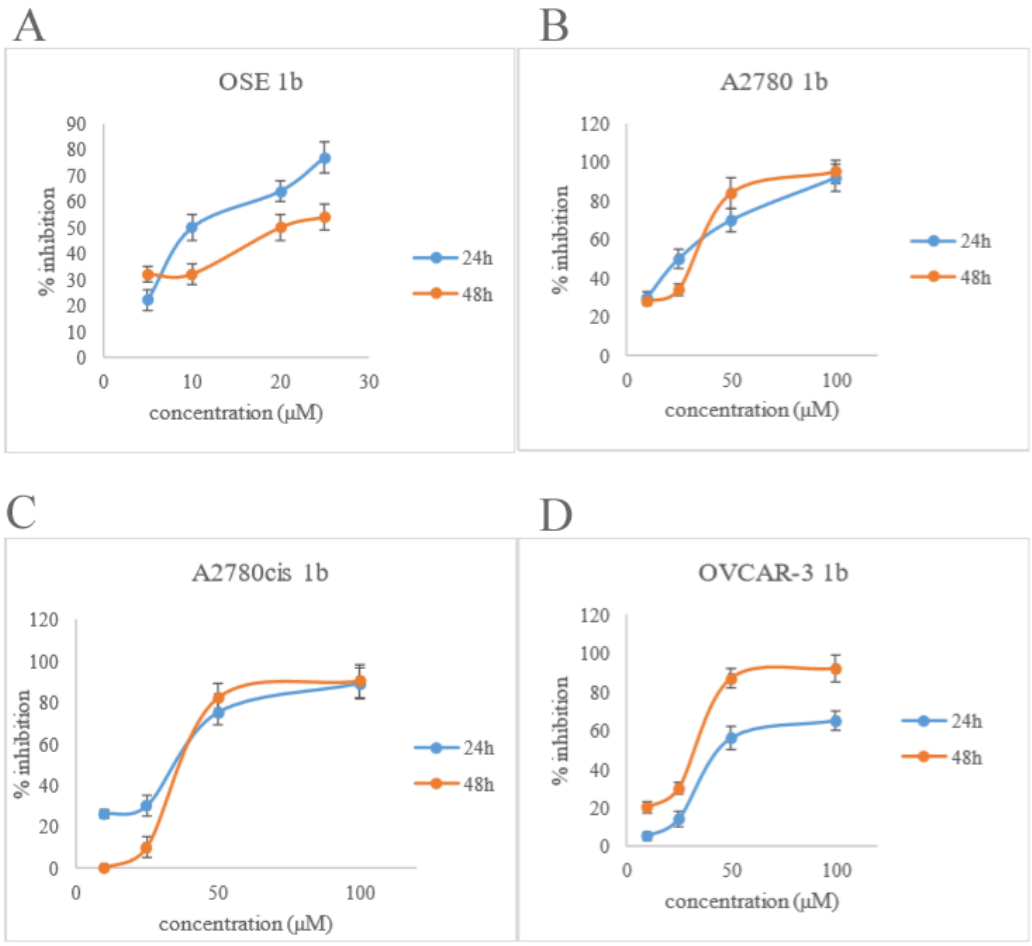

Figure 12 MTT results of complex 1b treatment in ovarian cell lines (A: OSE cell line with treatment of complex 1b for 24 h and 48 h; B: A2780 cell line with treatment of complex 1b for 24 h and 48 h; C: A2780cis cell line with treatment of 1b complex for 24 h and 48 h; D: OVCAR-3 cell line with treatment of complex 1b for 24 h and 48 h).

OSE cells that include complex 1b applied medium passed 50% inhibition after 10 µM at 24 h. Nevertheless, it was reached 50% inhibition at 20 µM at 48 h. Also, it was showed an increased cell number due to decreased drug toxicity at 48h (Figure 12A). Complex 1b applied A2780 cells displayed 70% inhibition at 50 µM at 24 h and 84% inhibition at 48 h (Figure 12B). A2780cis reached to the maximum inhibition value (89-90%) at 100 µM at 24 h and 48 h (Figure 12C). Complex 1b treatment in OVCAR-3 cells was highly effective, even at 48 h. It was reached to inhibition of 65% and 92% at 100 µM at 24 h and 48 h, respectively (Figure 12D).

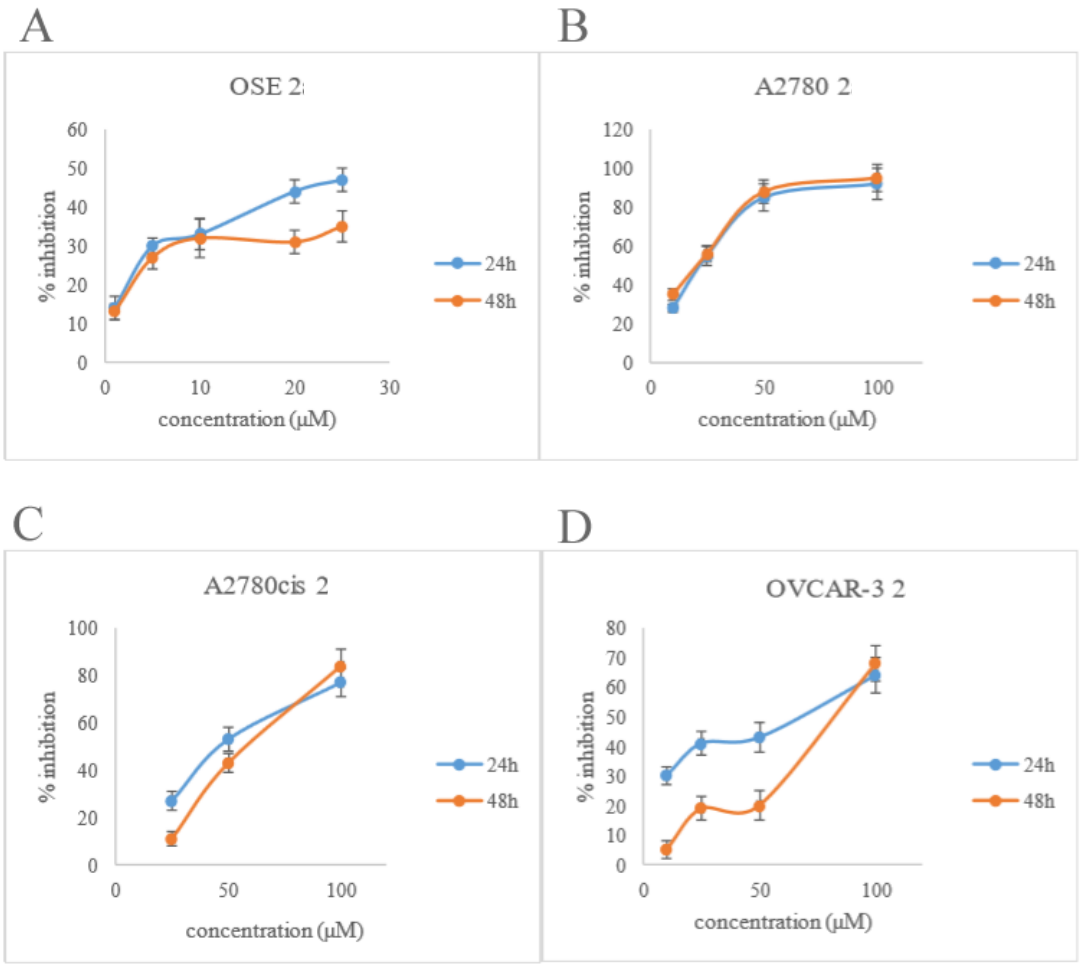

Figure 13 MTT results of complex 2 treatment in ovarian cell lines (A: OSE cell line with treatment of complex 2 for 24 h and 48 h; B: A2780 cell line with treatment of complex 2 for 24 h and 48 h; C: A2780cis cell line with treatment of complex 2 for 24 h and 48 h; D: OVCAR-3 cell line with treatment of complex 2 for 24 h and 48 h).

Complex 2 applied OSE cells failed to reach 50% inhibition between 1-25 µM doses, and toxicity was decreased at 48 h. The highest inhibition value (47%) was obtained at 25 µM at 24 h and 35% at 48 h for the same dose (Figure 13A). Nevertheless, A2780 cells reached a maximum inhibition value (92%) at 100 µM at 24 h and 94% at 48 h (Figure 13B). A2780cis cells passed 53% inhibition after 50 µM at 24 h and reached 84% inhibition at 100 µM at 48 h (Figure 13C). Complex 2 applied OVCAR-3 cells were able to reach 64% inhibition at 100 µM at 24 h and 68% at 48 h. However, complex 2 efficiency was generally lower at 48 h compared to 24 h in OVCAR-3 cell line (Figure 13D).

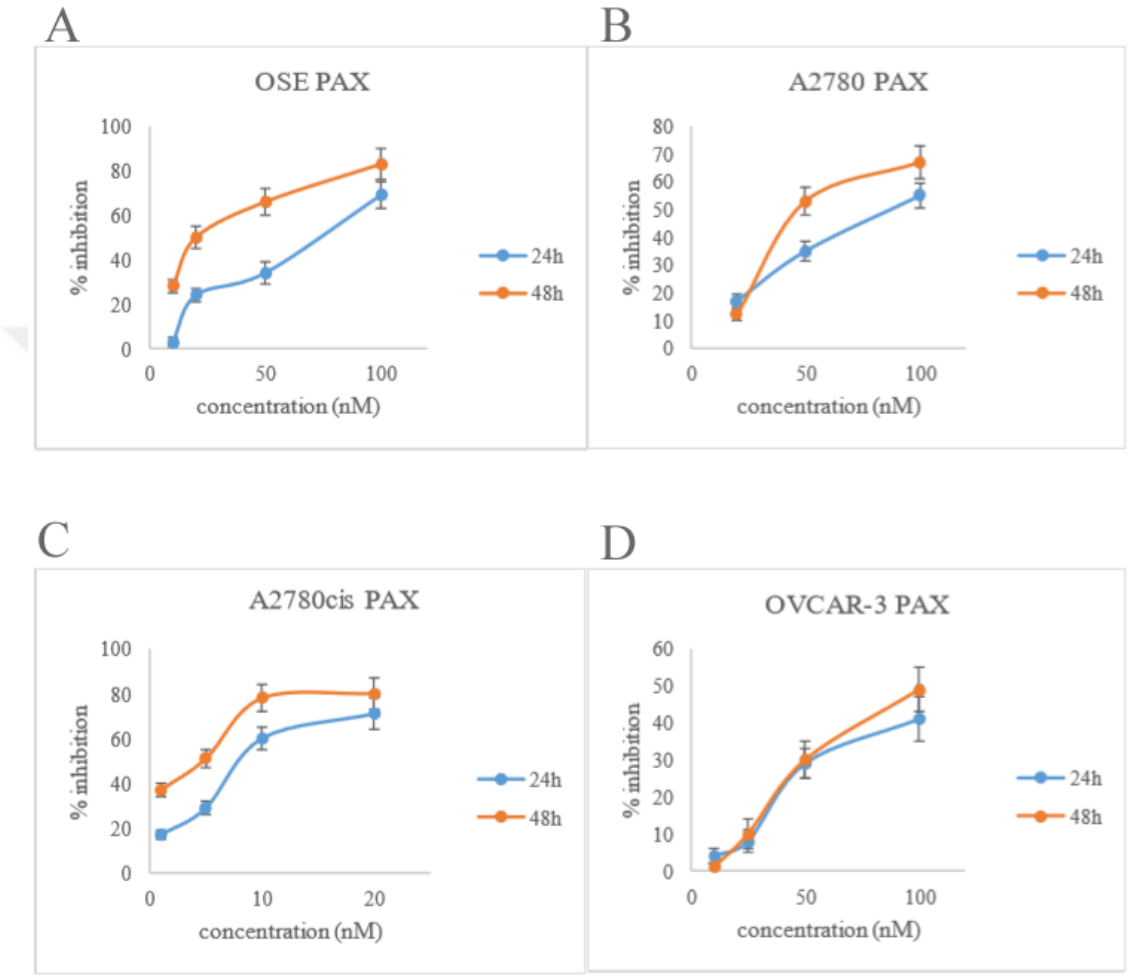

Figure 14 MTT results of PAX treatment in ovarian cell lines (A: OSE cell line with treatment of PAX for 24 h and 48 h; B: A2780 cell line with treatment of PAX for 24 h and 48 h; C: A2780cis cell line with treatment of PAX for 24 h and 48 h; D: OVCAR-3 cell line with treatment of PAX for 24 h and 48 h).

OSE cells showed a linear-like line after PAX treatment, and cytotoxicity was increased greatly at 48 h. It obtained 69% inhibition at 100 nM at 24 h and 83% inhibition at 48 h for the same dose (Figure 14A). A2780 cells with PAX treatment displayed 55% inhibition at 100 nM at 24 h and 53% inhibition at 50 nM at 48 h (Figure 14B). Inhibition value of PAX applied A2780cis cells was 71% inhibition at 20 nM at 24 h and 78% inhibition at 10 nM at 48 h (Figure 14C). On the other hand, OVCAR-3 showed 41% inhibition at 24 h and 49% at 48 h. (Figure 14D). In general, PAX displayed increased cytotoxicity to all cell lines at 48 h compared to 24 h.

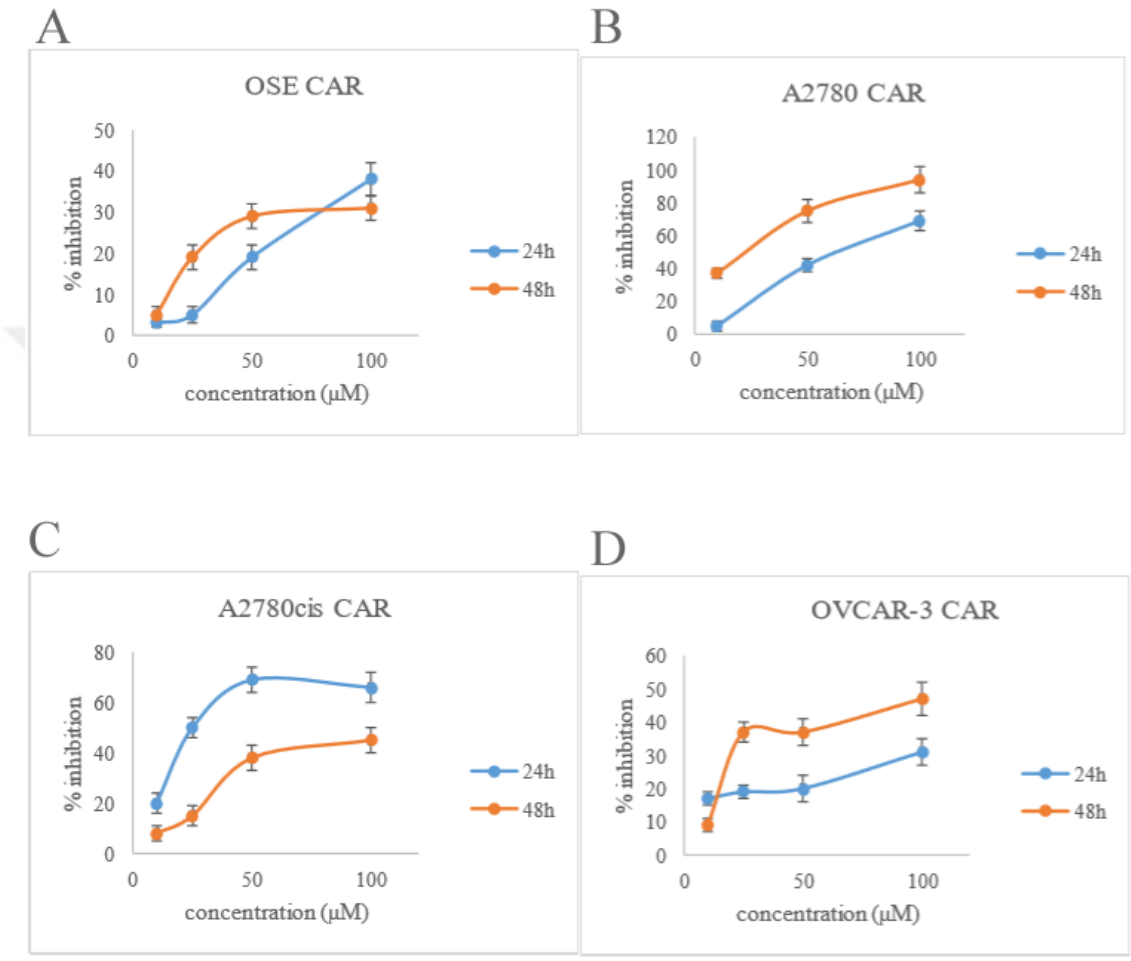

Figure 15 MTT results of CAR treatment in ovarian cell lines (A: OSE cell line with treatment of CAR for 24 h and 48 h; B: A2780 cell line with treatment of CAR for 24 h and 48 h; C: A2780cis cell line with treatment of CAR for 24 h and 48 h; D: OVCAR-3 cell line with treatment of CAR for 24 h and 48 h).

CAR applied OSE cells showed an increasing cytotoxicity profile and obtained 38% inhibition at 100 µM at 24 h while it was reached to maximum inhibition value (31%) at 48 h (Figure 15A). Although CAR treatment in A2780 cells showed only 69% inhibition at 100 µM at 24 h, it reached 94% inhibition at 48 h (Figure 15B). CAR application in A2780cis cells was more effective at 24 h than 48 h. The inhibition value was maximum (66%) at 100 µM at 24 h and 45% at 48 h (Figure 15C). As can be seen in Figure 15D, OVCAR-3 cells were more resistant to CAR, thus failed to reach to 50% inhibition value between 10-100 µM, although it was more effective at 48 h than 24 h. It was obtained %31 and %47 inhibition at 100 µM at 24 h and 48 h, respectively.

Table 5 demonstrates the IC50 values of compounds that we used in MTT assays for 24 h. The observed range of IC50 values for all compounds was between 11.6 ± 1.7 nM (PAX) and 234.5 ± 7.7 µM (CAR) towards tumor cell lines. Between all agents, PAX was the most toxic one and not only to cancer cells (A2780cis, OVCAR-3, and A2780) but also to a healthy cell line (OSE). Although PAX was highly effective towards A2780cis cell line (11.9 ± 1.7 nM), it was not equally effective against another cisplatin-resistant cell line, OVCAR-3 (116.2 ± 2.4 nM). PAX obtained high values against A2780 and OSE cell lines as well (82 ± 10.5 nM and 70.3 ± 0.6 nM, respectively).

Table 5 IC_{50} values of MTT Assay (µM: micromolar, nM: nanomolar, "-": no data)

MTT IC_{50} results		**Cell lines**			
		A2780cis	**OVCAR-3**	**A2780**	**OSE**
Ligands	**TSC^1**	80.5 ± 1.9 µM	210 ± 5.4 µM	-	-
	TSC^2	87.3 ± 1.1 µM	213 ± 6.1 µM	-	-
Drugs	**Complex 1a**	7.6 ± 2.0 µM	10.3 ± 0.7 µM	13.5 ± 1.3 µM	7.4 ± 2.2 µM
	Complex 1b	39.8 ± 1.1 µM	62.4 ± 9.4 µM	29.1 ± 1.5 µM	21.9 ± 8.6 µM
	Complex 2	46.2 ± 9.5 µM	60.9 ± 2.9 µM	24.2 ± 1.0 µM	33.6 ± 3.3 µM
	PAX	11.9 ± 1.7 nM	116.2 ± 2.4 nM	82 ± 10.5 nM	70.3 ± 0.6 nM
	CAR	41.7 ± 9.1 µM	234.5 ± 7.7 µM	70.7 ± 1.8 µM	41.6 ± 2.9 µM

Ligands of Ru agents were tested as a control to check whether the cause of cytotoxicity is from ligands or not. TSC^1 value for A2780cis was 80.5 ± 1.9 µM, and TSC^2 value for A2780cis was 87.3 ± 1.1 µM. TSC^1 value for OVCAR-3 was 210 ± 5.4 µM, and TSC^2 value for OVCAR-3 was 213 ± 6.1 µM. We showed that the cytotoxic effect was not from ligands of Ru agents. All Ru agents presented a higher anti-proliferative effect than ligands and generally lower cytotoxicity against healthy cell line (OSE) compared to carboplatin. Every compound exhibited distinctness in anti-proliferative activities.

Complex 1a was at least 3 to 5 times more effective than other Ru agents. Its IC_{50} values were 7.6 ± 2.0 µM for A2780cis, 10.3 ± 0.7 µM for OVCAR-3, 13.5 ± 1.3 µM for A2780, 7.4 ± 2.2 µM for OSE cell line. On the other hand, complex 1b showed lower toxicity than complex 1a

such as 39.8 ± 1.1 µM for A2780cis, 62.4 ± 9.4 µM for OVCAR-3, 29.1 ± 1.5 µM for A2780, 21.9 ± 8.6 µM for OSE cell line. For complex 2 IC50 values were 46.2 ± 9.5 µM for A2780cis, 60.9 ± 2.9 µM for OVCAR-3, 24.2 ± 1.0 µM for A2780, 33.6 ± 3.3 µM for OSE cell line.

Carboplatin showed the lowest cytotoxicity in particular to OVCAR-3 cell line (234.5 ± 7.7 µM) although it was highly toxic to OSE at most (41.6 ± 2.9 µM). OVCAR-3 cell line was the most resistant cell type against all agents and failed to respond well while A2780cis cell line obtained better results than others.

4.2. Intra and Extra-Glucose Levels of Ovarian Cell Lines

Glucose metabolism was increased in cisplatin-resistant cells (A2780cis), while it was generally suppressed in cisplatin-sensitive cells (A2780). As can be seen in Figure 16, A2780cis, compared to a healthy cell line (OSE), obtained the highest intracellular glucose amount and the fastest glucose use in the cell. Compared to control, PAX and CAR treated OSE cells showed the highest rate of glucose use between 0 h to 24 h while Ru agents, particularly complex 1b and 2, showed the fastest glucose use between 24 h to 48 h (Figure 16A). Even though all cancer cell lines showed a high demand for glucose, cisplatin-resistant cell lines such as OVCAR-3 and A2780cis demonstrated the most excellent glucose uptake. All treatments in A2780 cells accelerated the glucose uptake compared to control cells (Figure 16B). Extracellular glucose was decreased more than half in all ovarian cancer cell lines, while OSE showed less glucose uptake compared to others in the first 24 h. Extracellular glucose in A2780cis cells dramatically decreased in PAX, CAR, and complex 2 treated cells compared to complex 1a and 1b treatment (Figure 16C). Complex 1a and 1b treatment in OVCAR-3 slowed the glucose uptake compared to control and other agents (Figure 16D).

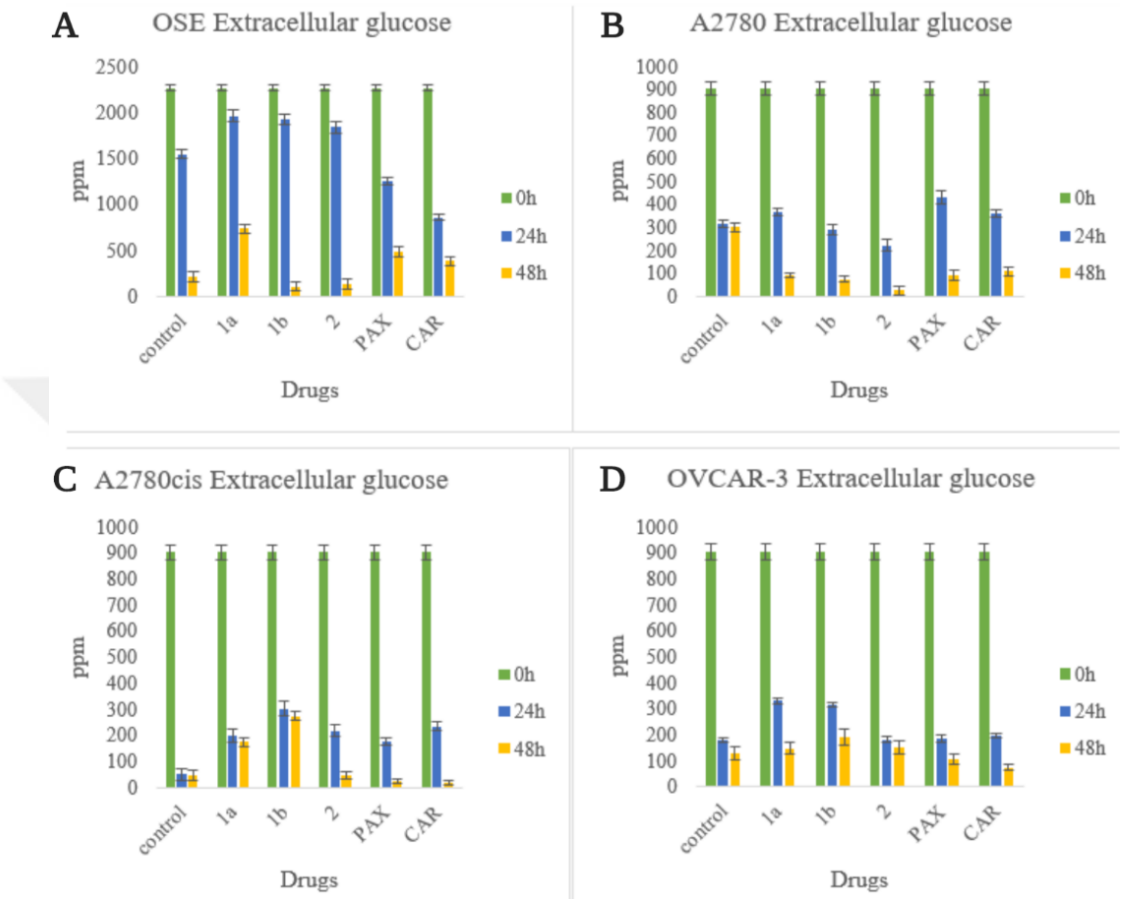

Figure 16 Change of extracellular glucose levels in ovarian cell lines (A: OSE cell line; B: A2780 cell line; C: A2780cis cell line; D: OVCAR-3 cell line).

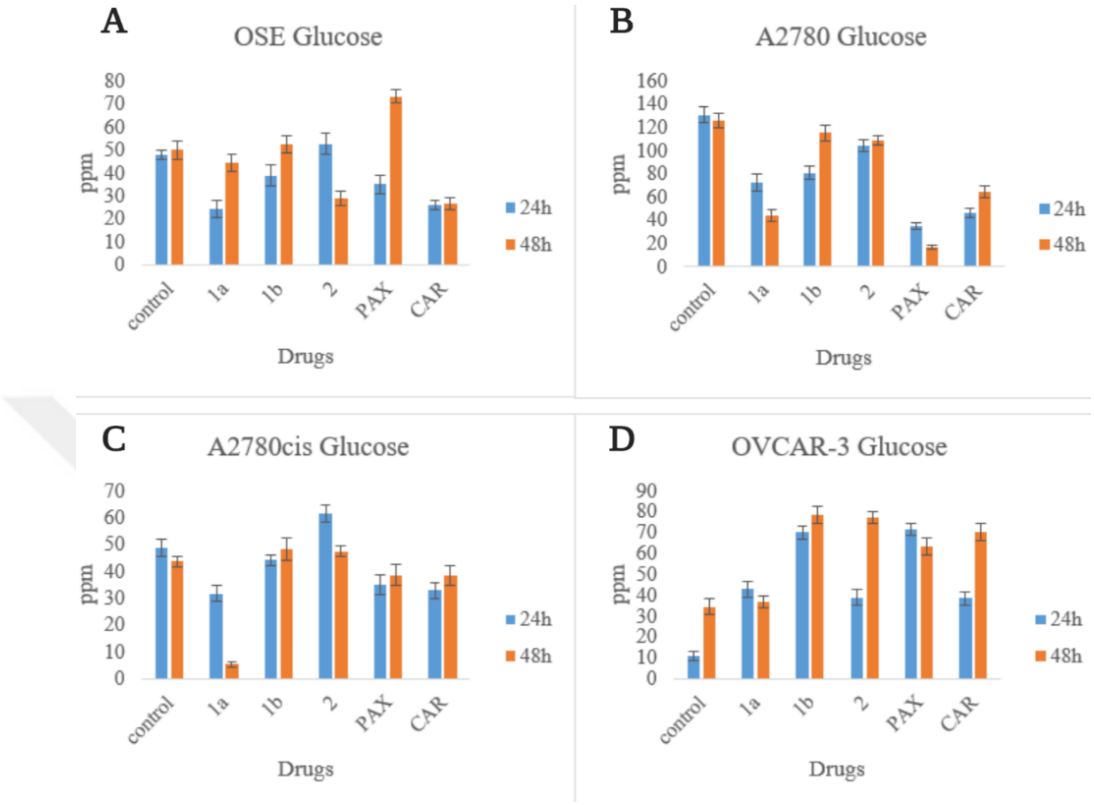

Figure 17 Change of intracellular glucose levels in ovarian cell lines (A: OSE cell line; B: A2780 cell line; C: A2780cis cell line; D: OVCAR-3 cell line).

Complex 1a, 1b, and PAX treated OSE cells showed an increase, while complex 2 showed a decrease in intracellular glucose between 24 h and 48 h compared to control (Figure 17A). Complex 1a and PAX treatment caused a decrease in intracellular glucose of A2780 cells compared to control. On the other hand, complex 1b, 2, and CAR treated A2780 cells showed an increased intracellular glucose value (Figure 17B). Glucose decreased dramatically in complex 1a treated A2780cis cells compare to others (Figure 17C). All treatments caused a high glucose uptake in OVCAR-3 cells, particularly complex 2 and CAR treatment, increased intracellular glucose levels two-fold higher between 24 h and 48 h (Figure 17D).

4.3. Intracellular TCA Cycle Metabolites of Ovarian Cell Lines

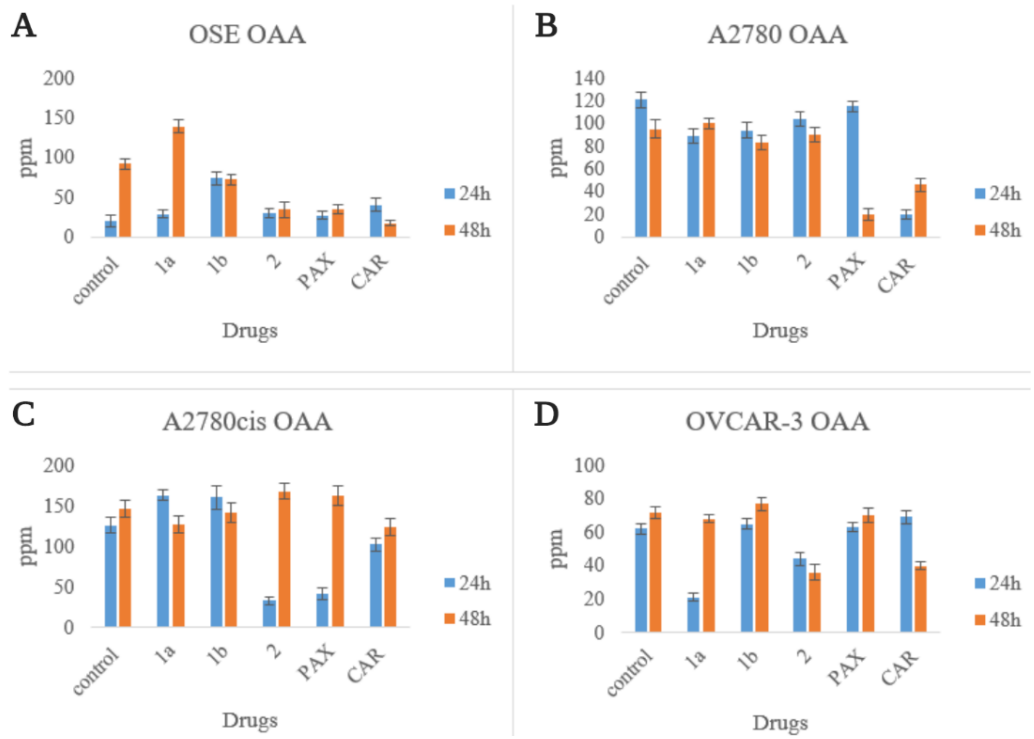

Figure 18 Change of OAA levels in ovarian cell lines (A: OSE cell line; B: A2780 cell line; C: A2780cis cell line; D: OVCAR-3 cell line).

Complex 1a increased OAA in OSE cells compared to control cells, while others were generally stable (Figure 18A). OAA reduced significantly in PAX treated A2780 cells between 24 h and 48 h while induced in CAR treated A2780 cells compared to control (Figure 18B). Although OAA amount was lower in CAR treated cells than in control cells, OAA increased significantly in complex 2, and PAX treated A2780cis cells while other agents slightly reduced it (Figure 18C). OVCAR-3 cells with complex 1a treatment caused a decrease in OAA values at 24 h, and CAR and complex 2 treatment also decreased OAA levels at 48 h compared to control (Figure 18D).

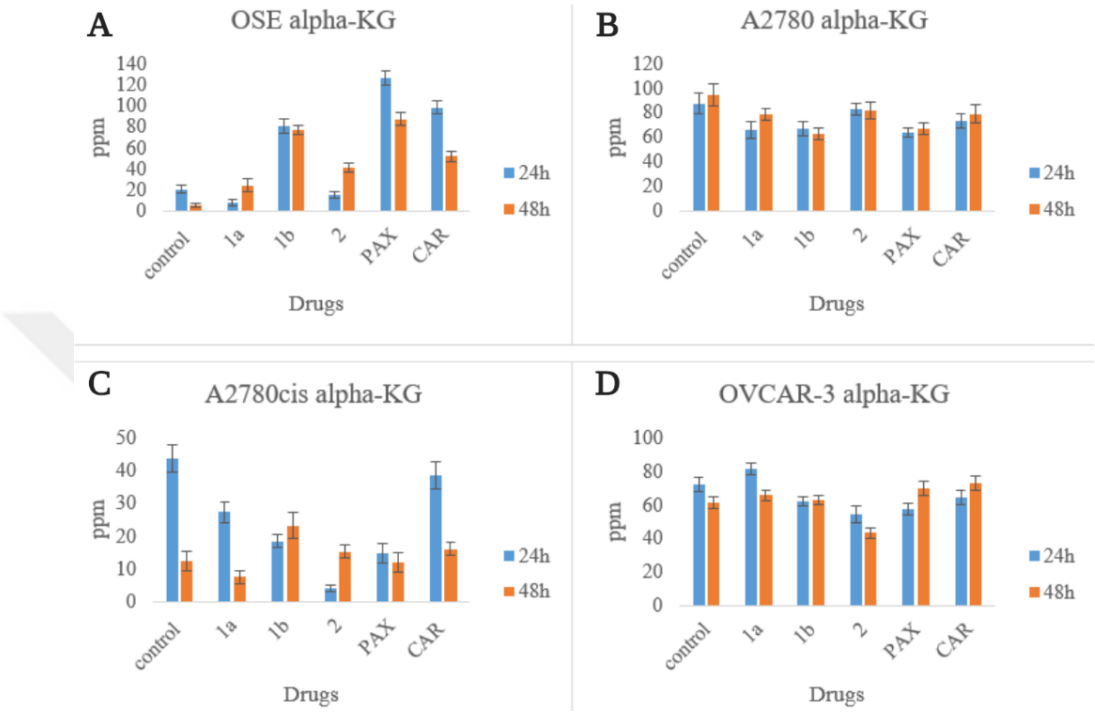

Figure 19 Change of alpha-KG levels in ovarian cell lines (A: OSE cell line; B: A2780 cell line; C: A2780cis cell line; D: OVCAR-3 cell line).

All treatments caused increased αKG levels in OSE cells compared to control, but a decrease in PAX and CAR treated OSE cells between 24 h and 48 h. On the contrary, complex 1a and 2 caused an increase compared to control (Figure 19A). Complex 1a treatment raised αKG levels of A2780 cells while other agents did not display much difference between 24 h and 48 h (Figure 19B). αKG levels in CAR and complex 1a treated A2780cis cells were significantly decreased while control was also decreased between 24 h and 48 h (Figure 19C). αKG levels in OVCAR-3 cells were stable mostly except complex 2 treated cells showed a decrease at 48 h (Figure 19D). In general, A2780cis cells had lower αKG levels than A2780 cells, and OVCAR-3 cells displayed stable αKG levels.

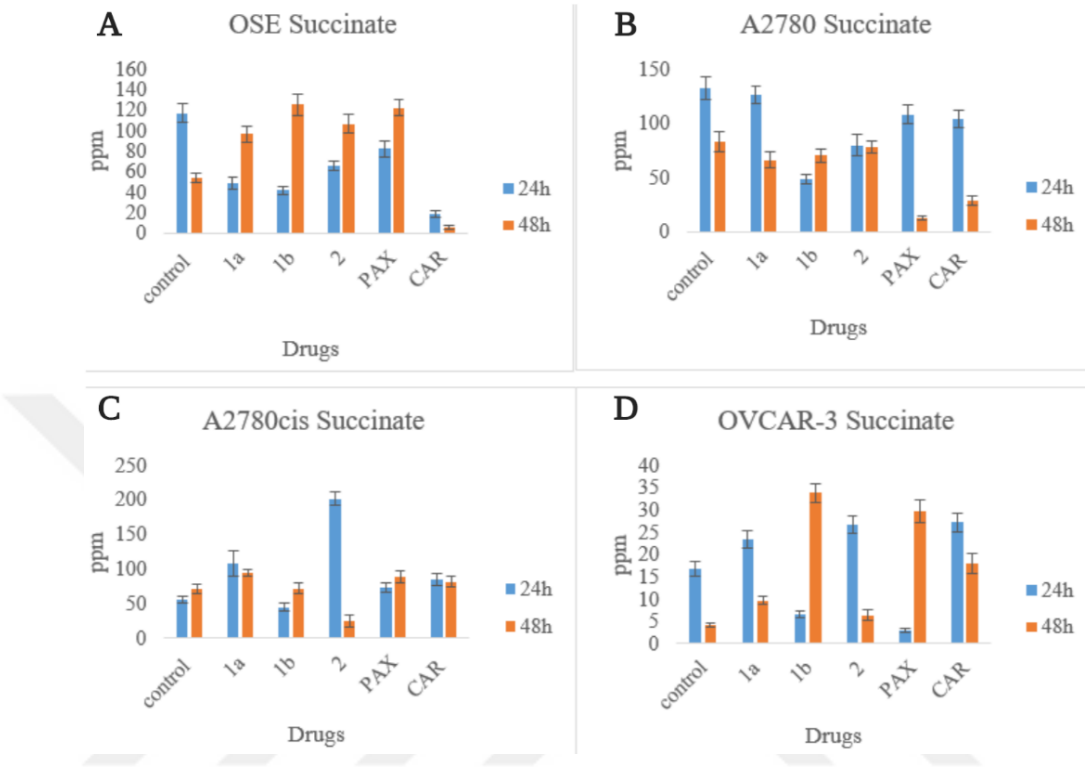

Figure 20 Change of succinate levels in ovarian cell lines (A: OSE cell line; B: A2780 cell line; C: A2780cis cell line; D: OVCAR-3 cell line).

Ru agents and PAX increased the succinate levels in OSE cells significantly while it was decreased in control cells. Also, CAR showed the lowest succinate levels in OSE cell line (Figure 20A). Complex 1a, PAX, and CAR treated A2780 cells displayed a significant reduction in succinate between 24 h and 48 h. On the other hand, complex 2 and 1b also lowered the succinate level at 24 h compared to control (Figure 20B). Complex 2 increased succinate levels in A2780cis cells at 24 h but then decreased dramatically at 48 h. Although other agents did not affect the succinate levels as much as the complex 2, complex 1a also increased while complex 1b decreased it at 24 h (Figure 20C). PAX and complex 1b treatment first decreased succinate levels at 24 h then increased in OVCAR-3 cells dramatically between 24 h and 48 h while complex 1a, 2, and CAR first increased it at 24 h then decreased it between 24 h and 48 h (Figure 20D). In general, succinate in cisplatin-resistant cells (OVCAR-3 and A2780cis) decreased more compared to cisplatin-sensitive cells (OSE and A2780).

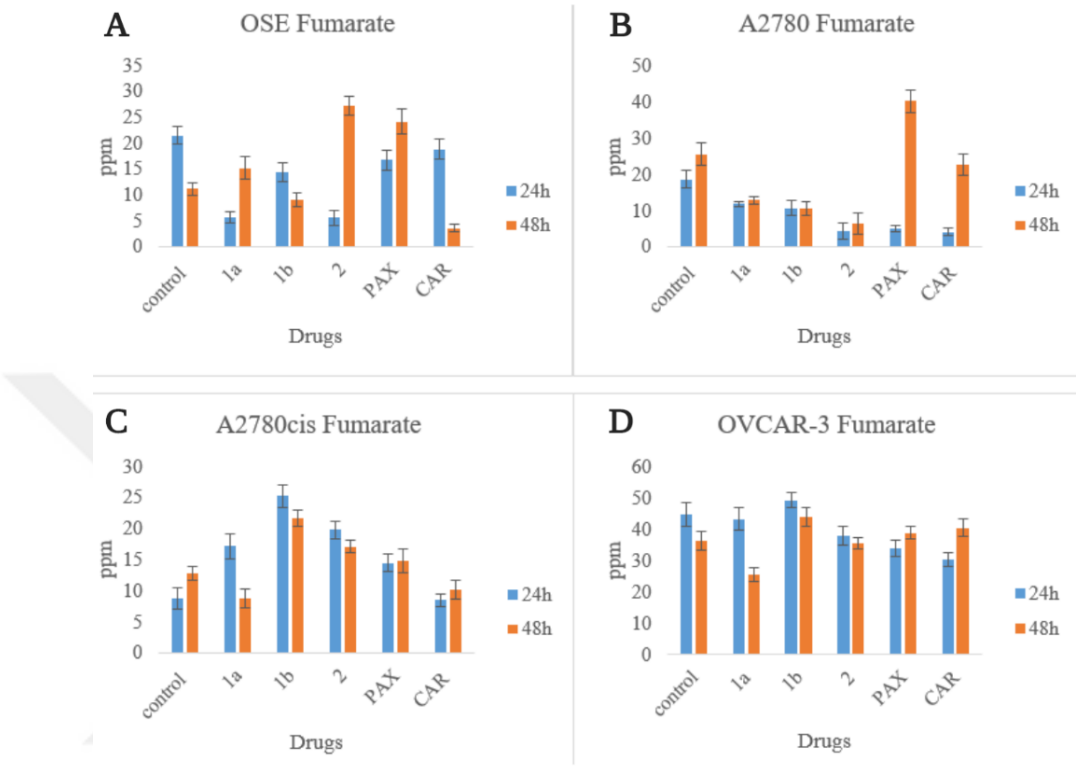

Figure 21 Change of fumarate levels in ovarian cell lines (A: OSE cell line; B: A2780 cell line; C: A2780cis cell line; D: OVCAR-3 cell line).

Fumarate levels in OSE reduced after complex 2, 1a, and 1b treatments at 24 h. However, complex 1a, 2, and PAX treated OSE cells increased fumarate levels at 48 h while CAR decreased it (Figure 21A). All Ru agents decreased fumarate levels in A2780 cells. PAX and CAR caused a decrease at 24 h but then increased notably at 48 h compared to control (Figure 21B). Complex 1a, 1b, 2 and PAX elevated fumarate levels in A2780cis cells at 24 h. Complex 1a and CAR decreased it at 48 h while complex 1b, 2, and PAX increased it compared to control (Figure 21C). Complex 2, PAX, and CAR lowered fumarate levels in OVCAR-3 cells at 24 h. Complex 1b, PAX, and CAR increased it at 48 h while complex 1a decreased it (Figure 21D). Generally, OVCAR-3 cells displayed mostly stable but significantly high fumarate levels compare to others.

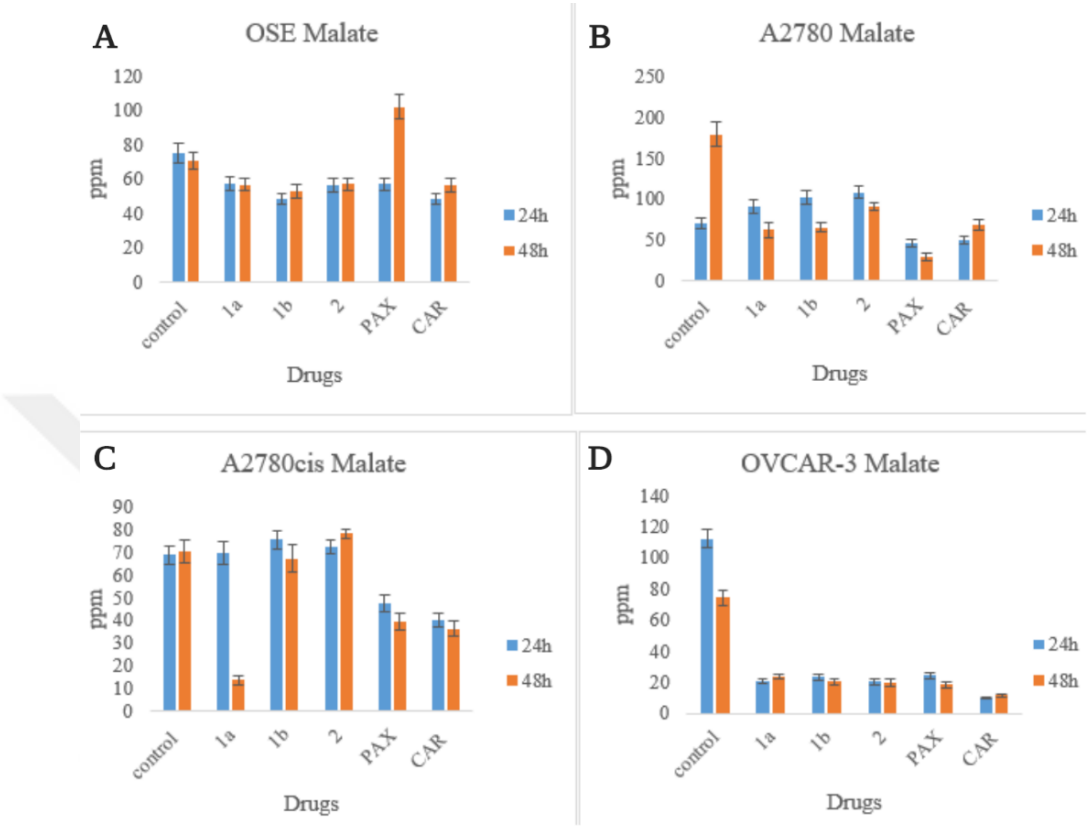

Figure 22 Change of malate levels in ovarian cell lines (A: OSE cell line; B: A2780 cell line; C: A2780cis cell line; D: OVCAR-3 cell line).

Ru agents and CAR treatments reduced malate levels in OSE cells compared to control, while PAX treatment lowered it significantly at 48 h (Figure 22A). PAX and CAR decreased malate levels in A2780 cells compared to control. Ru agents increased it at 24 h but then decreased it at 48 h compared to control cells (Figure 22B). Complex 1a, PAX, and CAR treatment decreased malate levels substantially in A2780cis cells, while others did not display any specific change (Figure 22C). All treatments, especially CAR, caused a dramatic decrease in malate levels of OVCAR-3 cells to compare to control (Figure 22D).

4.4. Lactic Acid Production in Ovarian Cell Lines

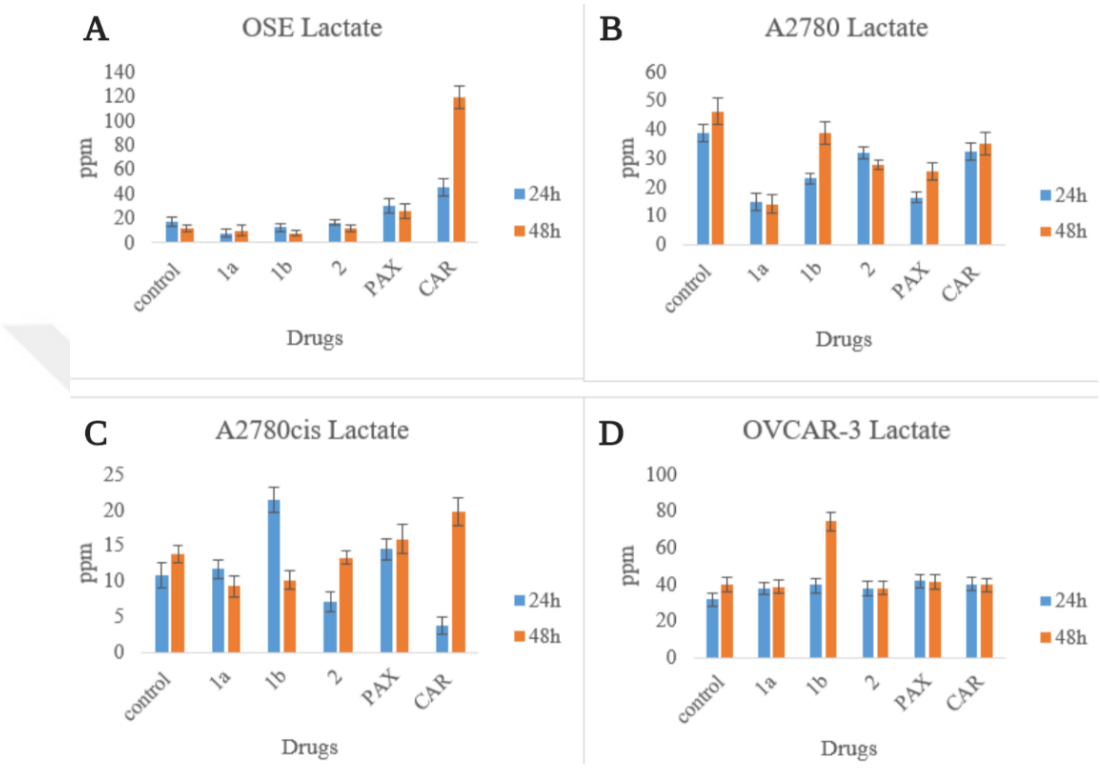

Figure 23 Change of lactate levels in ovarian cell lines (A: OSE cell line; B: A2780 cell line; C: A2780cis cell line; D: OVCAR-3 cell line).

Although other agents did not change lactate levels in OSE cells, PAX and CAR treatment considerably increased lactate levels in OSE cells compared to control (Figure 23A). All agents decreased lactate in A2780 cells, but especially complex 1a, 1b, and PAX reduced significantly at 24 h, but then PAX treated cells increased at 48h (Figure 23B). Complex 1b caused an increase in A2780cis cells at 24 h but reduced it at 48 h. On the other way, CAR and complex 2 treatment caused a decrease at 24 h but then increased it at 48 h. Complex 1a and PAX did not make any significant change in lactate levels of A2780cis cells compared to control cells (Figure 23C). Complex 1b increased lactate levels in OVCAR-3 cells at 48 h, while others did not change much. Generally, OVCAR-3 displayed stable lactate levels (Figure 23D).

5. DISCUSSION

Cell viability assessments showed that complex 1a has promising anti-proliferative attributes against cisplatin-resistant ovarian cancer cell lines, especially against OVCAR-3 cell line, which was the most resistant type among all. However, it was highly toxic to a healthy cell line (OSE), as well. Complex 2 displayed similar cytotoxicity levels with complex 1b, but its toxicity was lower against OSE cells, and also complex 2 was more efficient against A2780 cell line than complex 1b. Among three Ru agents that were used complex 1a was at least 3 to 5 times more effective than other ruthenium agents.

The cytotoxicity of Ru agents generally decreased at 48 h compared to 24 h values. As the cellular uptake experiments (Tavsan, Yaman, Subasi, & Ayar Kayalı, 2018) confirmed that Ru agents that were used were more efficient until 12 h because after that, it was started to throw out of the cells. Therefore complex 1b and 2 showed lower cytotoxicity in cells, and thus, cell proliferation was continued at 48 h. It was led to more glucose uptake and consumption in the cells. However, complex 1a showed an opposite aspect to other Ru agents; it was still highly toxic even after 24 h.

PAX was severely toxic to all cell lines, even with the concentration of nanomolar grade. Additionally, its toxicity gets higher at 48 h even with 5 nM dose. Although it was efficient against A2780cis (cisplatin-resistant ovarian cell line), it failed to demonstrate the same success towards OVCAR-3, which is another cisplatin-resistant cell line. Moreover, it was also harmful to a healthy cell line (OSE). However, CAR failed to kill OVCAR-3 cells while it was highly toxic towards OSE and A2780cis cells as much as complex 2.

Increased glycolysis is a crucial hallmark during cancer progression (Warburg, 1956b; Mathupala, Rempel, & Pedersen, 2001; Mathupala, Ko, & Pedersen, 2006). Increases in glucose and glutamine uptake are crucial for highly proliferating cells because they regularly require precursors for the synthesis of fatty acids and nucleic acids (Desideri, Vegliante, & Ciriolo, 2015).

It was reported that cisplatin-resistant ovarian cancer cells showed a high glucose demand (Catanzaro et al., 2015). HPLC analysis proved that the cisplatin-resistant cell line (A2780cis) possesses accelerated glucose metabolism than cisplatin-sensitive cells (A2780). Furthermore,

extracellular glucose levels were decreased more than half in all ovarian cancer cell lines, while OSE cell line exhibited reduced glucose uptake at 24 h. Extracellular glucose dramatically reduced in PAX, CAR, and complex 2 treated A2780cis cells compared to complex 1a and 1b. These conclusions may indicate a weakened glucose uptake in OVCAR-3 cells effected by complex 1a and 1b treatments.

Complex 1a and PAX treatments caused a decrease in intracellular glucose of A2780 cells compared to control. However, complex 1b, 2, and CAR applied A2780 cells exhibited increased intracellular glucose levels. Glucose decreased dramatically in complex 1a applied A2780cis cells compare to others. All agents caused high glucose uptake in OVCAR-3 cells; however, complex 2 and CAR treatments raised the intracellular glucose levels two-fold higher at 48 h compared to 24 h.

Each agent displayed a unique effect in cellular metabolism as each cell line responded differently to the agents. It is accepted that the TCA cycle oncometabolite accumulation triggers and supports the progression of cancer (Menendez, Alarcon, & Joven, 2014; Nam et al., 2014). Another study found that aberrations of the TCA cycle are related to various kinds of cancers (Montal et al., 2015; Pavlova & Thompson, 2016; Yuan et al., 2016). The mitochondrial aberrations lead to metabolic alteration in tumor cells and raise the rate of glycolysis, which supports cell survival and proliferation in turn (Frezza & Gottlieb, 2009; Gaude & Frezza, 2014).

Glycogen synthesis is commonly observed in cancer cells under hypoxic circumstances (Iida et al., 2012; Uekuri et al., 2013). Additionally, it is also related to resistance against chemotherapeutic drugs (Iida et al., 2012). Complex 1a applied A2780cis cells demonstrated accelerated glucose consumption but reduced TCA cycle metabolites in general. These results may imply the usage of glucose in glycogen synthesis or nucleotide synthesis through the pentose phosphate pathway or other pathways.

Glucose transporters are overexpressed to compensate for the increased glycolysis, and thus, glucose levels are elevated in cancer cells (Macheda, Rogers, & Best, 2005; Pelicano, Martin, Xu, & Huang, 2006). Interestingly, in cancer cells, lactate production via aerobic glycolysis does not shut down the OXPHOS pathway completely (Israelsen et al., 2013). In agreement with this,

A2780cis cell line with complex 2 treatment showed elevated lactic acid production as well as the αKG, OAA, and fumarate levels. These results may indicate a proof for a metabolic shift in cellular metabolism toward anaerobic respiration but also with accelerated TCA cycle. Furthermore, complex 1a applied A2780 cells showed lowered lactate levels with increased αKG and OAA levels. It may refer to a suppression of the lactic acid production in A2780 cell line with the acceleration of the TCA cycle.

Complex 1b applied OVCAR-3, and A2780 cells showed similar results as increased glucose uptake and lactate levels. These findings may imply a shift in cellular metabolism toward anaerobic respiration. In contrast to this, A2780cis cells displayed elevated TCA cycle intermediates with the same treatment, which might indicate an increased OXPHOS in cells.

In OVCAR-3 cells, PAX application caused an increase in TCA cycle intermediates and intracellular glucose levels. It may infer an accelerated OXPHOS in ovarian cancer cell lines by PAX treatment. Additionally, CAR treatment suppressed the TCA cycle in almost all cell lines and elevated the lactate levels in OSE cells, which may indicate an increase in anaerobic respiration along with suppressed OXPHOS.

One of the crucial intermediates of the TCA cycle is αKG because of its role in glutaminolysis, which is an energy-generating pathway in cellular metabolism. It is accepted that cancer cells overexpress the enzymes that are necessary for the conversion of glutamate from glutamine to supply additional energy sources under circumstances of glucose deprivation. Glutamate is used to create αKG, which can replenish the TCA cycle to synthesize ATP or amino acid (Yuneva et al., 2007; Weinberg et al., 2010; Altman, Stine, & Dang, 2016). Moreover, OAA can be overproduced to use in nucleotide synthesis via conversion to aspartate (Son et al., 2013).

αKG levels expected to be reduced in IDH-mutant cancer cells as a result of increased αKG conversion to 2-Hydroxyglutarate (2HG). However, it is rather elevated as a result of mitochondrial biosynthesis to compensate for the absent αKG (van Lith et al., 2014). In agreement with this study, αKG levels of OVCAR-3 cell line were generally stable but high. After treatment with agents, OSE cells showed a similar profile to OVCAR-3 cells and raised cellular αKG levels dramatically

compared to control. These results may suggest that OSE cells reprogrammed their metabolism to adapt to certain conditions like chemotherapy and imitated a profile alike to a resistant cell.

Another critical intermediate of the TCA cycle is succinate, which owns a significant part in producing ATP. Additionally, it is a fundamental modulator of the hypoxic response and has a significant role in tumorigenesis. SDH is responsible for transforming succinate to fumarate (King, Selak, & Gottlieb, 2006). Especially, mutations in the gene encoding SDH were involved in numerous cancers. Therefore, SDH is now considered a tumor suppressor (Bardella, Pollard, & Tomlinson, 2011). Also, SDH mutations cause an elevation in succinate levels and lead to HIF-1a (Hypoxia-inducible factor 1-alpha) stabilization, thus supporting tumor growth (Guzy et al., 2008).

Some studies reported that under nutrient deprivation or hypoxic conditions, cancer cells rely on fumarate respiration (Tomitsuka, Kita, & Esumi, 2010; Sakai et al., 2012). Fumarate respiration occurs by converting fumarate to succinate through the reverse reaction of SDH, which further generates ATP (Kita & Takamiya, 2002). Succinate, the outcome of fumarate respiration was observed remarkably high in tumor cells (Hirayama et al., 2009). In agreement with this, complex 1b treated OVCAR-3 and OSE cells, and complex 1a treated A2780cis cells exhibited high succinate levels.

Loss-of-function mutations in FH and SDH, increase the amount of fumarate and succinate, respectively (Pollard *et al.*, 2005), and gain-of-function mutations in IDH elevate D-2-hydroxyglutarate (D-2HG) levels (Ward et al., 2010; Dang et al., 2010). Another study proved that fumarate acts as an oncometabolite, which means metabolites that contribute to oncogenic processes (Sciacovelli & Frezza, 2016), and leading to over-expression of EMT-driving transcription factors. Complex 1a applied OSE cells demonstrated increased TCA cycle intermediates. However, they also showed lowered fumarate levels, which might be due to fumarate respiration in cells.

Complex 1a applied OVCAR-3 cells demonstrated decreased fumarate and OAA levels while OVCAR-3 cells with PAX treatment showed increased succinate, αKG, and fumarate levels. These results may imply a change in the energy metabolism of OVCAR-3 cells from fumarate respiration to OXPHOS respect to the alteration of treatment of complex 1a to PAX. All Ru agents

decreased fumarate levels in A2780 cells while Ru agents and PAX increased fumarate levels in A2780cis cells at 24 h.

In conclusion, we demonstrated the cytotoxic effects of Ruthenium complexes as well as carboplatin and paclitaxel drugs on the energy metabolism in ovarian cell lines. We found that Ruthenium complexes are promising candidates, and with further improvements, their potential might increase the efficiency of treatment against ovarian cancer. These results proved that each chemotherapeutic agent caused a characteristic metabolic respond in ovarian cell lines. This study may lead to further research for better therapeutics against cancer and improve the knowledge of the effects of chemotherapeutics on the energy metabolism of ovarian cells.

6. CONCLUSION AND RECOMMENDATIONS

Every cancer cell displays individual metabolic phenotypes of energy for two main reasons. First, it has been acknowledged that cancer is a heterogeneous disease, and it possesses genetic heterogeneity, which leads to metabolic heterogeneity in the cell (Marusyk & Polyak, 2010). Even in the same type of cancer, its constituent cell subtypes also exhibit characteristic variations in metabolic phenotype from one cell to another (Feron, 2009). Second, cancer cells constantly reprogram to adapt to challenging environmental alterations and nutrients deprivations. Therefore we understand that balances between glycolysis and OXPHOS or other pathways to produce ATP are continuously changing in cancer cells. Our results demonstrated that each cell line displayed a distinct metabolic response to the chemotherapeutic agents. Ruthenium complexes have promising attributes and can be highly beneficial in cancer treatment with further improvements. It is an essential step to unveil the effects of chemotherapeutic agents on cellular metabolism in order to discover new novel therapeutic approaches against cancer.

7. REFERENCES

Ahn, C. S., & Metallo, C. M. (2015). Mitochondria as biosynthetic factories for cancer proliferation. *Cancer Metab, 3*(1): 1. doi: 10.1186/s40170-015-0128-2

Alberts, D. S., Green, S., Hannigan, E. V., O'Toole, R., et al. (1992). Improved therapeutic index of carboplatin plus cyclophosphamide versus cisplatin plus cyclophosphamide: final report by the southwest oncology group of a phase III randomized trial in stages III and IV ovarian cancer. *Journal of Clinical Oncology, 10*(5): 706-717. doi: 10.1200/JCO.1992.10.5.706

Alderden, R. A. (2006). The Discovery and Development of Cisplatin. *Journal of Chemical Education, 83*(5): 728- 734. doi: 10.1021/ed083p728

Alessio, E., Mestroni, G., Bergamo, A., & Sava, G. (2004). Ruthenium Antimetastatic Agents. *Current Topics in Medicinal Chemistry, 4*(15): 1525-1535. doi: 10.2174/1568026043387421

Al-Sarraf, M., Metch, B., Kish, J., Ensley, J., Rinehart, J. J., et al. (1987). Platinum analogs in recurrent and advanced head and neck cancer: a southwest oncology group and Wayne state university study. *Cancer Treat Rep, 71*(7–8): 723-726. PMID: 3300967

Altman, B. J., Stine, Z. E., & Dang, C. V. (2016). From Krebs to clinic: glutamine metabolism to cancer therapy. *Nat Rev Cancer, 16*(10): 619-634. doi: 10.1038/nrc.2016.71

Amable, L. (2016). Cisplatin resistance and opportunities for precision medicine. *Pharmacol Res, 106*: 27-36. doi: 10.1016/j.phrs.2016.01.001

American Cancer Society: Cancer Facts and Figures 2019. Atlanta, Ga: American Cancer Society, 2019. Available from: https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2019.html. Accessed 07/06/2019.

Anderson, A. S., Roberts, P. C., Frisard, M. I., Hulver, M. W., & Schmelz, E. M. (2014).Ovarian tumor-initiating cells display a flexible metabolism. *Exp Cell Res, 328*(1): 44-57. doi: 10.1016/j.yexcr.2014.08.028

Ang, W. H., & Dyson, P. J. (2006). Classical and non- classical ruthenium- based anticancer drugs: towards targeted chemotherapy. *Eur J Inorg Chem, 20*: 4003-4018. doi: 10.1002/ejic.200690041

Aras, K., & Ersen, G. (1975). Ankara University Faculty of Dentistry Press. 2: p. 245-258.

Ayar Kayalı, H. (2005). Identification of some intermediate metabolite and enzymes efficiency on regulation of vancomycin antibiotic production by *Amycolatopsis orientalis*. Dokuz Eylül Unıversity Graduate School of Natural and Applied Sciences. Ph. D thesis.

Bajorin, D. F., Sarosdy, M. F., Pfister, D. G., Mazumdar, M., et al. (1993). Randomized trial of etoposide and cisplatin versus etoposide and carboplatin in patients with good-risk germ cell tumors: a multi-institutional study. *J Clin Oncol, 11*(4): 598-606. doi: 10.1200/JCO.1993.11.4.598

Banno, K., Yanokura, M., Iida, M., Adachi, M., et al. (2014). Application of microRNA in diagnosis and treatment of ovarian cancer. *BioMed research international*, 232817. doi: 10.1155/2014/232817

Bardella, C., Pollard, P. J., & Tomlinson, I. (2011). SDH mutations in cancer. *Biochim Biophys Acta, 1807*(11): 1432-1443. doi: 10.1016/j.bbabio.2011.07.003

Barry, N. P. E., & Sadler, P. J. (2013). Exploration of the medical periodic table: towards new targets. *Chem Commun, 49*(45): 5106-5131. doi: 10.1039/c3cc41143e

Bayley, J. P., & Devilee, P. (2010). Warburg tumours and the mechanisms of mitochondrial tumour suppressor genes. Barking up the right tree? *Curr Op in Genet Dev, 20*(3): 324-329. doi: 10.1016/j.gde.2010.02.008

Beral, V., Hermon, C., Peto, R., Reeves, G., Brinton, L., et al. (2012). Ovarian cancer and body size: individual participant meta-analysis including 25,157 women with ovarian cancer from 47 epidemiological studies. *Plos Med, 9*(4): e1001200. doi: 10.1371/journal.pmed.1001200

Berg, A. H., Combs, T. P., & Scherer, P. E. (2002). ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. *Trends Endocrinol Metab, 13*(2): 84-89. PMID: 11854024

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2002). *Biochemistry* (5th ed.). New York: W. H. Freeman Publishing, ISBN-10: 0-7167-3051-0.

Betanzos- Lara, S., Salassa, L., Habtemariam, A., Novakova, O., et al. (2012). Photoactivatable Organometallic Pyridyl Ruthenium(II) Arene Complexes. *Organometallics, 31*(9): 3466-79. doi: 10.1021/om201177y

Birsoy, K., Wang, T., Chen, W. W., Freinkman, E., et al. (2015). An Essential Role of the Mitochondrial Electron Transport Chain in Cell Proliferation Is to Enable Aspartate Synthesis. *Cell, 162*(3): 540-551. doi: 10.1016/j.cell.2015.07.016

Bokemeyer, C., Köhrmann, O., Tischler, J., Weissbach, L., Räth, U., et al. (1996). A randomized trial of cisplatin, etoposide and bleomycin (peb) versus carboplatin, etoposide and bleomycin (ceb) for patients with ‘good-risk' metastatic non-seminomatious germ cell tumors. *Ann Oncol, 7*(10): 1015-1021. doi: 10.1093/oxfordjournals.annonc.a010493

Brabec, V., & Kasparkova, J. (2005). Modifications of DNA by platinum complexes. Relation to resistance of tumors to platinum anti-tumor drugs. *Drug Resist Updat, 8*(3): 131-146. doi: 10.1016/j.drup.2005.04.006

Brammer, H. M. 3rd, Buck, J. L., Hayes, W. S., Sheth, S., & Tavassoli, F. A. (1990). From the archives of the AFIP. Malignant germ cell tumors of the ovary: radiologic-pathologic correlation. *RadioGraphics, 10*(4): 715-724. doi: 10.1148/radiographics.10.4.2165627

Brandon, M., Baldi, P., Wallace, D. C. (2006). Mitochondrial mutations in cancer. *Oncogene, 25*(34): 4647-4662. doi: 10.1038/sj.onc.1209607

Bugarcic, T., Habtemariam, A., Deeth, R. J., Fabbiani, F. P., Parsons, S., & Sadler, P. J. (2009). Ruthenium(II) arene anticancer complexes with redox- active diamine ligands. *Inorg Chem, 48(*19): 9444-9453. doi: 10.1021/ic9013366

Caneba, C. A., Yang, L., Baddour, J., Curtis, R., Win, J., Hartig, S., Marini, J., & Nagrath, D. (2014). Nitric oxide is a positive regulator of the Warburg effect in ovarian cancer cells. *Cell Death Dis, 5*: e1302. doi: 10.1038/cddis.2014.264

Catanzaro, D., Gaude, E., Orso, G., Giordano, C., Guzzo, G., et al. (2015). Inhibition of glucose-6-phosphate dehydrogenase sensitizes cisplatin resistant cells to death. *Oncotarget, 6*(30): 30102-30114. doi: 10.18632/oncotarget.4945

Chen, D., Milacic, V., Frezza, M., & Dou, Q. P. (2009). Metal Complexes, their Cellular Targets and Potential for Cancer Therapy. *Current Pharmaceutical Design, 15*(7): 777. doi: 10.2174/138161209787582183

Chen, V. W., Ruiz, B., Killeen, J. L., Cote, T. R., Wu, X. C., & Correa, C. N. (2003). Pathology and classification of ovarian tumors. *Cancer, 97*(10): 2631-2642. doi: 10.1002/cncr.11345

Choi, B. H., & Coloff, J. L. (2019). The Diverse Functions of Non-Essential Amino Acids in Cancer. *Cancers, 11*(5), 675. doi:10.3390/cancers11050675

Christofk, H. R., Vander Heiden, M. G., Harris, M. H., Ramanathan, A., et al. (2008). The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature, 452*(7184): 230-233. doi: 10.1038/nature06734

Clarke, M. J. (1989). *Ruthenium Chemistry Pertaining to the Design of Anticancer Agents*. In: Baulieu E. et al. (editors). Ruthenium and Other Non-Platinum Metal Complexes in Cancer Chemotherapy. Progress in Clinical Biochemistry and Medicine, Springer, Berlin, Heidelberg, vol 10, p. 25-39. doi: 10.1007/978-3-642-74760-1_2

Cragg, G. M. (1998). Paclitaxel (Taxol): a success story with valuable lessons for natural product drug discovery and development. *Med Res Rev, 18*(5): 315-331. PMID: 9735872

Dang, C. V. (2012). Links between metabolism and cancer. *Genes & development, 26*(9), 877-890. doi:10.1101/gad.189365.112

Dang, L., White, D. W., Gross, S., Bennett, B. D., Bittinger, M. A., et al. (2010). Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature, 465*(7300): 966. doi: 10.1038/nature09132

Dar, S., Chhina, J., Mert, I., Chitale, D., Buekers, T., Kaur, H., et al. (2017). Bioenergetic Adaptations in Chemoresistant Ovarian Cancer Cells. *Sci Rep, 7*(1): 8760. doi: 10.1038/s41598-017-09206-0

David, R. S., Mark, F. B., Luis, V., Daniel, L. C., Robert, A. B., et al. (2007). Phase III Randomized Trial of Intravenous Cisplatin Plus a 24- or 96-Hour Infusion of Paclitaxel in Epithelial Ovarian Cancer: A Gynecologic Oncology Group Study. *Journal of Clinical Oncology, 25*(28): 4466-4471. doi: 10.1200/JCO.2006.10.3846

de Souza, A. C., Justo, G. Z., de Araujo, D. R., & Cavagis, A. D. (2011). Defining the molecular basis of tumor metabolism: a continuing challenge since Warburg's discovery. *Cell Physiol Biochem, 28*(5): 771-792. doi: 10.1159/000335792

DeBerardinis, R. J., & Chandel, N. S. (2016). Fundamentals of cancer metabolism. *Science advances, 2*(5): e1600200. doi:10.1126/sciadv.1600200

Deberardinis, R. J., Sayed, N., Ditsworth, D., & Thompson, C. B. (2008). Brick by brick: Metabolism and tumor cell growth. *Curr Opin Genet Dev, 18*(1): 54-61. doi: 10.1016/j.gde.2008.02.003

Desideri, E., Vegliante, R., & Ciriolo, M. R. (2015). Mitochondrial dysfunctions in cancer: genetic defects and oncogenic signaling impinging on TCA cycle activity. *Cancer Lett, 356*(2 Pt A): 217-223. doi: 10.1016/j.canlet.2014.02.023

Einzig, A. I., Wiernik, P. H., Sasloff, J., Runowicz, C. D., & Goldberg, G. L. (1992). Phase II study and long-term follow-up of patients treated with taxol for advanced ovarian adenocarcinoma. *J Clin Oncol, 10*(11): 1748-1753. doi: 10.1200/JCO.1992.10.11.1748

Ferlay, J. S. H., Bray, F., Forman, D., Mathers, C., & Parkin, D. M. (2008). GLOBOCAN v 1.2: Cancer Incidence, Mortality, and Prevalence Worldwide: IARC Cancer Base No. 10. Lyon, France: International Agency for Research on Cancer, 2008. Available from: http://globocan.iarc.fr. Accessed 05/07/2019.

Fernandez, R., Melchart, M., Habtemariam, A., Parsons, S., & Sadler, P. J. (2004). Use of chelating ligands to tune the reactive site of half- sandwich ruthenium(II)- arene anticancer complexes. *Chemistry, 10*(20): 5173-5179. doi: 10.1002/chem.200400640

Feron, O. (2009). Pyruvate into lactate and back: from the Warburg effect to symbiotic energy fuel exchange in cancer cells. *Radiother Oncol, 92*(3): 329- 333. doi: 10.1016/j.radonc.2009.06.025

Franceschi, S., La Vecchia, C., Negri, E., Guarneri, S., Montella, M., et al. (1994). Fertility drugs and risk of epithelial ovarian cancer in Italy. *Hum Reprod, 9*(9): 1673-1675. doi: 10.1093/oxfordjournals.humrep.a138771

Frezza, C., & Gottlieb, E. (2009). Mitochondria in cancer: not just innocent bystanders. *Semin Cancer Biol, 19*(1): 4-11. doi: 10.1016/j.semcancer.2008.11.008

Gagliardi, R., Sava, G., Pacor, S., Mestroni, G., & Alessio, E. (1994). Antimetastatic action and toxicity on healthy tissues of Na[trans-RuCl4(DMSO)Im] in the mouse. Clin Exp Metastasis, 12(2): 93-100. PMID: 8306532

Gaitskell, K., Green, J., Pirie, K., Barnes, I., Hermon, C., et al. (2017). Histological subtypes of ovarian cancer associated with parity and breastfeeding in the prospective Million Women Study. *Int J Cancer, 142*(2): 281-289. doi: 10.1002/ijc.31063.

Galanski, M. (2006). Recent developments in the field of anticancer platinum complexes. *Recent Pat Anticancer Drug Discov, 1*(2): 285-295. PMID: 18221042

Galanski, M., Arion, V. B., Jakupec, M. A., & Keppler, B. K. (2003). Recent developments in the field of tumor inhibiting metal complexes. *Curr Pharm Des, 9*(25): 2078-2089. doi: 10.2174/1381612033454180

Galanski, M., Jakupec, M. A., & Keppler, B. K. (2005). Update of the pre-clinical situation of anticancer platinum complexes: novel design strategies and innovative analytical approaches. *Curr Med Chem, 12*(18): 2075-2094. doi: 10.2174/0929867054637626

Ganzera, M., Vrabl, P., Wörle, E., Burgstaller, W., & Stuppner, H. (2006). Determination of adenine and pyridine nucleotides in glucose-limited chemostat cultures of Penicillium simplicissimum by one-step ethanol extraction and ion-pairing liquid chromatography. *Analytical Biochemistry, 359*(1): 132–140. doi: 10.1016/j.ab.2006.09.012

Gaude, E., & Frezza, C. (2014). Defects in mitochondrial metabolism and cancer. *Cancer Metab, 2*: 10. doi: 10.1186/2049-3002-2-10

Glance, A. (2009). Ovarian cancer: an overview. *Am Fam Physician, 80*(6): 609-616. PMID: 19817326

Globocan http://gco.iarc.fr/today/online-analysis-map?v=2018&mode=population&mode_population=continents&population=900&populations=900&key=asr&sex=2&cancer=39&type=0&statistic=5&prevalence=0&population_group=0&ages_group%5B%5D=0&ages_group%5B%5D=17&nb_items=5&group_cancer=0&include_nmsc=1&include_nmsc_other=1&projection=natural-earth&color_palette=default&map_scale=quantile&map_nb_colors=5&continent=0&rotate=%255B10%252C0%255D. Accessed 19/07/2019.

Go, R. S., & Adjei, A. A. (1999). Review of the comparative pharmacology and clinical activity of cisplatin and carboplatin. *Journal of Clinical Oncology, 17*(1): 409-422. doi: 10.1200/JCO.1999.17.1.409

Goff, B. A., Mandel, L., Munt, H. G., & Melancron, C. H. (2000). Ovarian carcinoma diagnosis. *Cancer, 89*(10): 2068-2075. doi: 10.1002/1097-0142(20001115)89:10<2068::aid-cncr6>3.0.co;2-z

Goodman, L. S., Wintrobe, M. M., Dameshek, W., Goodman, M. J., Gilman, A., et al. (1984). Nitrogen Mustard Therapy: Use of Methyl-Bis(Beta-Chloroethyl)amine Hydrochloride and Tris(Beta-Chloroethyl)amine Hydrochloride for Hodgkin's Disease, Lymphosarcoma, Leukemia and Certain Allied and Miscellaneous Disorders. *JAMA, 251*(17): 2255-2261. doi: 10.1001/jama.1984.03340410063036

Griffin, A. M., Butow, P. N., Coates, A. S., Childs, A. M., Ellis, P. M., Dunn, S. M., & Tattersall, M. H. (1996). On the receiving end. V: Patient perceptions of the side effects of cancer chemotherapy in 1993. *Ann Oncol, 7*(2): 189- 195. doi: 10.1093/oxfordjournals.annonc.a010548

Guzy, R. D., Sharma, B., Bell, E., Chandel, N. S., Schumacker, P. T. (2008). Loss of the SdhB, but Not the SdhA, subunit of complex II triggers reactive oxygen species dependent hypoxia-inducible factor activation and tumorigenesis. *Mol Cell Biol, 28*(2): 718-731. doi: 10.1128/MCB.01338-07

Habtemariam. A., Melchart, M., Fernandez, R., Parsons, S., Oswald, I. D., et al. (2006). Structure-activity relationships for cytotoxic ruthenium(II) arene complexes containing N,N-, N,O-, and O,O-chelating ligands. *J Med Chem, 49*(23): 6858-68. doi: 10.1021/jm060596m

Hajek, R., Vorlicek, J., & Slavik, M. (1996). Paclitaxel (Taxol): a review of its antitumor activity in clinical studies Minireview. *Neoplasma, 43*(3): 141-154. PMID: 8841500

Hanahan D., & Weinberg R. A. (2011). Hallmarks of cancer: The next generation. *Cell, 144*(5): 646-674. doi: 10.1016/j.cell.2011.02.013

Hartge, P., Schiffman, M. H., Hoover, R., McGowan, L., Lesher, L., & Norris, H. J. (1989).A case-control study of epithelial ovarian cancer. *Am J Obstet Gynecol, 161*(1): 10-16. doi: 10.1016/0002-9378(89)90221-4

Hartinger, C. G., Jakupec, M. A., Zorbas-Seifried, S., Groessl, M., Egger, A., et al. (2008). KP1019, a new redox-active anticancer agent–preclinical development and results of a clinical phase I study in tumor patients. *Chem Biodivers, 5*(10): 2140-2155. doi: 10.1002/cbdv.200890195

Hatzivassiliou, G., Zhao, F., Bauer, D. E., Andreadis, C., Shaw, A. N., Dhanak, D., Hingorani, S. R., Tuveson, D.A., & Thompson, C. B. (2005). ATP citrate lyase inhibition can suppress tumor cell growth. *Cancer Cell, 8*(4): 311-321. doi: 10.1016/j.ccr.2005.09.008

Heffeter, P., Jungwirth, U., Jakupec, M., Hartinger, C., et al. (2008). Resistance against novel anticancer metal compounds: Differences and similarities. *Drug Resistance Updates, 11*(1-2): 1-16. doi: 10.1016/j.drup.2008.02.002

Heintz, A. P., Odicino, F., Maisonneuve, P., Quinn, M. A., Benedet, J. L., et al. (2006). Carcinoma of the ovary: FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. *Int J Gynaecol Obstet, 95*(1): 161-192. doi: 10.1016/S0020-7292(06)60033-7

Hennessy, B. T., Coleman, R. L., & Markman, M. (2009). Ovarian cancer. *Lancet, 374*(9698): 1371-1382. doi: 10.1016/S0140-6736(09)61338-6

Hensley, C. T., Wasti, A. T., & DeBerardinis, R. J. (2013). Glutamine and cancer: Cell biology, physiology, and clinical opportunities. *J Clin Invest, 123*(9): 3678-3684. doi: 10.1172/JCI69600

Herrinton, L. J., Stanford, J. L., Schwartz, S. M., & Weiss, N. S. (1994). Ovarian cancer incidence among Asian migrants to the United States and their descendants. *J Natl Cancer Inst, 86*(17): 1336-1339. doi: 10.1093/jnci/86.17.1336

Herzog, T. J. (2004). Recurrent ovarian cancer. *Am Assoc Cancer Res, 10*(22): 7439-7449. doi: 10.1158/1078-0432.CCR-04-0683

Hirayama, A., Kami, K., Sugimoto, M., et al. (2009). Quantitative metabolome profiling of colon and stomach cancer microenvironment by capillary electrophoresis time-of-flight mass spectrometry. *Cancer Res, 69*(11): 4918-4925. doi: 10.1158/0008-5472.CAN-08-4806

Hu, Y., Lu, W., Chen, G., Wang, P., et al. (2012). K-rasG12V transformation leads to mitochondrial dysfunction and a metabolic switch from oxidative phosphorylation to glycolysis. *Cell Res, 22*(2): 399-412. doi: 10.1038/cr.2011.145

Hudson, C. D., Savadelis, A., Nagaraj, A. B., Joseph, P., Avril, S., DiFeo, A., & Avril, N. (2016). Altered glutamine metabolism in platinum resistant ovarian cancer. *Oncotarget, 7*(27): 41637-41649. doi:10.18632/oncotarget.9317

Iida, Y., Aoki, K., Asakura, T., Ueda, K., et al. (2012). Hypoxia promotes glycogen synthesis and accumulation in human ovarian clear cell carcinoma. *Int J Oncol, 40*(6): 2122-30. doi: 10.3892/ijo.2012.1406

Ippolito, L., Marini, A., Cavallini, L., Morandi, A., Pietrovito, L., et al. (2016). Metabolic shift toward oxidative phosphorylation in docetaxel resistant prostate cancer cells. *Oncotarget, 7*(38): 61890-61904. doi: 10.18632/oncotarget.11301

Ishikawa, K., Takenaga, K., Akimoto, M., Koshikawa, N., et al. (2008). ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. *Science, 320*(5876): 661-664. doi: 10.1126/science.1156906

Israelsen, W. J., Dayton, T. L., Davidson, S. M., Fiske, B. P., Hosios A. M., et al. (2013). PKM2 isoform-specific deletion reveals a differential requirement for pyruvate kinase in tumor cells. *Cell, 155*(2): 397-409. doi: 10.1016/j.cell.2013.09.025

Jakupec, M. A., Arion, V. B., Kapitza, S., Reisner, E., Eichinger, A., et al. (2005). KP1019 (FFC14A) from bench to bedside: preclinical and early clinical development-an overview. *Int J Clin Pharmacol Ther, 43*(12): 595-596. doi: 10.5414/cpp43595

Johnstone, T. C., Suntharalingam, K., & Lippard, S. J. (2016). The Next Generation of Platinum Drugs: Targeted Pt(II) Agents, Nanoparticle Delivery, and Pt(IV) Prodrugs. *Chem Rev, 116(*5): 3436-3486. doi: 10.1021/acs.chemrev.5b00597

Jung, Y., & Lippard, S. J. (2007). Direct cellular responses to platinum-induced DNA damage. *Chem Rev, 107*(5):1387-1407. doi: 10.1021/cr068207j

Kampan, N. C., Madondo, M. T., McNally, O. M., Quinn, M., & Plebanski, M. (2015). Paclitaxel and its evolving role in the management of ovarian cancer. *BioMed Research International, 2015*: 413076. doi: 10.1155/2015/413076

Kellenberger, L. D., Bruin, J. E., Greenaway, J., Campbell, N. E., Moorehead, R. A., et al. (2010). The role of dysregulated glucose metabolism in epithelial ovarian cancer. *Journal of oncology*, 514310. doi:10.1155/2010/514310

Keppler, B. K., Rupp, W., Juhl, U. M., Endres. H., Niebl. R., & Balzer, W. (1987). Synthesis, molecular structure and tumor-inhibiting properties of trans-bis (imidazole) tetraehlororuthenate(III) and its methyl-substituted derivatives. *Inorg Chem, 26*(26): 4366-4370. doi: 10.1021/ic00273a018

King, A., Selak, M. A., & Gottlieb, E. (2006). Succinate dehydrogenase and fumarate hydratase: linking mitochondrial dysfunction and cancer. *Oncogene, 25*(34): 4675-4682. doi: 10.1038/sj.onc.1209594

Kita, K., & Takamiya, S. (2002). Electron-transfer complexes in Ascaris mitochondria. *Adv Parasitol, 51*: 95-131. PMID: 12238891

Kliewer, E. V., & Smith, K. R. (1995). Ovarian cancer mortality among immigrants in Australia and Canada. *Cancer Epidemiol Biomarkers Prev, 4*(5): 453-458. PMID: 7549799

Kulmacz, R. J. (1989). Concerted loss of cyclooxygenase and peroxidase activities from prostaglandin H synthase upon proteolytic attack. *Prostaglandins, 38*(3): 277-288. doi: 10.1016/0090-6980(89)90133-0

Kurman, R. J., & Shih, I-M. (2010). The Origin and pathogenesis of epithelial ovarian cancer-a proposed unifying theory. *The American journal of surgical pathology, 34*(3): 433. doi: 10.1097/PAS.0b013e3181cf3d79

Kvåle, G., Heuch, I., Nilssen, S., & Beral, V. (1988). Reproductive factors and risk of ovarian cancer: a prospective study. *Int J Cancer, 42*(2): 246-251. doi: 10.1002/ijc.2910420217

Lacey, J. V., Mink, P. J., Lubin, J. H., Sherman, M. E., Troisi, R., et al. (2002). Menopausal hormone replacement therapy and risk of ovarian cancer. *JAMA, 288*(3): 334-341. doi: 10.1001/jama.288.3.334

Langer, C. J., Leighton, J. C., Comis, R. L., O'Dwyer, P. J., et al. (1995). Paclitaxel and carboplatin in combination in the treatment of advanced non-small-cell lung cancer: a phase ii toxicity, response, and survival analysis. *J Clin Oncol, 13*(8): 1860-1870. doi: 10.1200/JCO.1995.13.8.1860

Li, J., Condello, S., Thomes-Pepin, J., Ma, X., Xia, Y., et al. (2017). Lipid Desaturation Is a Metabolic Marker and Therapeutic Target of Ovarian Cancer Stem Cells. *Cell Stem Cell, 20(*3): 303-314. doi: 10.1016/j.stem.2016.11.004.

Lokich, J., & Anderson, N. (1998). Carboplatin versus cisplatin in solid tumors: an analysis of the literature. *Ann Oncol, 9*(1): 13-21. doi: 10.1023/a:1008215213739

Lu, W., Hu, Y., Chen, G., Chen, Z., et al. (2012). Novel role of NOX in supporting aerobic glycolysis in cancer cells with mitochondrial dysfunction and as a potential target for cancer therapy. *PLoS Biol, 10*(5): e1001326. doi: 10.1371/journal.pbio.1001326

Macheda, M. L., Rogers, S., & Best, J. D. (2005). Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. *J Cell Physiol, 202*(3): 654-662. doi: 10.1002/jcp.20166

Manfredi, J. J., & Horwitz, S. B. (1984). Taxol: an antimitotic agent with a new mechanism of action. *Pharmacol Ther, 25*(1): 83-125. doi: 10.1016/0163-7258(84)90025-1

Marusyk, A., & Polyak, K. (2010). Tumor heterogeneity: causes and consequences. *Biochim Biophys Acta, 1805*(1): 105- 117. doi: 10.1016/j.bbcan.2009.11.002

Mathupala, S. P., Ko, Y. H., & Pedersen, P. L. (2006). Hexokinase II: cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria. *Oncogene, 25*(34): 4777-4786. doi: 10.1038/sj.onc.1209603.

Mathupala, S. P., Rempel, A., & Pedersen, P. L. (2001). Glucose catabolism in cancer cells: identification and characterization of a marked activation response of the type II hexokinase gene to hypoxic conditions. *J Biol Chem, 276*: 43407-43412. doi: 10.1074/jbc.M108181200.

Mayers, J. R., & Vander Heiden, M. G. (2015). Famine versus feast: understanding the metabolism of tumors in vivo. *Trends Biochem Sci, 40*(3): 130-140. doi: 10.1016/j.tibs.2015.01.004

McEvoy, G. K. (editor). (1987). American Hospital Formulary Service Drug Information. Bethesda, Maryland: American Society of Health-System Pharmacists, Inc.

McGowan, L., Norris, H. J., Hartge, P., Hoover, R., & Lesher, L. (1988). Risk factors in ovarian cancer. *Eur J Gynaecol Oncol, 9*(3): 195-199. PMID: 3391190

McGuire, W. P., Hoskins, W. J., Brady, M. F., Kucera, P. R., et al. (1996). Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. *N Engl J Med, 334*(1): 1-6. doi: 10.1056/NEJM199601043340101

McGuire, W. P., Rowinsky, E. K., Rosenshein, N. B., Grumbine, F. C., et al. (1989). Taxol: a unique antineoplastic agent with significant activity in advanced ovarian epithelial neoplasms. *Ann Intern Med, 111*(4): 273-279. doi: 10.7326/0003-4819-111-4-273

McLaughlin, J. R., Risch, H. A., Lubinski, J., Moller, P., Ghadirian, P., et al. (2007). Reproductive risk factors for ovarian cancer in carriers of BRCA1 or BRCA2 mutations: A case-control study. *Lancet Oncol, 8*(1): 26-34. doi: 10.1016/S1470-2045(06)70983-4.

Menendez, J. A., Alarcon, T., & Joven, J. (2014). Gerometabolites: the pseudohypoxic aging side of cancer oncometabolites. *Cell Cycle, 13*(5): 699-709. doi: 10.4161/cc.28079

Menendez, J.A., & Lupu, R. (2007). Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nat Rev Cancer, 7*(10): 763-777. doi: 10.1038/nrc2222

Mestroni, G., Alessio, E., & Sava, G. (1998). International Patent PCT C 07F 15/00. A61 K 31/28, WO. 98/0043.

Metallo, C. M., Gameiro, P. A., Bell, E. L., Mattaini, K. R., Yang, J., et al. (2012). Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. *Nature, 481*(7381): 380-384. doi: 10.1038/nature10602

Mitchell, P. (1961). Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature, 191*: 144-148. doi: 10.1038/191144a0

Montal, E. D., Dewi, R., Bhalla, K., Ou, L., et al. (2015). PEPCK coordinates the regulation of central carbon metabolism to promote cancer cell growth. *Mol Cell, 60*(4):571e583. doi: 10.1016/j.molcel.2015.09.025

Montes, A. F., Gómez, J. G., Viejo, M. N., Bermejo, M. A., et al. (2012). Epidemiology and Etiology of Ovarian Cancer, Ovarian Cancer – Basic Science Perspective, Dr. Samir Farghaly (Ed.), ISBN: 978-953-307-812-0, InTech, doi: 10.5772/27679. Available from:

http://www.intechopen.com/books/ovarian-cancer-basic-science-perspective/etiology-and-epidemiology-ofovar-an-cancer. Accessed 29/08/2019.

Morris, C. R., Sands, M. T., & Smith, L. H. (2010). Ovarian cancer: predictors of early-stage diagnosis. *Cancer Causes Control, 21*(8): 1203-1211. doi: 10.1007/s10552- 010-9547-0.

Mosgaard, B. J., Lidegaard, Ø., Kjaer, S. K., Schou, G., & Andersen, A. N. (1997). Infertility, fertility drugs, and invasive ovarian cancer: a case control study. *Fertil Steril, 67*(6): 1005-1012. doi: 10.1016/s0015-0282(97)81431-8

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods, 65*(1–2): 55-63. doi: 10.1016/0022-1759(83)90303-4.

Muggia, F. (2009). Platinum compounds 30 years after the introduction of cisplatin: implications for the treatment of ovarian cancer. *Gynecol Oncol, 112*(1): 275- 281. doi: 10.1016/j.ygyno.2008.09.034

Mullen, A. R., Wheaton, W. W., Jin, E. S., Chen, P. H., Sullivan, L. B., et al. (2012). Reductive carboxylation supports growth in tumour cells with defective mitochondria. *Nature, 481*(7381): 385-388. doi: 10.1038/nature10642

Mutch, D. G., & Prat, J. (2014). FIGO staging for ovarian, fallopian tube and peritoneal cancer. *Gynecol Oncol, 133*(3): 401-404. doi: 10.1016/j.ygyno.2014.04.013

Nam, H., Campodonico, M., Bordbar, A., Hyduke, D. R., et al. (2014). A systems approach to predict oncometabolites via context-specific genome-scale metabolic networks. *PLoS Comput Biol, 10*(9): e1003837. doi: 10.1371/journal.pcbi.1003837

National Cancer Institute. (2017). Ovarian, Fallopian Tube, and Primary Peritoneal Cancer, https://www.cancer.gov/types/ovarian. Accessed 29/07/2019.

Neijt, J. P., ten Bokkel Huinink, W. W., van der Burg, M. E., van Oosterom, A. T., et al. (1984). Randomised trial comparing two combination chemotherapy regimens (Hexa-CAF vs

CHAP-5) in advanced ovarian carcinoma. *Lancet, 2*(8403): 594-600. doi: 10.1016/s0140-6736(84)90594-4

Nicholls, D. G., & Budd, S. L. (2000). Mitochondria and neuronal survival. *Physiol Rev, 80*(1): 315-360. doi: 10.1152/physrev.2000.80.1.315

Oliveira, L., Caquito, J. M. Jr., & Rocha, M. S. (2018). Carboplatin as an alternative to Cisplatin in chemotherapies: New insights at single molecule level. *Biophys Chem, 241:* 8-14. doi: 10.1016/j.bpc.2018.07.004.

Olson, S. H., Mignone, L., Nakraseive, C., Caputo, T. A., et al. (2001). Symptoms of ovarian cancer. *Obstet Gynecol, 98*(2): 212-217. doi: 10.1016/s0029-7844(01)01457-0

Omura, G., Blessing, J. A., Ehrlich, C. E., Miller, A., et al. (1986). A randomized trial of cyclophosphamide and doxorubicin with or without cisplatin in advanced ovarian carcinoma. A Gynecologic Oncology Group study. *Cancer, 57*(9): 1725-1730. doi: 10.1002/1097-0142(19860501)57:9<1725::aid-cncr2820570903>3.0.co;2-j

Ott, I., & Gust, R. (2007). Non platinum metal complexes as anti-cancer drugs. *Arch Pharm (Weinheim), 340*(3): 117-126. doi: 10.1002/ardp.200600151

Pavlova, N. N., & Thompson, C. B. (2016). The emerging hallmarks of cancer metabolism. *Cell Metab, 23*(1): 27-47. doi: 10.1016/j.cmet.2015.12.006

PDQ® Adult Treatment Editorial Board. PDQ Endometrial Cancer Treatment. Bethesda, MD: National Cancer Institute. Updated 01/02/2019. Available at: https://www.cancer.gov/types/uterine/hp/endometrial-treatment-pdq. Accessed 27/06/2019.

Pelicano, H., Martin, D. S., Xu, R. H., & Huang, P. (2006). Glycolysis inhibition for anticancer treatment. *Oncogene, 25*(34): 4633-4646. doi: 10.1038/sj.onc.1209597

Petros, J. A., Baumann, A. K., Ruiz-Pesini, E., Amin, M. B., et al. (2005). mtDNA mutations increase tumorigenicity in prostate cancer. *Proceedings of the National Academy of Sciences of the United States of America, 102*(3): 719-724. doi: 10.1073/pnas.0408894102

Pfeiffer, T., Schuster, S., & Bonhoeffer, S. (2001). Cooperation and competition in the evolution of ATP-producing pathways. *Science, 292*(5516): 504-507. doi: 10.1126/science.1058079

Pieper, T., Borsky, K., & Keppler, B. K. (1999). Non-platinum antitumor compounds. *Top Biol Inorg Chem, 99*: 171-199.

Pollard, P. J., Brière, J. J., Alam, N. A., Barwell, J., Barclay, E., et al. (2005). Accumulation of Krebs cycle intermediates and over-expression of HIF1 alpha in tumours which result from germline FH and SDH mutations. *Hum Mol Genet, 14*(15): 2231-2239. doi: 10.1093/hmg/ddi227

Prat, J. (2012). Ovarian carcinomas: five distinct diseases with different origins, genetic alterations, and clinicopathological features. *Virchows Arch, 460*(3): 237-249. doi: 10.1007/s00428-012-1203-5

Prat, J. (2015). FIGO Committee on Gynecologic Oncology. FIGO's staging classification for cancer of the ovary, fallopian tube, and peritoneum: abridged republication. J Gynecol Oncol, 26(2): 87-89. doi: 10.3802/jgo.2015.26.2.8758.

Rademaker-Lakhai, J. M., van den Bongard, D., Pluim, D., Beijnen, J. H., & Schellens, J. H. (2004). A Phase I and pharmacological study with imidazolium-trans-DMSO-imidazole-tetrachlororuthenate, a novel ruthenium anticancer agent. *Clin Cancer Res, 10*(11): 3717-3727. doi: 10.1158/1078-0432.CCR-03-0746

Reedijk, J. (1987). The Mechanism of Action of Platinum Antitumor Drugs. *Pure & Appl Chem, 59*(2): 181- 192. doi: 10.1351/pac198759020181

Richardson, G. S., Scully, R. E., Nikrui, N., & Nelson, J. H. (1985). Common epithelial cancer of the ovaries. *N Engl J Med, 312*: 415-424. doi: 10.1056/NEJM198502143120706

Riman, T., Dickman, P. W., Nilsson, S., Correia, N., et al. (2002). Hormone replacement therapy and the risk of invasive epithelial cancer in Swedish women. *J Natl Cancer Inst, 94*(7): 497-504. doi: 10.1093/jnci/94.7.497

Risch, H. A., Marrett, L. D., & Howe, G. R. (1994). Parity, contraception, infertility, and the risk of epithelial ovarian cancer. *Am J Epidemiol, 140*(7): 585-597. doi: 10.1093/oxfordjournals.aje.a117296

Roberts, P. C., Mottillo, E. P., Baxa, A. C., Heng, H. H., Doyon-Reale, N., et al. (2005). Sequential molecular and cellular events during neoplastic progression: A mouse syngeneic ovarian cancer model. *Neoplasia, 7*(10): 944-956. doi: 10.1593/neo.05358

Rockwell, S., Dobrucki, I. T., Kim, E. Y., Marrison, S. T., & Vu, V. T. (2009). Hypoxia and radiation therapy: past history, ongoing research, and future promise. *Curr Mol Med, 9*(4): 442-458. doi: 10.2174/156652409788167087

Roesch, A., Vultur, A., Bogeski, I., Wang, H., Zimmermann, K. M., et al. (2013). Overcoming intrinsic multidrug resistance in melanoma by blocking the mitochondrial respiratory chain of slow-cycling JARID1B (high) cells. *Cancer Cell, 23*(6): 811-825. doi: 10.1016/j.ccr.2013.05.003

Sakai, C., Tomitsuka, E., Esumi, H., Harada, S., & Kita, K. (2012). Mitochondrial fumarate reductase as a target of chemotherapy: from parasites to cancer cells. *Biochim Biophys Acta, 1820*(5): 643-651. doi: 10.1016/j.bbagen.2011.12.013

Sava, G., & Bergamo, A. (2000). Ruthenium-based compounds and tumour growth control (review). *Int J Oncol, 17*(2): 353-365. doi: 10.3892/ijo.17.2.353

Sava, G., & Bergamo, A. (2009). *Ruthenium Drugs for Cancer Chemotherapy: An Ongoing Challenge to Treat Solid Tumours.* In: Bonetti, A., Leone, R., Muggia, F. M., Howell, S. B. (editors). Platinum and other heavy metal compounds in cancer chemotherapy. Cancer Drug Discovery and Development. Humana Press, Totowa, NJ, p. 57-66. doi: 10.1007/978-1-60327-459-3_8

Sava, G., Alessio, E., Bergamo, A., & Mestroni, G. (1999). *Sulfoxide Ruthenium Complexes: Non-Toxic Tools for the Selective Treatment of Solid Tumour Metastases.* In: Clarke, M. J., Sadler, P. J. (editors) Metallopharmaceuticals I. Topics in Biological Inorganic Chemistry, vol 1. Springer, Berlin, Heidelberg. p. 143-169. doi: 10.1007/978-3-662-03815-4_6

Sava, G., Pacor, S., Bergamo, A., Cocchietto, M., Mestroni, G., et al. (1995). Effects of ruthenium complexes on experimental tumors: irrelevance of cytotoxicity for metastasis inhibition. *Chem Biol Interact, 95*(1-2): 109-126. doi: 10.1016/0009-2797(94)03350-1

Sava, G., Pacor, S., Mestroni, G., & Alessio, E. (1992). Na[trans-RuCl4(DMSO)Im], a metal complex of ruthenium with antimetastatic properties. *Clin Exp Metastasis, 10*(4): 273-280. PMID: 1617835

Sava, G., Zorzet, S., Giraldi, T., Mestroni, G., & Zassinovich, G. (1984). Antineoplastic activity and toxicity of an organometallic complex of ruthenium(II) in comparison with cis-PDD in mice bearing solid malignant neoplasms. *Eur J Cancer Clin Oncol, 20*(6): 841-847. doi: 10.1016/0277-5379(84)90223-2

Schluga, P., Hartinger, C. G., Egger, A., Reisner, E., Galanski, M, et al. (2006). Redox behavior of tumor inhibiting ruthenium(III) complexes and effects of physiological reductants on their binding to GMP. *Dalton Trans, 14*: 1796-1802. doi: 10.1039/b511792e

Sciacovelli, M., & Frezza, C. (2016). Oncometabolites: unconventional triggers of oncogenic signalling cascades. *Free Radic Biol Med, 100*: 175-181. doi: 10.1016/j.freeradbiomed.2016.04.025

Shih, I. M., & Kurman, R. J. (2004). Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. *Ame J Pathol, 164*(5): 1511-1518. doi: 10.1016/s0002-9440(10)63708-x

Siegel, R. L., Miller, K. D., & Jemal, A. (2018). Cancer statistics, 2018. *CA Cancer J Clin, 68*(1): 7- 30. doi: 10.3322/caac.21442

Slonczewski, J. L., & Foster, J. W. (2011). *Microbiology: An Evolving Science* (2nd Ed.). New York: W.W. Norton and Company Inc. p. 166.

Son, J., Lyssiotis, C. A., Ying, H., Wang, X., et al. (2013). Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. *Nature, 496*: 101-105. doi: 10.1038/nature12040

Stewart, D. J. (2007). Mechanisms of resistance to cisplatin and carboplatin. *Crit Rev Oncol/Hematol, 63*(1): 12-31. doi: 10.1016/j.critrevonc.2007.02.001

Stronach, E. A., Cunnea, P., Turner, C., Guney, T., et al. (2015). The role of interleukin-8 (IL-8) and IL-8 receptors in platinum response in high grade serous ovarian carcinoma. *Oncotarget, 6*(31): 31593-603. doi: 10.18632/oncotarget.3415

Suffness, M. (1994). Is taxol a surrogate for a universal regulator of mitosis? *In Vivo, 8*(5): 867-878. PMID: 7727737

Swenerton, K., Jeffrey, J., Stuart, G., Roy, M., Krepart, G., et al. (1992). Cisplatin-cyclophosphamide versus carboplatin-cyclophosphamide in advanced ovarian cancer: a randomized phase III study of the national cancer institute of Canada clinical trials group. *J Clin Oncol, 10*(5): 718-726. doi: 10.1200/JCO.1992.10.5.718

Tavsan, Z., Yaman, P. K., Subasi, E., & Ayar Kayalı, H. (2018). Screening organometallic thiophene containing thiosemicarbazone ruthenium(II/III) complexes as potential anti-tumour agents. *J Biol Inorg Chem, 23*: 425. doi: 10.1007/s00775-018-1549-5

Thigpen, J. T., Blessing, J. A., Ball, H., Hummel, S. J., et al. (1994). Phase II trial of paclitaxel in patients with progressive ovarian carcinoma after platinum-based chemotherapy: a Gynecologic Oncology Group study. *J Clin Oncol, 12*(9): 1748-53. doi: 10.1200/JCO.1994.12.9.1748

Todd, R. C., & Lippard, S. J. (2009). Inhibition of transcription by platinum antitumor compounds. *Metallomics: integrated biometal science, 1*(4): 280-291. doi: 10.1039/b907567d

Tomitsuka, E., Kita, K., & Esumi, H. (2010). The NADH-fumarate reductase system, a novel mitochondrial energy metabolism, is a new target for anticancer therapy in tumor microenvironments. *Ann N Y Acad Sci, 1201*(1): 44-49. doi: 10.1111/j.1749-6632.2010.05620.x

Trimble, E. L., Adams, J. D., Vena, D., Hawkins, M. J., et al. (1993). Paclitaxel for platinum-refractory ovarian cancer: results from the first 1,000 patients registered to National Cancer Institute Treatment Referral Center 9103. *J Clin Oncol, 11*(12): 2405-10.

Uekuri, C., Shigetomi, H., Ono, S., Sasaki, Y., et al. (2013). Toward an understanding of the pathophysiology of clear cell carcinoma of the ovary (Review). *Oncol Lett, 6*: 1163-73. doi: 10.3892/ ol.2013.1550.

van Lith, S. M., Navis, A. C., Verrijp, K., Niclou, S. P., et al. (2014). Glutamate as chemotactic fuel for diffuse glioma cells: are they glutamate suckers? *Biochim Biophys Acta - Rev Cancer, 1846*(1): 66-74. doi: 10.1016/j.bbcan.2014.04.004

Vander Heiden, M. G., Cantley, L. C., & Thompson, C. B. (2009). Understanding the Warburg effect: The metabolic requirements of cell proliferation. *Science, 324*(5930): 1029- 1033. doi: 10.1126/science.1160809

Vander Heiden, M. G., Locasale, J. W., Swanson, K. D., Sharfi, H., Heffron, G. J., et al. (2010). Evidence for an alternative glycolytic pathway in rapidly proliferating cells. *Science, 329*(5998): 1492- 1499. doi: 10.1126/science.1188015

Wallace, D. C. (2013). A mitochondrial bioenergetic etiology of disease. *J Clin Invest, 123*(4): 1405-1412. doi: 10.1172/JCI61398

Warburg, O. (1956a). On respiratory impairment in cancer cells. *Science, 124*(3215): 269-270.

Warburg, O. (1956b). On the origin of cancer cells. *Science, 123*(3191): 309-314. doi: 10.1126/science.123.3191.309

Warburg, O., Posener, K., & Negelein, F. (1924). Uber den stoffwechsel der carcinomzelle. *Biochem Z, 152*: 319-44.

Warburg, O., Wind, F., & Negelein, E. (1927). THE METABOLISM OF TUMORS IN THE BODY. *The Journal of general physiology, 8*(6), 519-530. doi: 10.1085/jgp.8.6.519

Ward, P. S., Patel, J., Wise, D. R., Abdel-Wahab, O., et al. (2010). The common feature of leukemia associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. *Cancer Cell, 17*(3): 225-234. doi: 10.1016/j.ccr.2010.01.020.

Weinberg, F., Hamanaka, R., Wheaton, W. W., Weinberg, S., Joseph, J., et al. (2010). Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity. *Proc Natl Acad Sci USA, 107*(19): 8788-8793. doi: 10.1073/pnas.1003428107

Wellen, K. E., Lu, C., Mancuso, A., Lemons, J. M., Ryczko, M., Dennis, J. W., et al. (2010). The hexosamine biosynthetic pathway couples growth factor induced glutamine uptake to glucose metabolism. *Genes Dev, 24*(24): 2784-2799. doi: 10.1101/gad.1985910

Wentzensen, N., Poole, E. M., Trabert, B., White, E., Arslan, A. A., Patel, A. V., et al. (2016). Ovarian Cancer Risk Factors by Histologic Subtype: An Analysis from the Ovarian Cancer Cohort Consortium. *J Clin Oncol, 34*(24): 2888-2898. doi: 10.1200/JCO.2016.66.8178

Wheate, N. J., Walker, S., Craig, G. E., & Oun, R. (2010). The status of platinum anticancer drugs in the clinic and in clinical trials. *Dalton Transactions, 39*(35): 8113-8127. doi: 10.1039/c0dt00292

Whittemore, A. S., Harris, R., & Itnyre, J. (1992). Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. II. Invasive epithelial ovarian cancers in white women. *Collaborative Ovarian Cancer Group. Am J Epidemiol, 136*(10): 1184-1203. doi: 10.1093/oxfordjournals.aje.a116427

Williams, C. J., Mead, G. M., Macbeth, F. R., Thompson, J., et al. (1985). Cisplatin combination chemotherapy versus chlorambucil in advanced ovarian carcinoma: mature results of a randomized trial. *J Clin Oncol, 3*: 1455-1462. doi: 10.1200/JCO.1985.3.11.1455

Wiltshaw E, & Kroner T. (1976). Phase II study of cis-dichlorodiammineplatinum(II) (NSC-119875) in advanced adenocarcinoma of the ovary. *Cancer Treat Rep, 60*(1): 55-60.

Wise, D. R., & Thompson, C. B. (2010). Glutamine addiction: a new therapeutic target in cancer. *Trends in biochemical sciences, 35*: 427-433. doi: 10.1016/j.tibs.2010.05.003

Wise, D. R., DeBerardinis, R. J., Mancuso, A., Sayed, N., et al. (2008). Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. *Proc Natl Acad Sci U S A, 105*: 18782-18787. doi: 10.1073/pnas.0810199105

Wise, D. R., Ward, P. S., Shay, J. E., Cross, J. R., Gruber, J. J., Sachdeva, U. M., et al. (2011). Hypoxia promotes isocitrate dehydrogenase-dependent carboxylation of a-ketoglutarate to citrate to support cell growth and viability. *Proc Natl Acad Sci, 108*: 19611-19616. doi: 10.1073/pnas.1117773108

World Health Organization (WHO). https://www.who.int/news-room/fact-sheets/detail/cancer. Accessed <18/06/2019>.

Wu, W., & Zhao, S. (2013). Metabolic changes in cancer: beyond the Warburg effect. *Acta Biochimica et Biophysica Sinica, 45*(1): 18-26. doi: 10.1093/abbs/gms104

Xu, Y., Gao, W., Zhang, Y., Wu, S., et al. (2018). ABT737 reverses cisplatin resistance by targeting glucose metabolism of human ovarian cancer cells. *International Journal of Oncology, 53*: 1055-1068. doi: 10.3892/ijo.2018.4476.

Yaman, P. K., Şen, B., Karagöz, C. S., & Subaşı, E. (2017). Half-sandwich ruthenium-arene complexes with thiophen containing thiosemicarbazones: Synthesis and structural characterization. *J Organomet Chem, 832*: 27-35. doi: 10.1016/j.jorganchem.2017.01.013

Yuan, C., Clish, C. B., Wu, C., Mayers, J. R., et al. (2016). Circulating metabolites and survival among patients with pancreatic cancer. *J Natl Cancer Inst, 108*:djv409. doi: 10.1093/jnci/djv409

Yuneva, M., Zamboni, N., Oefner, P., Sachidanandam, R., & Lazebnik, Y. (2007). Deficiency in glutamine but not glucose induces MYC-dependent apoptosis in human cells. *J Cell Biol, 178*: 93-105. doi: 10.1083/jcb.200703099

Zhang, C., Zhang, D., & Cheng, J. X. (2015). Coherent Raman Scattering Microscopy in Biology and Medicine. *Annu Rev Biomed Eng, 17*: 415-45. doi: 10.1146/annurevbioeng-071114-040554

Zhao, Y., Butler, E. B., & Tan, M. (2013). Targeting cellular metabolism to improve cancer therapeutics. *Cell Death Dis, 7*(4): e532. doi: 10.1038/cddis.2013.60

Zhou, Y., Tozzi, F., Chen, J., Fan, F., et al. (2012). Intracellular ATP levels are a pivotal determinant of chemoresistance in colon cancer cells. *Cancer Res*, *72*(1): 304-314. doi: 10.1158/0008-5472.CAN-11-1674

8. APPENDIX

8.1. Curriculum Vitae

Gülnur Çırak

Birth Year:	1996
Address:	İzmir Uluslararası Biyotıp ve Genom Enstitüsü (iBG) Dokuz Eylül Üniversitesi Sağlık Yerleşkesi Balçova 35340 İzmir
e-mail:	gulnurcirak@gmail.com

EDUCATION

Country	University	Faculty/Institute	Field of Study	Degree	Graduation Year
TR	Dokuz Eylul University	Izmir International Biomedicine and Genome Institute	Molecular Biology and Genetics	MSc	2019
TR	Istanbul University	Faculty of Science	Molecular Biology and Genetics	BA	2017

ACADEMIC EXPERIENCES

University	Country	City	Department	Type	Date
Izmir Institute of Technology	TR	Izmir	Molecular Biology and Genetics	Internship	July-August 2016
Ege University	TR	Izmir	Bioengineering, BILTEM	Internship	January-February 2015
Ege University	TR	Izmir	Faculty of Medicine, Department of Medical Biology	Internship	July 2014

RESEARCH FIELDS

Molecular Cancer Biology, Energy Metabolism, Retrotransposons, Gene editing